# Ultrastructure of the uterus of *Xiphinema pinoides* (Nematoda)

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**Summary** — A study of the ultrastructure of the uterus of *Xiphinema pinoides* showed that the basic features are similar to those reported for *X. meridianum* and *X. theresiae*. However, it differs in some details, and in the extreme rarity of crystalloids and absence of spines. Apparently the glandular cells of the *pars dilatata uteri* produce large amounts of secretion which forms a thick layer covering the inner plasma membrane of the cells bordering the lumen of the uterus. From this layer, membranous structures project into the lumen that connect with globular inclusions of the Z-differentiation. The latter seem to be derived from the same secretion, plus some engulfed material.

**Résumé** — *Ultrastructure de l'utérus de* Xiphinema pinoides *(Nematoda)* — L'étude de l'ultrastructure de l'utérus de *Xiphinema pinoides* montre que sa structure de base est identique à celle précédemment rapportée pour *X. meridianum* et *X. theresiae*. Cependant, certains détails sont différents, de même que l'extrême rareté des cristalloïdes et l'absence d'épines. Apparemment, les cellules glandulaires de la *pars dilatata uteri* produisent une quantité importante d'une sécrétion formant une couche épaisse recouvrant la membrane plasmatique interne des cellules bordant la lumière de l'utérus. A partir de cette couche, des structures membraneuses font saillie dans la lumière, reliant ainsi sa couche interne aux inclusions globuleuses de la différentiation Z. Ces dernières paraissent constituées à partir de cette même sécrétion et de matériel englobé.

**Key-words** : *Xiphinema*, ultrastructure, nematodes.

This paper is one of a series on the structure of the female reproductive system in the genus *Xiphinema*. The entire system has been investigated in *X. pinoides*, but as all other parts are completely comparable to those in *X. meridianum* and *X. theresiae* (Van de Velde *et al.*, 1990*a*, *b*), this study deals only with the uterus. The terminology used follows Coomans (1965) and Van de Velde *et al.* (1990*a*, *b*).

## Materials and methods

Soil samples were collected in South Africa 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 picked out of the extraction medium and placed in an ice bath for a few minutes in order to stretch the specimens. Then they were killed and fixed in ice-cooled fixative, composed of 0.75 % acrolein, 1.5 % glutaraldehyde and 0.75 % paraformaldehyde in 0.1 M sodium cacodylate buffer. After approximately 15 h of fixation at 7 °C, they were rinsed in 0.2 M sodium cacodylate buffer for 8 h. During this rinse the nematodes were cut into pieces of roughly 200-300 μm, to facilitate penetration.

Postfixation took place 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 cut on a Reichert OMU-2 ultramicrotome and picked up on formvar-coated slotted grids. The sections were post-stained in a LBK ultrastainer, for 30 min in uranyl acetate at 40 °C and 5 min in lead stain at 20 °C.

## Results

The uterus is comprised of a wide proximal part, the *pars dilatata uteri (p.d.u.)* and a tubular part that is distally connected to the ovejector. Inside the tubular part, a short distance from the *p.d.u.* occurs the Z-differentiation with four irregularly shaped inclusions (Fig. 1).

The wall of the whole uterus consists of an outer basal lamina, a muscle layer and an inner lining. A prominent glandular epithelium occurs in the *p.d.u.*

The tubular part has a wide lumen and a relatively thin wall (Fig. 2 A). The outer basal lamina (250-500 nm thick) covers the outer plasma membrane of the muscle cells (Fig. 2 B). The muscle filaments run predominantly transverse and are arranged in bundles separated by thin strands of cytoplasm. The filaments occupy a 1-2 μm thick zone throughout most of the length of the tubular part, but in the short (proximal) area between the *p.d.u.*
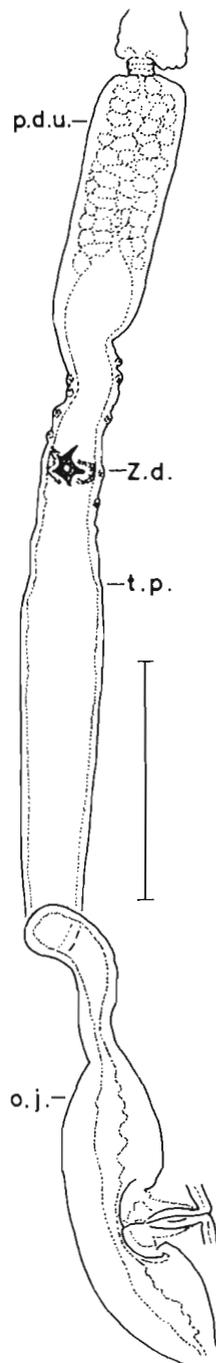
**Fig. 1.** Anterior uterus of *Xiphinema pinoides*.

List of abbreviations : *b.l.* : basal lamina; *cr.* : crystalloid; *g.e.r.* : granular endoplasmic reticulum; *g.i.* : globular inclusion; *i.l.* : inner lining; *m.l.* : muscle layer; *m.v.* : microvilli; *N* : nucleus of muscle cell; *N'* : nucleus of gland cell; *n* : nucleolus; *o.j.* : ovejector; *p.d.u.* : *pars dilatata uteri*; *s.c.* : sperm cells; *s.v.* : secretory vacuole; *t.p.* : tubular part of uterus; *Z.d.* : Z-differentiation. *(Bar = 60 µm.)*

and the Z-differentiation (Fig. 2 C) the zone is 3-5 µm thick. The nuclei of the muscle cells are quite prominent, irregular in shape, with finely granular nucleoplasm, very electron-dense chromatin and a somewhat less electron-dense nucleolus. The nuclei usually occur near the outer cell membrane, bulge out of the uterus contour, and are located peripheral to the muscle filaments (Fig. 2 A-C). Occasionally the nuclei may occur inside the inward protrusions of the muscle cells where they appear to lie between the muscle filaments and the lumen wall (Fig. 2 A, lower nucleus). The inner cell membrane is covered by a 250-500 nm thick, amorphous substance, that together form the inner, folded lining of the lumen. The folds are variable in shape and length, they enclose cytoplasm and occasionally a nucleus (see above). The lumen contains a fine granular material within which are scattered some small electron-dense globules and very few small crystalloids (Fig. 2 A).

The wall of the Z-differentiation has essentially the same structure as that of the tubular part, but is slightly thicker due to the greater number of muscle filaments that occupy a 4-8 µm thick zone (Figs 2 C, 3 A). The amorphous material on the inner plasma membrane forms thin membrane-like extensions into the lumen that sometimes encompass parts of the lumen (Figs 2 D, 3 B, C). Some of these projections enlarge, seem to engulf granular and other material from the uterine lumen and connect with globular inclusions (Figs 3 B, C). The latter consist of the same amorphous material that forms the inner lining, and of vacuolar spaces with different kinds of usually electron-dense material. These vacuoles are formed when the amorphous material encompasses material from the lumen; they vary considerably in size and content, but crystalloids were not observed in any of them (Figs 2 C, 3 A, C).

The *pars dilatata uteri*, which is about 50-70 µm long and 15-18 µm wide, is highly glandular throughout most of its length, but compared to the main part of the *p.d.u.* the cells near the distal end are smaller. These gland cells contain well developed granular endoplasmic reticulum and have abundant secretory material stored in a variety of inclusions ranging from small granules to huge vacuoles (Figs 4 A, B). The secretory material, which is moderately electron-dense, contains various inclusions that are sometimes reminiscent of degenerated cell organelles (Fig. 4 C). At the borderline of the large vacuoles, the endoplasmic reticulum appears partly broken up. The nuclei of the gland cells are larger and less electron-dense than those of the muscle cells; the nucleolus is generally bigger than that in the muscle cell nuclei (Fig. 4 B). The gland cells occupy most of the *p.d.u.* Towards the centre of the lumen, the apical membranes of these cells may be highly folded or give rise to long microvilli-like projections (Fig. 4 B). The lumen becomes more evident distally where it connects with the lumen of the tubular part. In between the gland
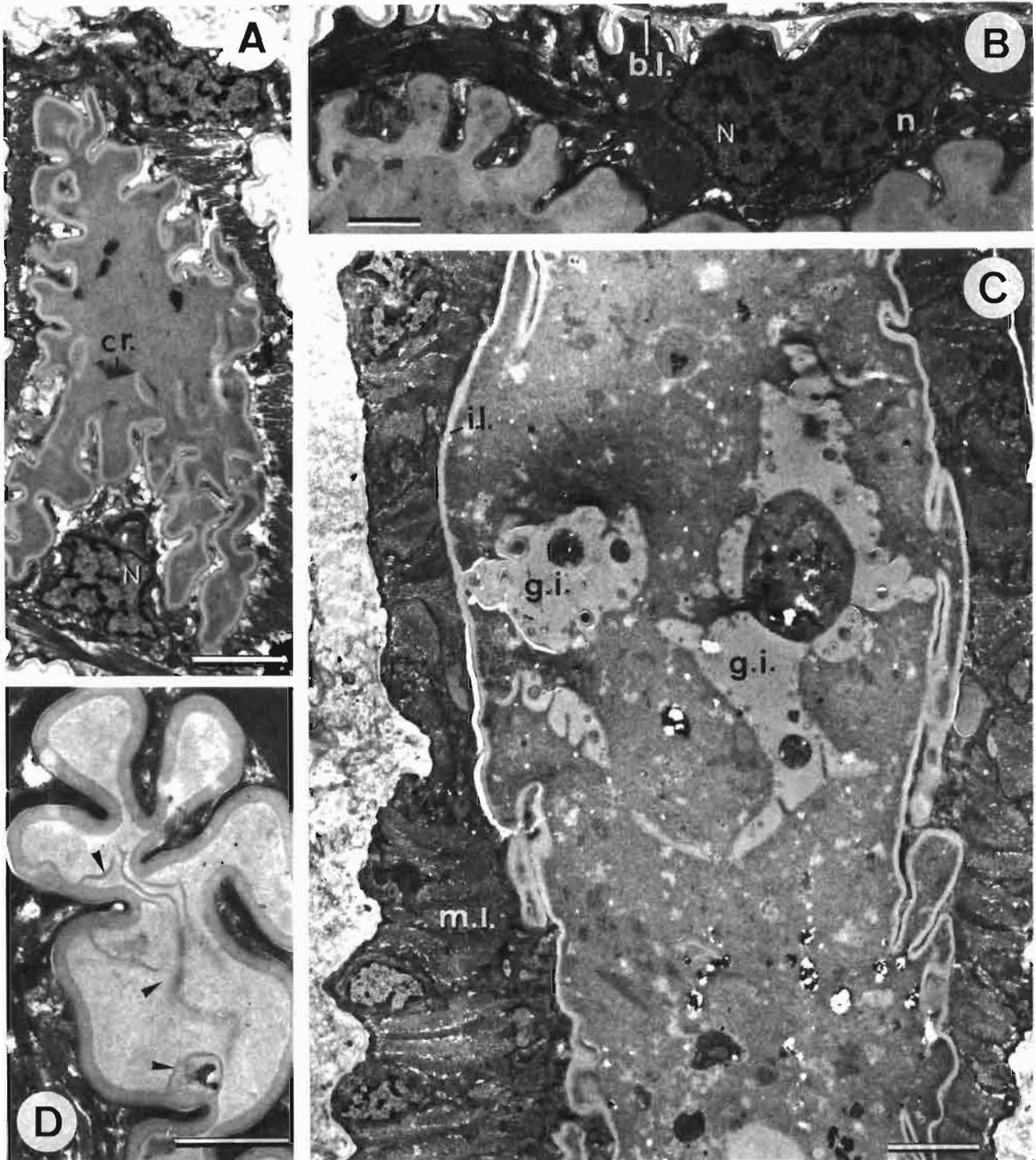
**Fig. 2.** A : Slightly oblique transverse section through the tubular part of the uterus; B : Detail of the wall of the tubular part, transverse section; C : Longitudinal section through the Z-differentiation; D : Inner lining with membrane-like extensions (arrowheads) near Z-differentiation, transverse section. *(Bar equivalent : A = 2 µm; B = 1 µm; C = 2 µm; D = 1 µm.)* For abbreviations, see Fig. 1.
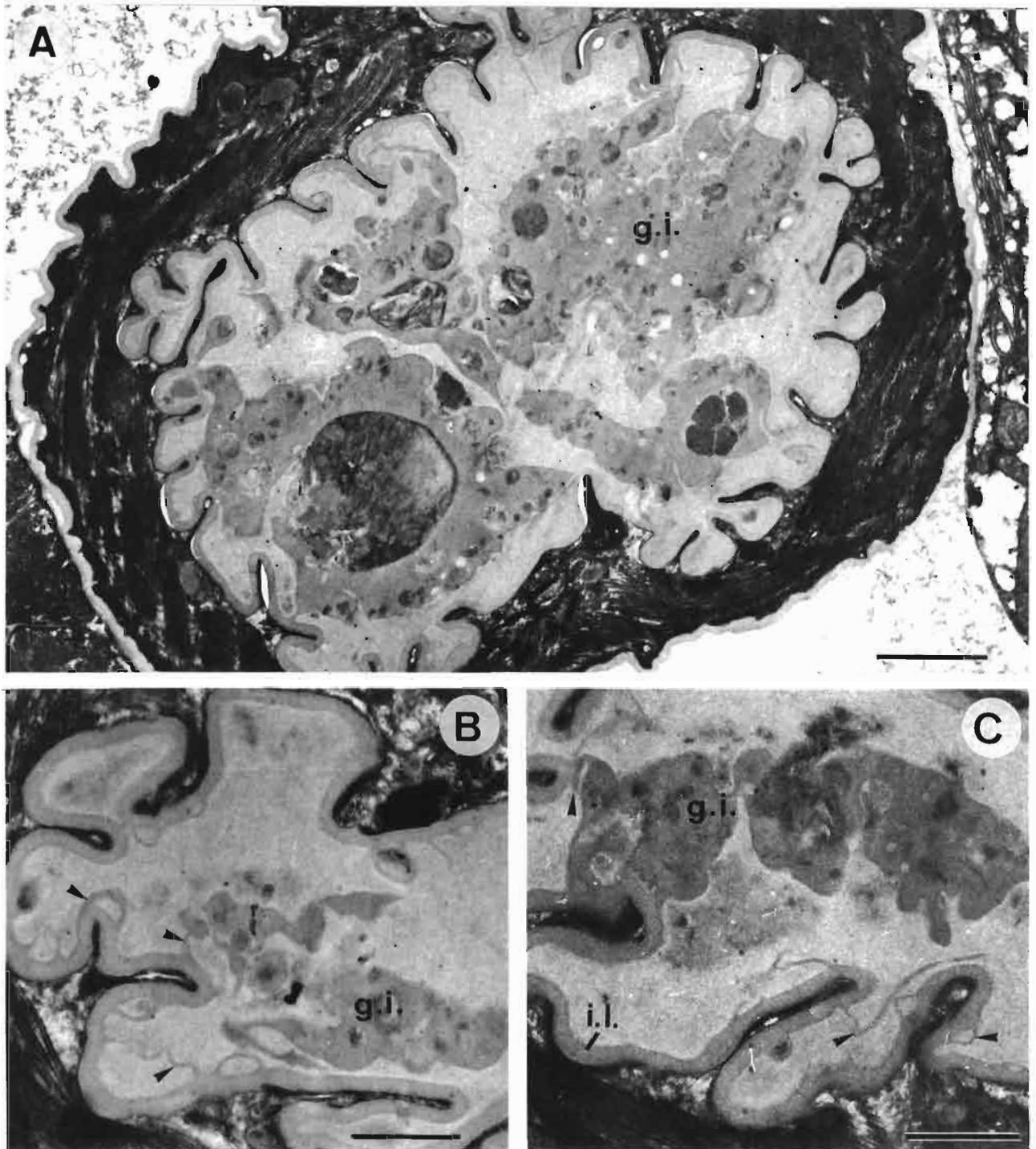
**Fig. 3.** A : Transverse section through Z-differentiation. B-C : Details of connections between inner lining, membrane-like extensions (arrowheads) and globular inclusions in the Z-differentiation, transverse section. *(Bar equivalent : A = 2 μm; B = 1 μm; C = 1 μm.)* For abbreviations, see Fig. 1.
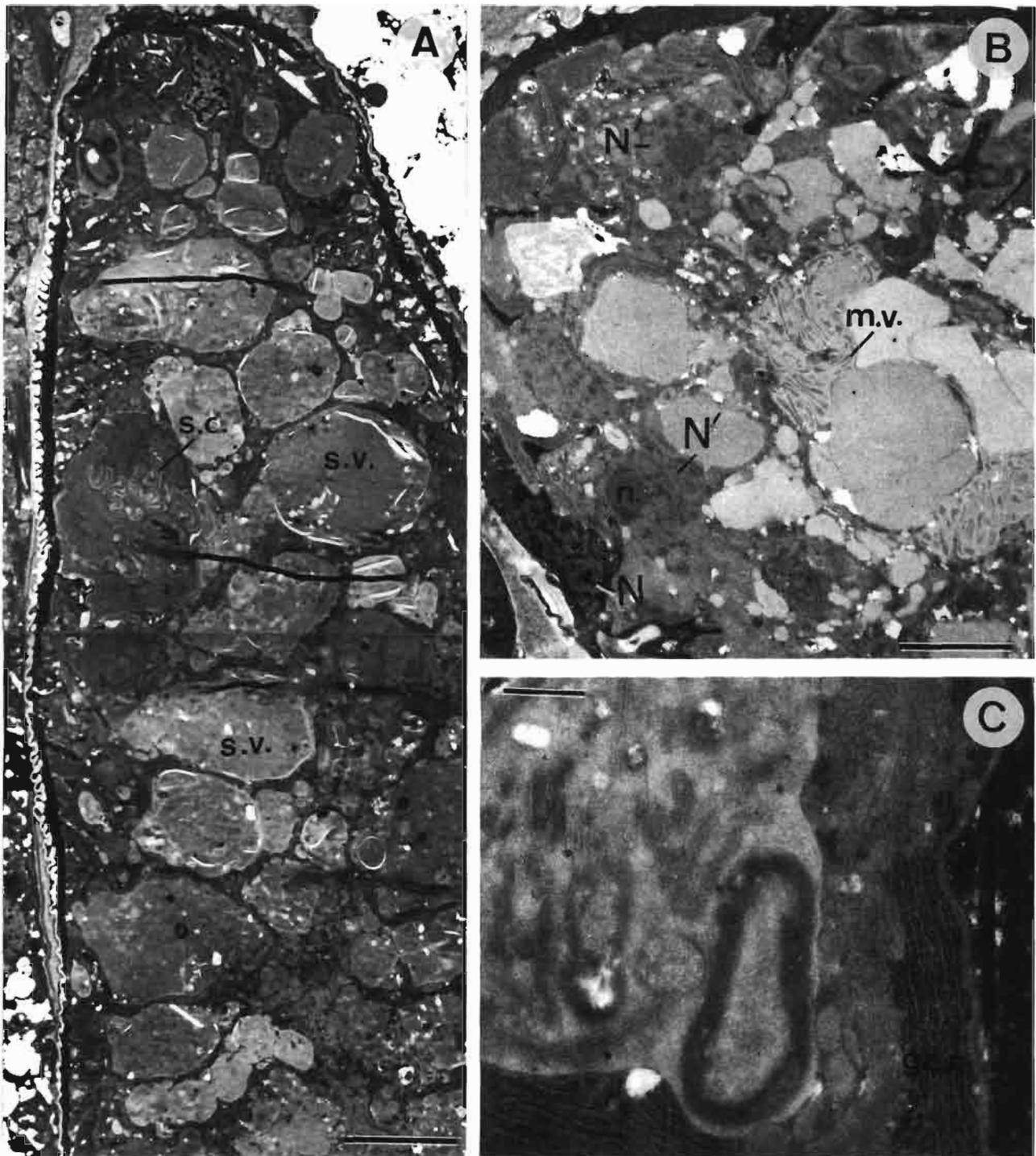
**Fig. 4.** A : Longitudinal section of the glandular part of the *pars dilatata uteri;* B : Transverse section of the same; C : Details of the cytoplasm and inclusions of a gland cell. *(Bar equivalent : A = 4 µm; B = 2 µm; C = 0.5 µm.)* For abbreviations, see Fig. 1.

cells, and more so distally than proximally, sperm cells are usually stored in clusters of five to about twenty (Fig. 4 A).

At the base of the glandular epithelium the inner cell membrane of the muscle cells forms narrow infolds filled with cytoplasm and sporadically wider folds enclosing nuclei. However, most of the muscle cell nuclei are inside the main part of the wall, i.e. below the glandular epithelium, centrally from the muscle filaments. The latter are mainly transverse, but also oblique in orientation and form a 0.65-1.3 µm thick layer. The outer basal lamina is 250-500 nm thick; it forms many short (1-2 µm) outward protrusions (Fig. 4 A).

## Discussion

The structure of the uterus in *X. pinoides* resembles that described for *X. meridianum* (Van de Velde *et al.*, 1990*a*) and *X. theresiae* (Van de Velde *et al.*, 1990*b*) except in some details, such as the extreme rarity of crystalloids and the absence of spines.

Large and active glandular cells in the *p.d.u.* apparently produce large amounts of a secretion which, once in the uterine lumen, covers the entire inner plasma membrane of the bordering cells to form a thick inner lining of the lumen. The same secretion also seems to form the globular inclusions of the Z-differentiation since — apart from the " engulfed " material — these inclusions have the same electron density and are in direct contact with the lining. Why the globular inclusions form in the Z-differentiation area and not elsewhere is not known. However, the membrane-like projections from the inner lining in that area may " catch " or act as accretion centres for some of the secretion that enters the uterine lumen. The globular inclusions could then form around such centres by further deposition of secretion, thereby also engulfing other substances present in the lumen. This would explain the differences in electron-density and configuration of these inclusions.

The amorphous secretion on the inner plasma membrane of the muscle cells presumably protects these cells from damage during egg passage. Such a function does not exclude the previously proposed hypothesis that the secretion contributes to the formation of an outer layer of the egg shell (Coomans, 1965; Bird, 1976; McClure & Bird, 1976; Bleve-Zacheo *et al.*, 1976), but is complementary to it. In a number of species the secretions

also form the globular or variously shaped inclusions, called the Z-differentiation. We are still unable to offer any proof of their function. Slowing down the egg passage may be a result of their presence, but is not necessarily a primary function. The globules may be simply by-products of the secretory activity of the *p.d.u.* The same could be true for the crystalloids found in a number of species.

The secretion process in the gland cells of the *p.d.u.* is to some extent enigmatic. Among gland cells there have been clear signs of organelle degeneration including fragmentation of endoplasmic reticulum at the margin of or inside the large secretion-filled vacuoles. Although the possibility of artifacts cannot be excluded, the constant presence of these phenomena in all five specimens studied with TEM points to the occurrence of an apparently autophagous mechanism and a possible apocrine (cf. also Van de Velde *et al.*, 1990*a*) or even holocrine type of secretion.

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