

Comparative morpho-anatomical studies of the female gonoduct within the Pratylenchidae (Nematoda: Tylenchina)

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Summary – The cellular morphology of the gonoduct of six *Pratylenchus* species, three *Pratylenchoides* species, *Radopholus similis*, *Zygotylenchus guevarai*, *Hirschmanniella loofi* and *Nacobbus aberrans* was revealed by dissection and light microscopy. Except for *Nacobbus aberrans*, all studied species show an overall similarity in gonoduct construction, i.e., an ovary often ending with a ring of cells, an oviduct formed from two rows of four cells and a 12-celled spermatheca followed by a tricolumella containing 16-24 cells. *Pratylenchoides magnicauda* and *Z. guevarai* did not diverge from the other Pratylenchidae in this respect, although their gonoduct differs from that of *Amplimerlinius* and *Meloidogyne*, both formerly postulated as related genera. The spermatheca structure observed in *N. aberrans* has not been reported elsewhere in the Nematoda, although the uterus is similar to that reported within the Heteroderinae and Meloidogyninae and the uterus comprises more than 300 cells, enlarging from a tricolumella to a polycolumella. Transmission electron microscopy of *Z. guevarai* revealed details of the cytoplasmatic contact between epithelial cells and the germ cells; a finger-like ovarian wall cell extension was found penetrating the oocyte. The oviduct lacks a preformed lumen and comprises eight cells with highly plicated cell membranes. The spermatheca is constructed from flattened wall cells and is followed by columnar uterus cells where evidence of eggshell formation was demonstrated.

Keywords – gonad, *Hirschmanniella*, morphology, *Nacobbus*, *Pratylenchus*, *Pratylenchoides*, TEM, ultrastructure, *Zygotylenchus*.

Light microscopic studies of the female reproductive system of some Pratylenchidae were reported by Seinhorst (1968), Roman and Hirschmann (1969); Geraert (1973) and Chizhov and Berezina (1988). The gonad development of *Pratylenchus crenatus* Loof, 1960 was studied using light microscopy (LM) by Dickerson (1962) while on the ultra-structural level the female gonad of *Pratylenchus penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941 was examined in elaborate detail by Endo *et al.* (1997, 1999) with the transmission electron microscope (TEM).

Identification of root-lesion nematodes is difficult because of little morphological variation and overlapping morphometrical characters (Roman & Hirschmann, 1969) whilst many phylogenetic questions within the Pratylenchidae remain unanswered: the position of *Pratylenchoides magnicauda* (Thorne, 1935) Baldwin, Luc & Bell, 1983 (Baldwin *et al.*, 1983; Ryss & Sturhan, 1994) and *Nacobbus* (Baldwin & Cap, 1992) is questionable; parsimony analyses of 26S rDNA of *Pratylenchus* spp. showed that the genus represented a paraphyletic assemblage (Al-

Banna *et al.*, 1997); *Zygotylenchus* has been suggested as the evolutionary link to the Meloidogyninae (Geraert, 1997; Pourjam *et al.*, 2000) and, in a recent phylogenetic analysis of 18 SSU rDNA, the Pratylenchidae did not prove to be a monophyletic group (De Ley & Blaxter, 2002).

In view of the importance of the female reproductive system in nematode systematics (Geraert, 1981, 1983), we examined the cellular gonoduct architecture of several species and genera within the Pratylenchidae. The female gonoduct of *Pratylenchus* spp., *Pratylenchoides* spp., *Zygotylenchus guevarai* (Tobar Jiménez, 1963) Braun & Loof, 1966, *Radopholus similis* (Cobb, 1893) Thorne, 1949, *Hirschmanniella loofi* Sher, 1968 and *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen 1944 was studied by means of LM after dissection of the reproductive system. *Pratylenchus penetrans* and *Z. guevarai* were also examined using TEM. As the comprehensive studies on female *P. penetrans* gonad ultra-structure by Endo *et al.* (1997, 1999) agreed with our findings, we only present the TEM results of *Z. guevarai* in this paper.

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Material and methods

NEMATODE MATERIAL

A list of the examined species is presented in Table 1. The populations were extracted from soil samples or carrot disk cultures. *Pratylenchus penetrans* and *Z. guevarai* were extracted from soil samples, surface sterilised with 2000 ppm streptomycin sulphate (Sigma, St. Louis, MO, USA) for 12 h and rinsed three times with sterile distilled water. Five to ten individuals of each population were used to establish monoxenic cultures on carrot disks (Moody *et al.*, 1973).

TERMINOLOGY

The terminology of the reproductive system is based on Geraert (1983) who followed the interpretation of Chitwood and Chitwood (1950). The genital system consists of an ovary (=gonad) and gonoduct. The oviduct is a constricted region between the ovary and the spermatheca or uterus. Here the term uterus is restricted to the columnar region of the gonoduct. A uterine sac follows the uterus. The term tricolumella (Hirschmann & Triantaphyllou, 1968) describe the spatial arrangement of the uterus cells. The terms distal and proximal are used to describe the position of whatever part in relation to the vulva position.

LIGHT MICROSCOPY

The method used to study the cellular structure of the female reproductive system was based on Geraert (1973). Gonads were first extruded, four to six young females of every population being put in a drop of water on a glass slide and then cut with an eye-knife, to expel the gut and gonad. This preparation was either stained with acetic orcein (2% aqueous solution of orcein in acetic acid) or observed directly under LM. Both methods were applied. Using stains results in a stronger differentiation of the nuclei whereas general cell morphology is better preserved without staining. Classical stains were unsatisfactory for staining the *Nacobbus* gonoduct cells. To visualise the nuclei, a drop of 4,6-diamidino-2-phenylindole (DAPI, Sigma) at a concentration of 1 µg/ml in PBS with 0.1% Triton-X100 was added to the dissected gonads.

The cellular structure of the ovary was only partly studied with LM as it was difficult to observe all structures; only the ripening zone of the ovary contained distinct cells which could be visualised with the techniques used.

TRANSMISSION ELECTRON MICROSCOPY

Adult females were picked out and placed on ice to relax. They were killed and fixed in ice-cooled Karnovsky's (1965) fixative, composed of 2% paraformaldehyde, 2.5% glutaraldehyde and 0.5% CaCl₂ in 0.134 M sodium cacodylate buffer. The nematodes were bisected to improve permeability. After approximately 15 h of fixation at 4°C, they were rinsed in 0.134 M sodium cacodylate buffer for 8 h. Post-fixation took place in reduced osmium, a mixture of 1 ml OsO₄ (4%), 3 ml Na cacodylate (0.134 M) and 66 mg K₃Fe(CN)₆, for 36 h at pH 7.4. After rinsing with double distilled water the specimens were dehydrated in 50, 75, 90% and absolute ethanol to which CuSO₄ bars were added to remove any remaining water. The specimens were subsequently infiltrated with a low-viscosity embedding medium (Spurr, 1969). Ultrathin (50-90 nm) longitudinal sections were cut on a Reichert ultracut ultramicrotome (Leica, Vienna, Austria) with a diamond knife (Diatome Ltd., Biel, Switzerland) and mounted on formvar coated single slot copper grids (Agar Scientific, Stansed, UK). The sections were stained (EM stain, Leica) with uranyl acetate and lead citrate and viewed with a Jeol JEM-1010 (Jeol Ltd, Tokyo, Japan) transmission electron microscope operating at 60 kV.

Results

A schematic overview of the results is presented in Fig. 3.

PRATYLENCHUS FILIPJEV, 1936 (FIG. 1)

The ripening zone of the ovary comprises 16 cells. At the transition to the oviduct in young females, eight cells form a sphincter-like structure, although in older specimens those cells were more dispersed. Eight randomly arranged cells precede this circle. The oviduct forms a clear constriction between ovary and spermatheca and is composed of eight flattened cells in two rows. In some specimens of *P. scribneri* Steiner, 1943 an additional pair of smaller cells could be observed between the oviduct and the ovary (Fig. 1F). These cells looked like oviduct cells, but contained smaller and darker nuclei.

In all species observed, the spermatheca was formed from a total of 12 cells, the arrangement of which is species specific. The spermatheca of *P. crenatus* comprises ten, more or less rounded, cells in variable positions with two, more elongated, cells making the connection to the uterus (Fig. 1A). The spermatheca of *P. coffeae*

Table 1. Host, origin and source of studied *Pratylenchidae* populations. All populations were extracted from carrot disk cultures, except for the field populations and *Nacobbus aberrans* (from tomato).

Species	Original host or sampling site	Origin of population	Source
<i>Hirschmanniella loofi</i>	Reed (<i>Phragmites australis</i>)	Bourgoyen-Ossemers, Ghent, Belgium	field population
<i>Nacobbus aberrans</i>	Tomato	not known	G. Karssen, Plant Protection Service, Wageningen, The Netherlands
<i>Pratylenchus penetrans</i>	Apple orchard	Kerkom, Belgium	Agricultural Research Centre, Department Crop Protection, Ghent, Belgium (CLO-Gent)
	Not known	Azores, Portugal	
	Apple orchard	Eksaarde, Belgium	field population
	Grass land	Bourgoyen-Ossemers, Ghent, Belgium	field population
	Grass land with plum tree	Ingelmunster, Belgium	field population
<i>P. coffeae</i>	Banana	not known	CLO-Gent
<i>P. crenatus</i>	Maize-field	Eksaarde, Belgium	field population
<i>P. flakkensis</i>	Grassland (dominated by <i>Arrhenatherus elatius</i> and <i>Holcus lanatus</i>)	Eksaarde, Belgium	field population
<i>P. scribneri</i>	Maize	Vero Beach, FL, USA	CLO-Gent
<i>P. thornei</i>	Chick-pea	Santaella, Córdoba, Spain	CLO-Gent
<i>P. vulnus</i>	Unknown	Unknown	CLO-Gent
<i>P. zaeae</i>	Maize	South Africa	CLO-Gent
<i>Pratylenchoides magnicauda</i>	River bank (dominated by <i>Trifolium</i> sp., <i>Quercus</i> sp. and <i>Salix</i>)	Redu, Ardennes, Belgium	A. Ryss, Russian Academy of Sciences, St Petersburg, Russia
<i>Pratylenchoides ritteri</i>	Wheat	Apulia, Italy	A. Troccoli, Istituto di Nematologia Agraria, Bari, Italy
<i>Pratylenchoides crenicauda</i>	Lawn, in the vicinity of a willow tree (<i>Salix matsudana</i> Koidz)	Botanical garden, Ghent University, Belgium	field population
<i>Radopholus similis</i>	Banana	Sennar, Sudan	G. Elbadri, Crop Protection, Wad Medani, Sudan
<i>Zygotylenchus guevarai</i>	Pistachio	Kerman, Iran	E. Pourjam, Tarbiat Modares University, Iran

(Zimmerman, 1898) Filipjev & Schuurmans Stekhoven, 1941 is not clearly differentiated from the uterus. Twelve cells were counted, but an unambiguous cellular architecture could not be described (Fig. 1B). The spermatheca of *P. thornei* Sher & Allen, 1953 is partly offset, the offset portion comprising on average four cells, the spermatheca cells being more or less round and equally sized (Fig. 1C). The spermatheca of *P. penetrans* is asymmetrical and the oviduct is not connected to the spermatheca entirely axi-

ally. The slightly protruding corner of the spermatheca on the oviduct side comprises small cells of various shape, two large rounded cells making the connection to the uterus (Fig. 1D). The oviduct of *P. zaeae* Graham, 1951 is partly enveloped by the spermatheca, which has six cells variably arranged in the region close to the oviduct, followed by four slightly larger cells with two cells making the connection to the uterus (Fig. 1E). The spermatheca of *P. scribneri* is asymmetrical and slightly offset due to the

protruding and larger spermatheca cells at one side of the spermatheca. Four spermatheca cells make the connection to the uterus (Fig. 1F). The spermatheca of *P. flakkensis* Seinhorst, 1968 and *P. vulnus* Allen & Jensen, 1951 also seems to comprise 12 cells, but the number of successful dissections was too low to obtain reliable results.

The uterus of all studied *Pratylenchus* species consists of three rows of cells (=a tricolumella), each row being four cells long. The uterine sac follows the columnar uterus.

ZYGOTYLENCHUS GUEVARAI

Light microscopy

The ovary connects to the oviduct via a ring of eight cells (Fig. 2A). Two rows of four cells comprise the oviduct. The spermatheca has 12 cells, the two cells connecting it to the uterus being clearly larger. The sperm cells are variously shaped and often swollen. They are also found at the beginning of the uterus which is formed from three rows of four cells.

Transmission electron microscopy

The description below is based on the posterior branch of the didelphic reproductive system (Fig. 4A), with the exception of one picture of the spermatheca taken from the anterior branch (Fig. 6).

The ovarian wall cells form a thin layer around the germ cells and have extensions between some of the germ cells. These cytoplasmatic, finger-like extensions can even enter the oocytes (Fig. 4B). In the germinal zone the oocytes, arranged in a single file, have a clear cell membrane and the large, variably shaped nucleus contains a dark spherical nucleolus. As the oocytes become older they increase in size and contain more and more lipid droplets and yolk.

The oviduct (Fig. 5) consists of irregularly shaped cells having highly plicated cell membranes. In one longitudinal section four adjacent oviduct nuclei could be seen; two nuclei at the same level being observed close to the spermatheca. No preformed lumen was observed. The oviduct cells contain unknown secretions and nuclei with irregularly shaped nuclear membranes lined with dense chromatin.

The spherical spermatheca (Fig. 6) occupies almost the whole body cavity as observed in a transverse section. The spermatheca wall cells are highly flattened, their nuclei being oval with dispersed dense chromatin. The variably shaped sperm cells have a thick head portion containing the nucleus and a thinner, more elongated,

tail. The nucleus lacks a membrane and is seen as a prominent mass of chromatin surrounded by clusters of mitochondria.

The columnar uterus cells (Fig. 7A, B) have plicated membranes. The cytoplasm is filled with mitochondria, numerous ribosomes and some inclusions with both heavily and slightly stained zones. The nucleus contains a prominent nucleolus and some chromatin adjoins the nuclear membrane. A preformed uterus lumen was not observed, the columnar cells being pressed against each other and forming 'membrane-junctions' (Fig. 7B) as described by Endo *et al.* (1997, 1999).

The columnar uterus is followed by a uterine sac (Fig. 8) filled with a finely granular substance. The cuticula of the vagina is continuous with the ventral lining of the uterus sac while a flattened cellular wall lines the rest of the uterine sac.

PRATYLENCHOIDES WINSLOW, 1958 (FIG. 2B, C, D)

The ripening zone of the ovary consists of 16 cells with a ring formed from a variable number of cells connecting the ovary to the oviduct. The oviduct of *P. magnicauda* (Fig. 2C) and *P. ritteri* Sher, 1970 (Fig. 2D) comprises two rows of four cells. In *P. crenicauda* Winslow, 1958 (Fig. 2B) only three cells were observed in each row for the majority of the observed specimens, although an oviduct with two rows of four cells has also been observed. The spermatheca and oviduct of an excised gonad forms the corner of an angle between ovary and uterus. The spermatheca of the studied *Pratylenchoides* species comprises 12 variously shaped cells, the spermatheca cells of *P. ritteri* being more rounded and protruding. The uterus is constructed from three rows of six to seven columnar cells.

RADOPHOLUS SIMILIS (FIG. 2E)

The ripening zone of the ovary has four groups of four cells. The oviduct comprises two rows of four, flattened, cells. The spermatheca always contains 12, variably positioned, cells and is followed by a tricolumella comprising 12 cells.

HIRSCHMANNIELLA LOOFI (FIG. 2F)

The ripening zone could not be distinguished from the remainder of the ovary. The oviduct comprises two rows of four cells. The spermatheca cells are indistinctly delimited and their cell borders are twisted. The number

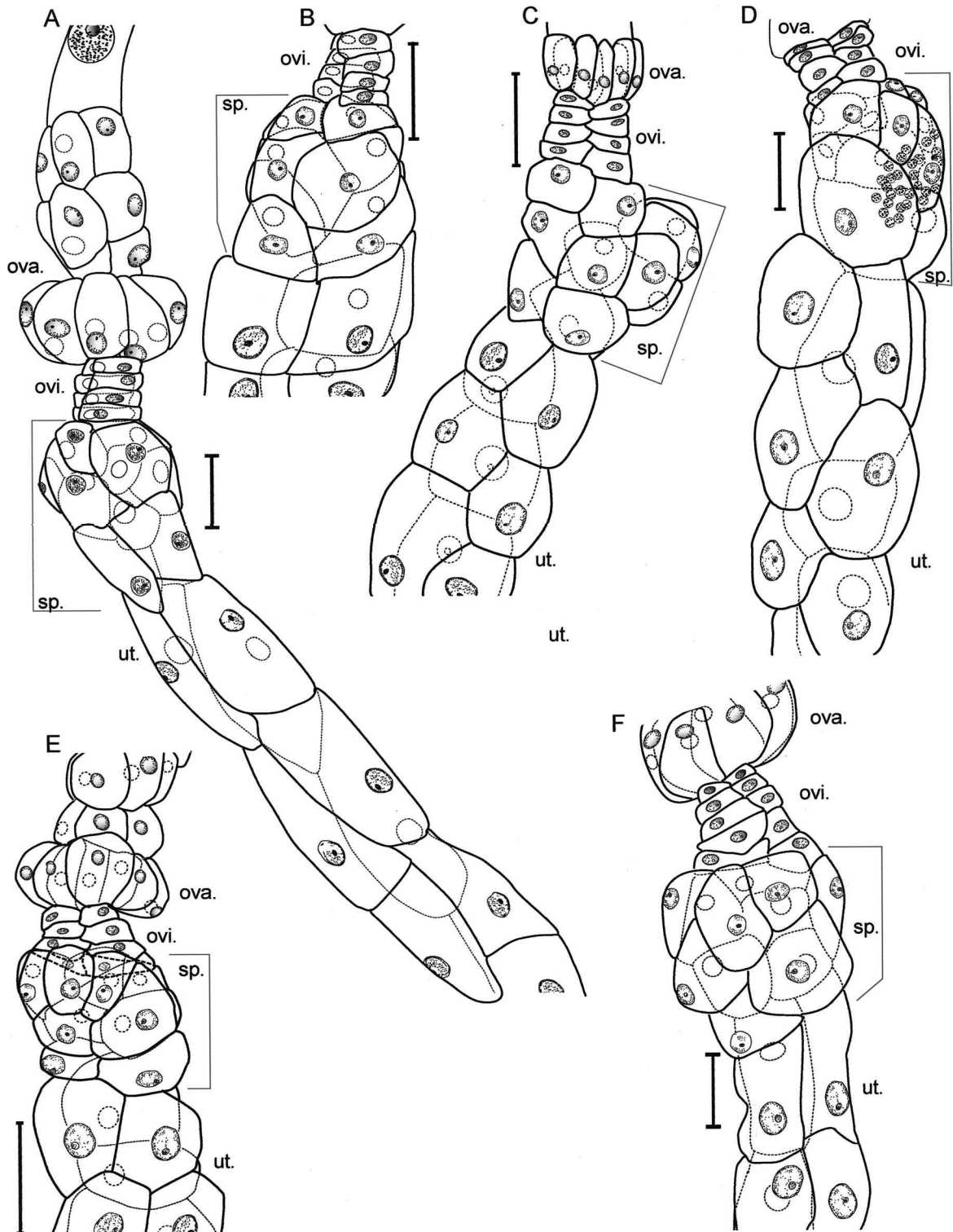


Fig. 1. Partial female reproductive systems of *Pratylenchus* spp. A: *Pratylenchus crenatus*; B: *P. coffeae*; C: *P. thornei*; D: *P. penetrans*; E: *P. zeae*; F: *P. scribneri*. End of ovary (ova.), oviduct (ovi.), spermatheca (sp.) and uterus (ut.). (Scale bars = 10 μ m.)

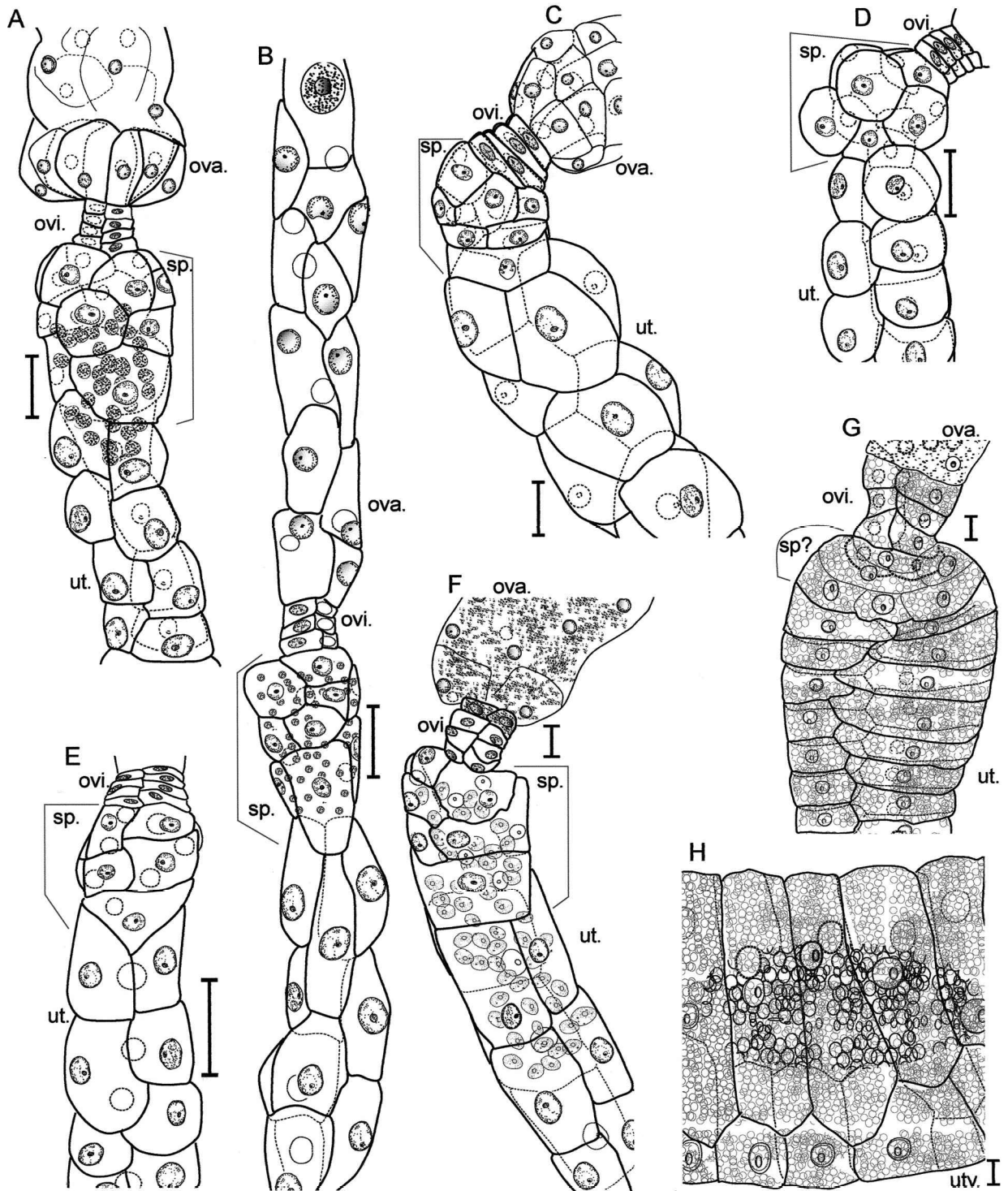


Fig. 2. Partial female reproductive systems of *Pratylenchidae*. A: *Zegotylenchus guevarai*; B: *Pratylenchoides crenicauda*; C: *P. magnicauda*; D: *P. ritteri*; E: *Radopholus similis*; F: *Hirschmanniella loofi*; G: *Nacobbus aberrans* spermatheca region; H: *Nacobbus aberrans* uterus closer to the vulva. End of ovary (ova.), oviduct (ovi.), spermatheca (sp.), uterus (ut.) and uterus closer to the vulva (utv.). (Scale bars = 10 µm.)

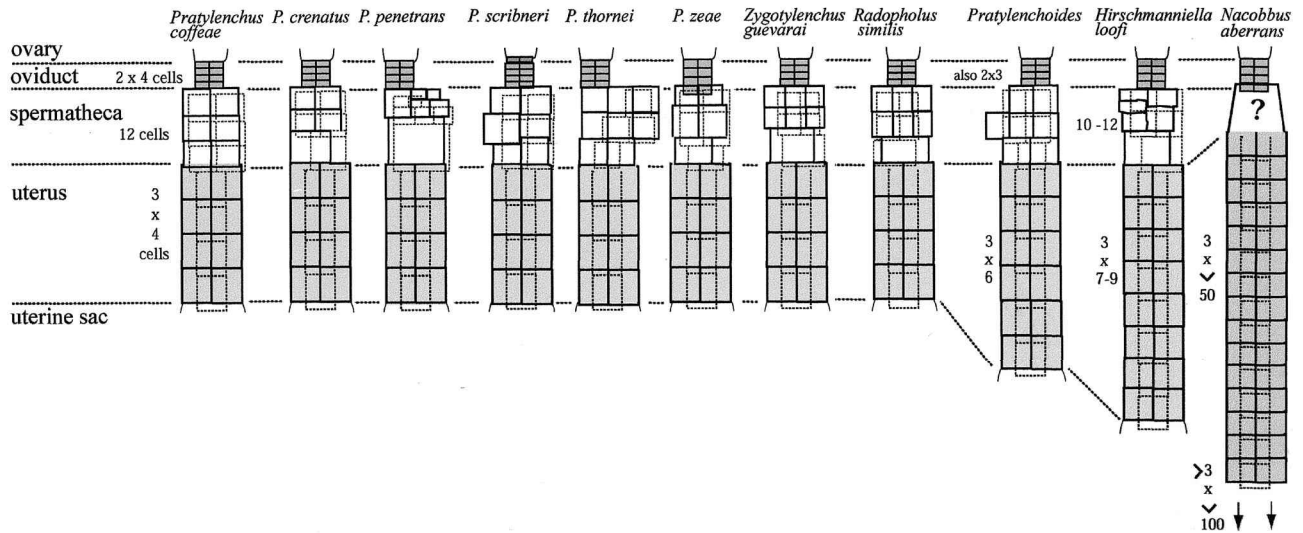


Fig. 3. Schematic presentation of the cellular structure of the female gonoduct within the studied Pratylenchidae.

of spermatheca cells varies from ten to 12. The uterus is constructed from three rows of seven or eight elongated cells. Sperm was not only observed in the spermatheca, but also occurred in the uterus up to the level of the second triplet of uterus cells.

NACOBBUS ABERRANS (FIG. 2G, H)

The oviduct consists of two rows of four cells, which are partly enveloped by the spermatheca. The spermatheca cell boundaries are only partly visible and the spermatheca region is difficult to distinguish from the uterus. After DAPI staining, ten to 14 nuclei could be counted in the spermatheca-region, although it was impossible to determine the exact number of cells. The uterus adjacent to the spermatheca is formed by a long tricolumella with, on average, 64 cells in each row; the cells being larger distally. The uterus portion closer to the vulva enlarges to form an irregular polycolumella; the exact number of cells (more than 100) being indeterminable.

Discussion

Except for *N. aberrans*, all species studied show a striking overall similarity in gonoduct construction *i.e.*, an ovary often ending with a ring of cells, two rows of four cells forming the oviduct and a spermatheca with typically 12 cells followed by a tricolumella with 16 to 24 cells. This overall similarity is an indication

in favour of the coherence of the family Pratylenchidae (with the exception of *N. aberrans*). However, the cellular gonoduct structure provides no arguments to support consideration of this family as a monophyletic group as a similar genital structure was found in some members of the Belonolaimidae and Hoplolaimidae (Geraert, 1981; Chizhov & Berezina, 1988).

OVARY

In the germinal zone of the ovary, the wall cells form a thin sheath around the germinal cells with extensions between them. Cytoplasmic contact between germ cells and epithelial cells appears to be minimal for the *P. penetrans* studied herein and for the specimens studied by Endo *et al.* (1999). In *Zygotylenchus*, on the other hand, an ovarian wall cell extension was found penetrating the germ cell and making cytoplasmic contact. We assume that these differences between *Pratylenchus* and *Zygotylenchus* are only a matter of observation. Hilgert (1976) attributed a feeding function of the ovarian wall for the germ cells *via* cytoplasmic contact. Our results corroborate this hypothesis for *Zygotylenchus*. These finger-like wall extensions presumably have a comparable function to the central cytoplasmic mass extending into the ovary of *Xiphinema theresiae* (Van De Velde & Coomans, 1988) or the typical rachis-like structure found, for instance, in *Meloidogyne javanica* (Bird & Bird, 1991). Transport to the oocytes results in the storage of more and more lipid and yolk and these oocytes

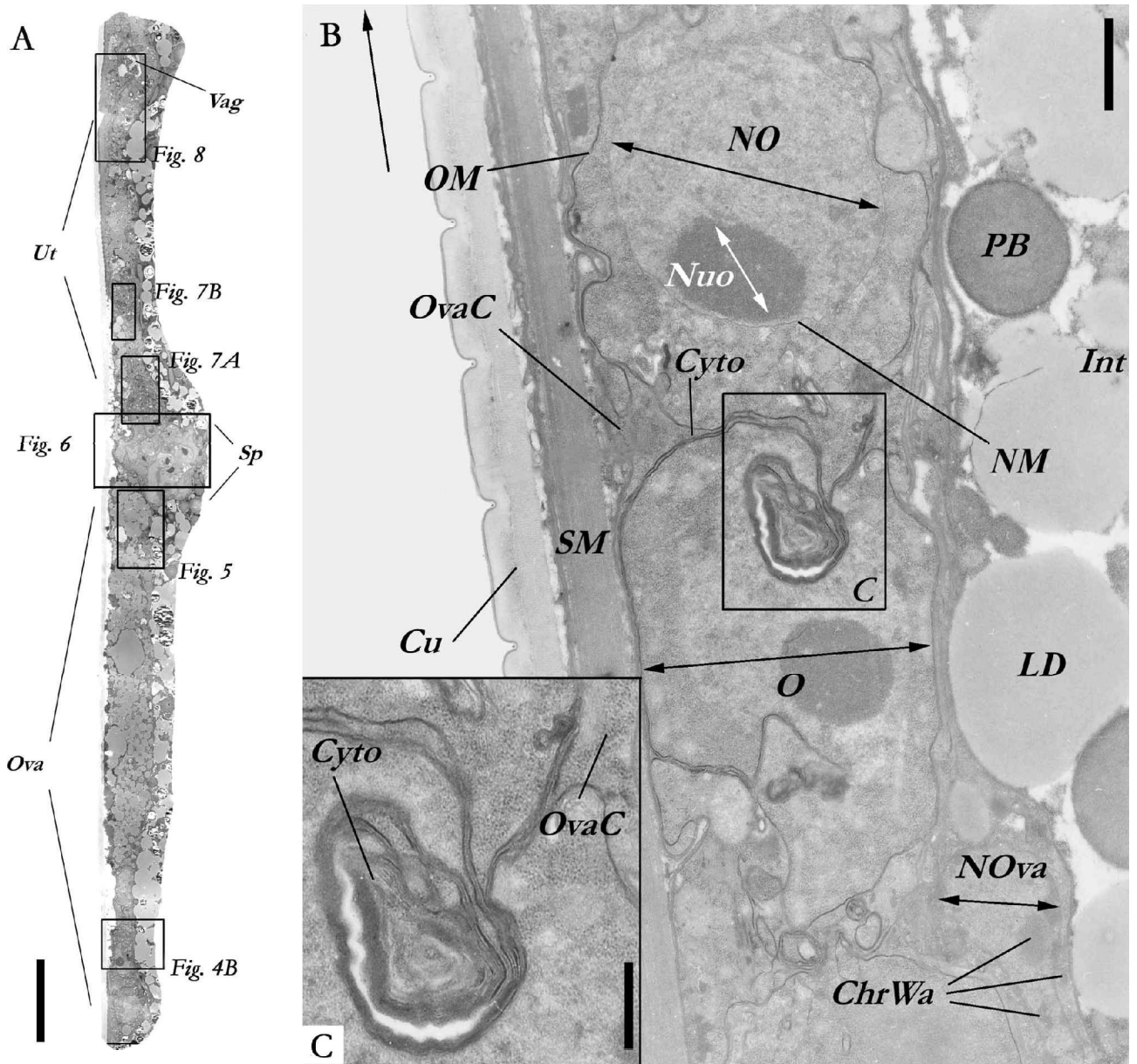


Fig. 4. A: Overview TEM study of posterior gonoduct branch of *Zygotylenchus guevarai*, frames approximately locate the TEM photographs (the spermatheca picture was taken from the anterior branch); B: Longitudinal section through ovary of *Zygotylenchus guevarai*; two oocytes surrounded by ovarian wall cells. C: Detail of ovarian wall cell extension entering an oocyte. Abbreviations: Cu = cuticle, ChrWa = chromatin of nucleus ovarian wall cell, Cyto = cytoplasmic ovarian wall extension, Int = intestine, LD = lipid droplet, NM = nuclear membrane, NO = nucleus of oocyte, Nuo = nucleolus of oocyte, NOva = nucleus ovarian wall cell, O = oocyte, OM = oocyte membrane, Ova = longitudinal section through ovary, OvaC = ovarian wall cell, PB = protein body, SM = somatic muscular tissue, Sp = spermatheca, Ut = uterus, Vag = Vagina. (Scale bars: A = 10 µm, B = 1 µm, C = 500 nm.)

finally end up in the often empty proximal part of the ovary. For most of the studied Pratylenchidae this region was morphologically divergent and comprised 16 slightly

protruding cells most easily observed with LM. Endo *et al.* (1999) described the distal part of the ovary as an oviduct with irregularly shaped cells having plicated

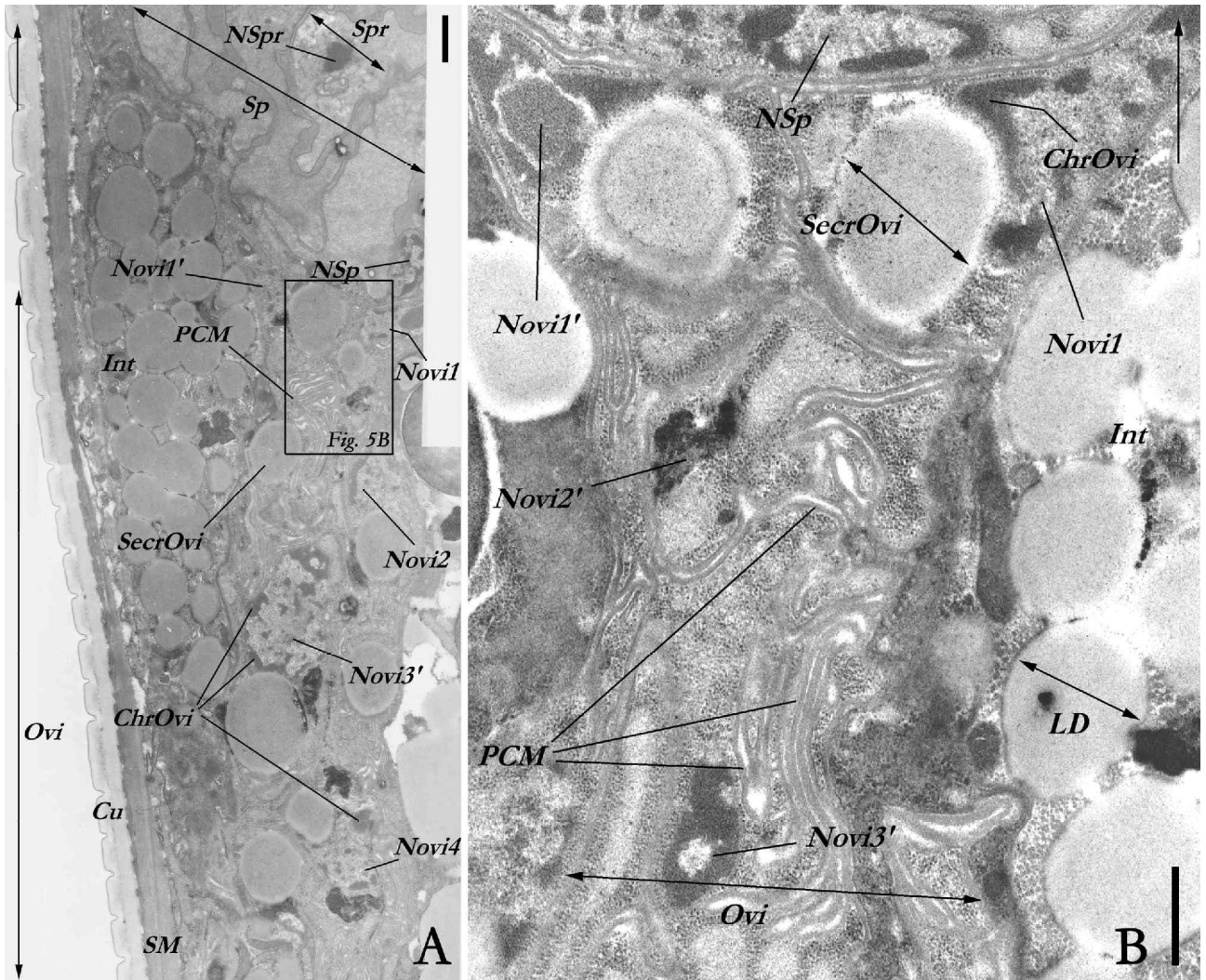


Fig. 5. A: Longitudinal section through oviduct of *Zygotylenchus guevarai*; four adjacent oviduct nuclei are visible, two being at the same level close to the spermatheca; B: Detail of oviduct, a secretion is visible in the two oviduct cells, which connect to the spermatheca. Abbreviations: Cu = cuticle, ChrOvi = chromatin of nucleus oviduct cell, Int = intestine, LD = lipid droplet, Novi (1, 2, 4) = nuclei of oviduct cells of right cell row, Novi (1', 3'): nuclei of oviduct cells of left cell row, NSpr = nucleus sperm cell, NSp = nucleus spermatheca cell, Ovi = oviduct, PCM = plicated cell membrane, SecrOvi = secretion within oviduct cell, SM = somatic muscular tissue, Sp = spermatheca, Spr = sperm cell. (Scale bars: A = 1 μ m, B = 500 nm.)

plasma membranes and muscle filaments. What they described as 'closely arranged cells' (which appear to function as a valve), the oviduct *sensu* Endo *et al.* (1999) must be the ring of eight ovarian wall cells as seen in our LM studies (Figs 1, 2). In their apparent transverse section (see Fig. 12 of Endo *et al.*, 1999) a circle of cells is visible and eight nuclei can be counted.

OVIDUCT

The oviduct, a relatively short constriction between ovary and spermatheca and without a visible lumen, consists of two rows of four cells in all the species studied.

The oviduct itself is defined differently in the literature (for an overview see Geraert, 1976). We apply the oviduct definition *sensu* Geraert (1976), *i.e.*, the few cells that

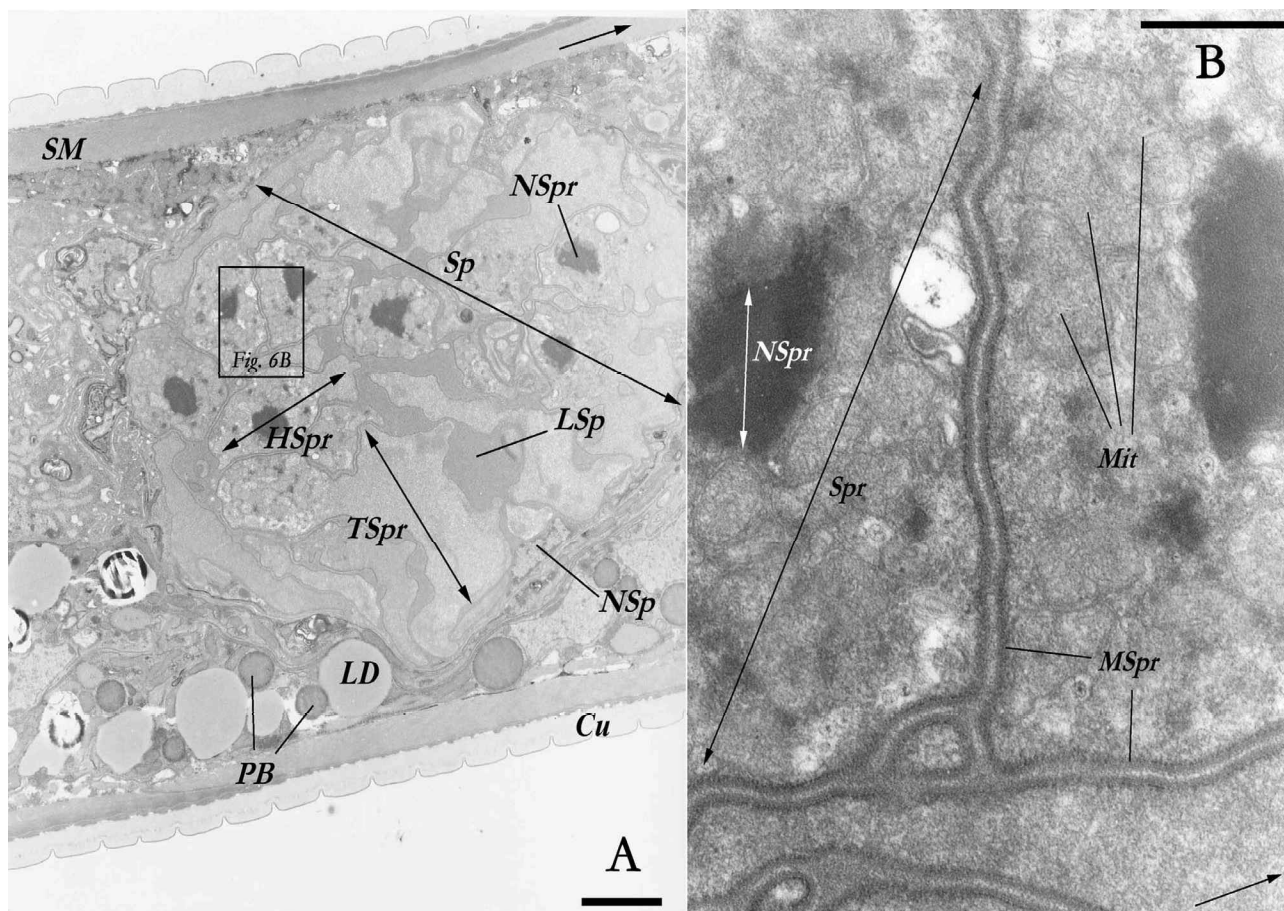


Fig. 6. A: Longitudinal section through spermatheca of *Zygotylenchus guevarai*; B: Detail of two adjacent sperm cells. Abbreviations: Cu = cuticle, HSpr = head portion sperm cell, LD = Lipid droplet, LSp = lumen spermatheca, Mit = mitochondria, MSpr = membrane sperm cell, NSp = nucleus spermatheca cell, NSpr = nucleus sperm cell, PB = protein bodies, SM = somatic muscular tissue, Sp = spermatheca, Spr = sperm cell, TSpr = tail portion sperm cell. (Scale bars: A = 2 μ m, B = 500 nm.)

form the constriction between ovary and spermatheca. Endo *et al.* (1999) considered the ripening zone of the ovary as the oviduct. Huettel and Dickson (1981) however, illustrated in *Radopholus similis* an oviduct with approximately 20 cells, presumably representing the combined oviduct and the ring formed by the ovary wall cells. Even though these distal ovary cells can form a sphincter-like structure, this seems to be dependent on nematode maturity, being found more often in young females. The oviduct *sensu* Geraert (1976) is a remarkably stable structure with a homogeneous morphology in all studied specimens.

SPERMATHECA

The number of spermatheca cells appears to be nearly constant within the Pratylenchidae and independent of

whether the species is monosexual (e.g., *P. zeae*) or bisexual (e.g., *P. penetrans*). Geraert (1973) described for *Pratylenchus* sp. a 12-celled spermatheca and we confirmed this for other *Pratylenchus* species. Chizhov and Berezina (1988) found 12 to 14 cells in the spermatheca of *Hirschmanniella* sp., while we found a variable, but slightly lower, number. Roman and Hirschmann (1969) drew only ten nuclei in the spermatheca of *Pratylenchus coffeae* and *P. scribneri*. These observations were not made on dissected gonads, but on orcein stained *in toto* mounts. Pourjam *et al.* (2000) described, from an Iranian *Zygotylenchus* population mounted in glycerine, a spermatheca apparently consisting of numerous cells reminiscent of *Meloidogyne* and, as a consequence, a relationship with the root-knot nematodes was suggested. Our LM studies, however, showed that the sperm cells often have a

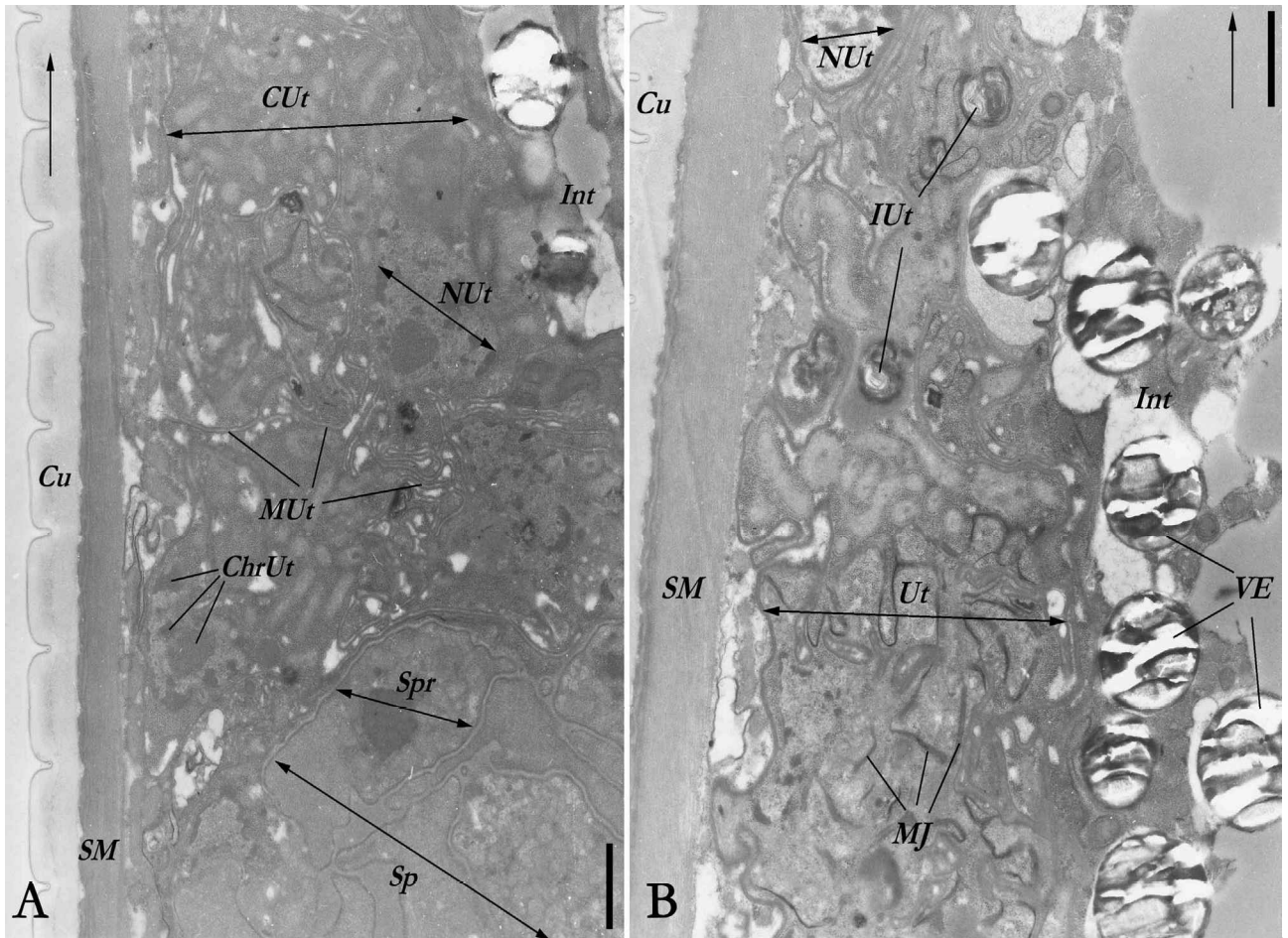


Fig. 7. A: Longitudinal section through columnar uterus of *Zegotylenchus guevarai* at spermatheca-uterus transition; B: Longitudinal section through columnar uterus of *Zegotylenchus guevarai*; the columnar cells are pressed against each other and form 'membrane-junctions' sensu Endo et al. (1997, 1999). Abbreviations: Cu = cuticle, CUt = columnar cells of uterus, ChrUt = chromatine in nucleus of uterus cell, Int = intestine, IUt = inclusion in uterus, MJ = membrane junction, MUt = membrane uterus cell, NUt = nucleus uterus cell, SM = somatic muscular tissue, Sp = spermatheca, Spr = sperm cell, Ut = uterus, VE = vesicles with undefined content. (Scale bars: 1 µm.)

swollen appearance while TEM showed these sperm cells to be remarkably long and variably shaped. As a result it is possible that Pourjam *et al.* (2000) could have interpreted the very large sperm cells as spermatheca wall cells.

The genital structure of all *Pratylenchoides* species studied was similar and no differences were found for *P. magnicauda*, a species whose systematic position has been questioned. *Amplimerlinius*, the assumed close relative of *P. magnicauda*, was found to have a slightly different genital structure, the axial spermatheca being bell-shaped with 12 to 14 cells with slightly interlaced cell borders (Bert, unpubl.). Consequently, our results support the inclusion of *P. magnicauda* in the Pratylenchidae.

The shape of the spermatheca has been used in species identification within the genus *Pratylenchus* (see Seinhorst, 1968; Loof, 1991; Ryss, 2002). Our results suggest the potential use of spermatheca cell morphology in species differentiation, although we only studied the inter-population variability of the spermatheca for *P. penetrans*. For this species the possession of an asymmetrical spermatheca with a protruding corner containing small cells on the ovary side was a constant character.

UTERUS

The uterus in all species studied consists of three rows of cells and is hence named a tricolumella. This is in con-

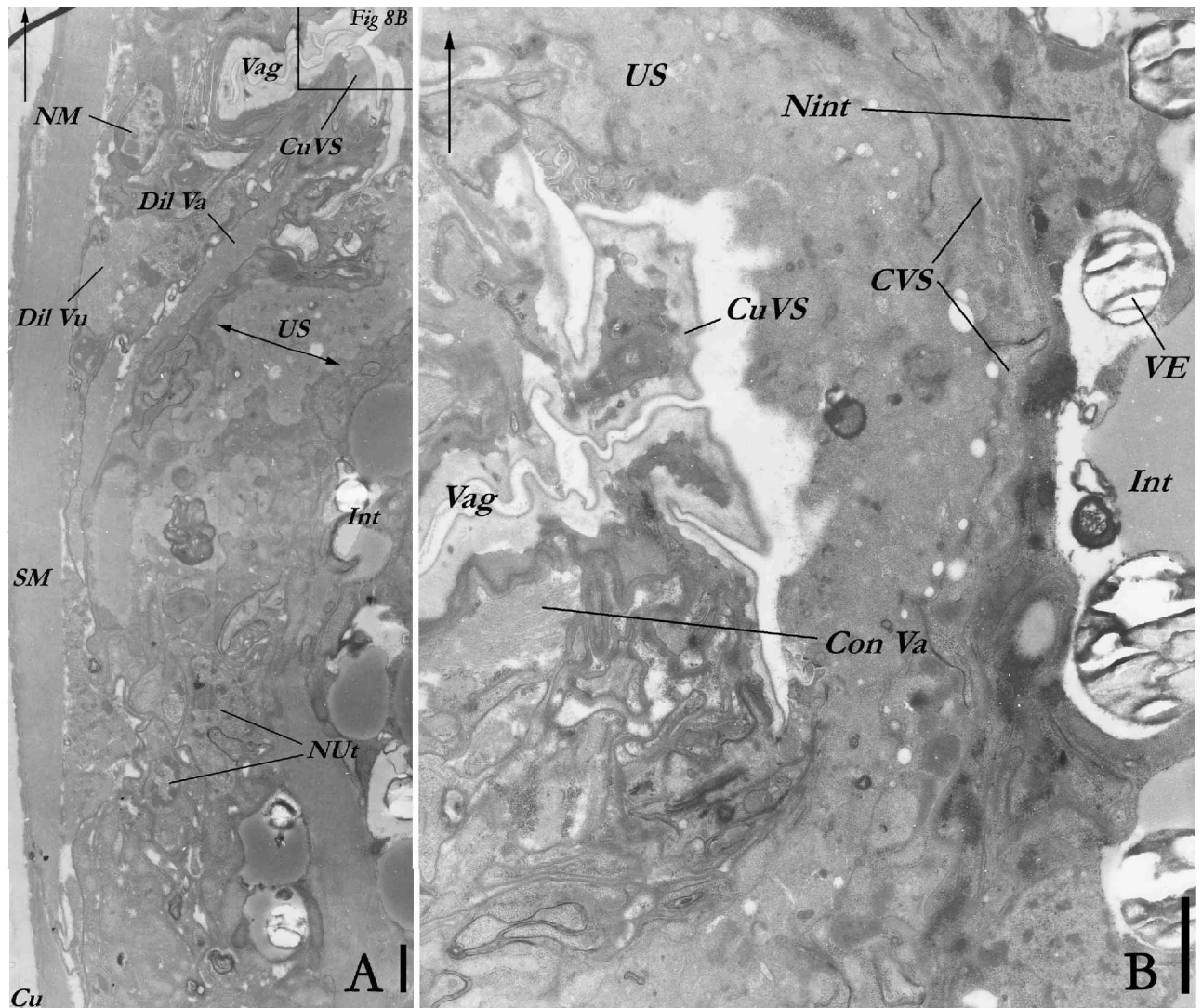


Fig. 8. A: Longitudinal section through columnar uterus, uterine sac and vagina of *Zygotlenchus guevarai*; B: Detail of vagina and uterine sac. Abbreviations: Cu = cuticle, Con Va = constrictor vaginae, CVS = cellular outline of uterus, CuVS = cuticular ventral outline of uterine sac, Dil Va = dilator vaginae, Dil Vu = dilator vulvae, Int = intestine, Nint = nucleus intestinal cell, NM = nucleus muscle cell, NUt = nucleus uterine cell, SM = somatic muscular tissue, US = uterine sac, Vag = vagina, VE = vesicle with undefined content. (Scale bars: 1 μ m.)

cordance with the majority of the literature describing the cellular arrangement of the uterus within the suborder Hoplolaimina (*sensu* Siddiqi, 2000). On the other hand, Seinhorst (1968) and Endo *et al.* (1999) mention a quadricolumella in their gonad studies of *Pratylenchus*. This is not based on their own observations, however, but was respectively taken from Wu (1967) and Coomans (1962). The account by Dickerson (1962) of the development of a 20-celled quadricolumella in *Pratylenchus crenatus* is strik-

ing, but although his illustration suggests the presence of four cell rows in the uterus, only three rows were actually drawn.

In the studied Pratylenchidae species the number of columella cells is restricted (max. 24 cells) except in *Nacobus aberrans*, where the number of uterus cells exceeds 300. This could be an adaptation of the mode of reproduction associated with a sedentary way of life. In this aspect *N. aberrans* is similar to the Heteroderinae and

Meloidogyninae (Bert *et al.*, 2002) although the structure of the complete genital system does not match with any representative of the Heteroderinae or Meloidogyninae. A similar spermatheca-like structure has not been observed or reported in the literature for any other Nematoda. The differing gonoduct structure of *Nacobbus* in contrast to the overall similarity of the other studied genera is an argument in favour of dividing the Pratylenchidae into only two subfamilies (Nacobbinae and Pratylenchinae) as was done by Luc (1987), and not into four subfamilies (Nacobbinae, Pratylenchinae, Hirschmanniellinae and Radopholinae) as outlined by Siddiqi (2000).

Our TEM observation suggests that the columella cells might have a functional role in providing secretions that contribute to egg shell formation (Coomans, 1962; Bird & Bird, 1991; Endo *et al.*, 1999). We observed the production of secretions in the uterus and the presence of secretory granules which appear to merge with the dark-appearing outer layer of the egg shell, although the intermediate excretion phase as described by Hilgert (1976) has not been established.

LM OF DISSECTED ORGANS VS TEM

Defining and specifying the different gonoduct units was based on LM of dissected gonads (Geraert, 1976, 1981). This technique is relatively easy and the three dimensional structure is well preserved. However, interpretation may be speculative as morphological discrimination between the cells of adjacent gonoduct parts can be difficult. On a sub-cellular level, and with the aid of TEM, these morphological differences are much more evident and it seems that the interpretation based on LM is justified.

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