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The Comparative Cellular Architecture of the Female Gonoduct Among Tylenchoidea (Nematoda: Tylenchina)

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Abstract: The cellular architecture of the female gonoduct of 68 nematode populations representing 42 species belonging to Tylenchidae, Belonolaimidae, Hoplolaimidae and *Meloinema* is shown to have an overall similarity in cellular gonoduct structure. The oviduct consists of two rows of four cells; the spermatheca is comprised of 10 to 20 cells, and the uterus cells, except in the case of *Psilenchus*, are arranged in four (Tylenchidae) or three (Belonolaimidae, Hoplolaimidae and *Meloinema*) regular rows. Although the genus *Meloinema* is classified within Meloidogynidae, its spermatheca is clearly hoplolaimid-like and lacks the spherical shape with lobe-like protruding cells typical of *Meloidogyne*. Detailed morphology of expelled gonoducts may provide a valuable character set in phylogenetic analysis, and the cellular morphology of the spermatheca appears to be a distinguishing feature at species level, especially in the genera *Tylenchus* and *Geocenamus*. Ultrastructural data on the oviduct-spermatheca region of *Meloidogyne incognita* complement light-microscopic (LM) results. The combination of LM of expelled organs and transmission electron microscopy (TEM) on selected sections is put forward as a powerful tool to combine three-dimensional knowledge with ultrastructural detail.

Key words: Belonolaimidae, electron microscopy, gonoduct, Hoplolaimidae, *Meloinema*, morphology, taxonomy, TEM, Tylenchidae, ultrastructure

The female reproductive system has been shown to be important in nematode systematics (Geraert, 1981, 1983), and the cellular morphology with respect to number and spatial arrangement of cells seems to be specific and constant for many nematodes species. Detailed examinations of the female reproductive system of free-living and plant-parasitic, as well as insect-parasitic, nematodes have been undertaken (Geraert, 1973, 1976; Geraert et al., 1980a, 1980b; Chizhov, 1981; Chizhov and Swiliam, 1986; Chizhov and Berezina, 1988a, 1988b). Recently, Bert et al. (2002, 2003) focused on the gonoduct structure of endoparasitic nematode families Pratylenchidae, Heteroderinae and the genus Meloidogyne. Analyzing the structure of expelled gonoducts explores informative new morphological comparative characters. Although molecular-based Tylenchoidea phylogenies are advancing (Subbotin et al., 2006), morphological characters may provide a valuable independent character set to be mapped on molecular-based branches or to construct total-evidence phylogenies. Whereas the vastly superior resolution of an electron microscope has been shown to provide an improved phylogenetic signal (e.g., Zhang and Baldwin, 2000; Baldwin et al., 2001), the herein presented dissection technique generates new morphological data on a relatively less time expensive base compared to transmission electron microscopy. Furthermore, additional morphological characters are essential for a more substantiated differentiation of plant-parasitic nematode taxa which often posses a deceptively similar anatomical pattern.

The objectives of this study were to achieve (i) a light-microscopic detailed gonoduct analysis of 68 populations of 42 species of Tylenchidae, Belonolaimidae and Hoplolaimidae in order to complete the knowledge of the female gonoduct structure within the Tylenchoidea, and to develop (ii) a complementary ultrastructural study of the oviduct-spermatheca region of *Meloidogyne incognita* for improved insight in our LM-obtained data and to clarify relationships.

Material and Methods

Nematode species were obtained from soil samples or cultures (Table 1). Extraction and examination of the female reproductive system was based on the method of Geraert (1973), i.e., bisecting the specimen at the vulva region with a small scalpel-induced expulsion of gut and gonad. Nematodes were further processed without removing nonreproductive tissue to avoid damage. The gonoduct expulsion procedure was repeated until at least 20 preparations could be observed for each population (except as noted). Preparations were either stained with acetic orcein (UCB, Leuven, Belgium, 2% aqueous solution of orcein in acetic acid) or observed directly in temporary mounts with the light microscope. By staining, a stronger differentiation of the nuclei is achieved, whereas without staining, the general cell morphology is better preserved. The cellular morphology of the ovary was only partly (or not) studied with light microscopy, as it turned out to be difficult to observe all structures; only the ripening zone of the ovary contains distinct cells which could be visualised with the techniques used here. Two types of light microscopes were used during this study: a Reichert Zetopan (Reichert-Leica, Vienna, Austria) and an Olympus BX 51 DIC (Olympus optical, Tokyo, Japan). Measurements and illustrations were prepared using a camera lucida; the drawings were prepared using Illus-

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TABLE 1. List of species studied (and their authorities), description of sampling site or culture and location

Species	Sampling site/Population origin	Location/Source
Tylenchus davainei Bastian, 1865	Sand dune covered with *Hippophae rhamnoides* L. (sea buckthorn)	Knokke, Belgian coast
T. elegans de Man, 1876	Sand mound on fallow, vegetation dominated by *Urtica dioica* L. and *Glechoma hederaceae* L., loamy sand	Ghent, Belgium
T. arcuatus Siddiqi, 1963	Apple orchard, sandy loam soil	Vliermaal, Belgium
	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden Ghent University, Belgium
	Mosses on rocks	Idem
Filenchus vulgaris (Brzeski, 1963a) Lownsbery & Lownsbery, 1985	Grassland on former dump site	Ghent, Belgium
	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden, Ghent University, Belgium
F. thornei (Andrássy, 1953) Andrássy, 1963	Apple orchard, sandy loam soil	Vliermaal, Belgium
F. quartus (Szczygiel, 1969) Lownsbery & Lownsbery, 1985	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden, Ghent University, Belgium
F. facultativus (Szczgiel, 1969) Raski & Geraert, 1987	Meadow in the vicinity of apple tree	Pleaux, France
Filenchus cf. *facultativus* (Szczygiel, 1969) Raski & Geraert, 1987	Rhizosphere of willow above stony drain	34°13.508'N, 117°03.361'W, Route 18, California, US
Filenchus cf. *orbus* (Andrássy, 1954) Meyl, 1961	Canal bank	Ghent, Belgium
Filenchus cf. *terrestris* Raski & Geraert, 1987	Grassland	Bourgoyen-Ossemeersen, Ghent, Belgium
Coslenchus costatus (de Man, 1921) Siddiqi, 1978	Moervaart canal bank	Lokeren, Belgium
	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden Ghent University, Belgium
C. polonicus Brzeski, 1982	Wetland, sandy loam soil with a high peat content	Bourgoyen-Ossemeersen, Ghent, Belgium
C. andrassyi Brzeski, 1987	Border of a football pitch	Heusden, Destelbergen, Belgium
	Lawn, sandy loam soil	Sint-Amands, Antwerp province, Belgium
Coslenchus cf. *polygyrus* Bajaj & Bharta, 1983	Potato field	Schorisse, Flemish Ardennes, Belgium
Aglenchus agricola (de Man, 1880) Meyl, 1961	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden Ghent University, Belgium
	Apple orchard, sandy loam soil	Vliermaal, Belgium
	Football pitch	Heusden, Destelbergen, Belgium
	Lawn, loamy sand soil	Geel, Antwerp province, Belgium
Basiria duplexa (Hagemeyer & Allen, 1952) Geraert, 1968	Lawn, light sandy loam soil	Sint-Amands, Antwerp province, Belgium
B. graminophila Siddiqi, 1959	Potato field	Schorisse, Flemish Ardennes, Belgium
	Grassland on former dump site	Ghent city, Belgium
	Rhizosphere of *Brassica rapa* L. (rape)	Lalitpur district, Nepal
B. gracilis (Thorne, 1949) Siddiqi, 1963	Grassland on former dump site	Ghent city, Belgium
Boleodorus thylactus Thorne, 1941	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz), sandy loam soil	Botanical garden, Ghent University, Belgium
	unknown	Spain
Neopsilenchus magnidens (Thorne, 1949) Thorne & Malek, 1968	Apple orchard, sandy loam soil	Vliermaal, Limburg province, Belgium
Psilenchus aestuarius Andrássy, 1962	Grassland on former dump site	Ghent city, Belgium
Cephalenchus leptus Siddiqi, 1963	Apple orchard, sandy loam soil	Vliermaal, Limburg province, Belgium
	Tropical rainforest	Kakamega, Kenya
C. hexalineatus (Garaert, 1962) Geraert & Goodey, 1964	Uncovered soil in the vicinity of *Hedera helix* L. (common ivy) and *Betula* sp. (birch)	Landegem, East-Flanders province, Belgium
Geocenamus brevidens (Allen, 1955) Brzeski, 1991	Grassy riverbank, vegetation dominated by *Arrhenatherion elatius* (L.) Presl and *Holcus lanatus* L.	Moervaart canal, Lokeren, Belgium
Geocenamus nothus (Allen, 1955) Brzeski, 1991	Grassy riverbank, vegetation dominated by *Arrhenatherion elatius* and *Holcus lanatus*	Moervaart canal, Lokeren, Belgium
	Pistacio	Kerman, Iran[a]
Geocenamus quadrifer (Andrássy, 1954) Brzeski, 1991	Grassy riverbank, vegetation dominated by *Arrhenatherion elatius* and *Holcus lanatus*	Moervaart canal, Lokeren, Belgium
Geocenamus cf. *nurserus* (Eroshenko & Volkova, 1987) Fortuner & Luc, 1989	Rhizosphere of willow above stony drain	34°13.508'N, 117°03.361'W, Route 18, California, US
Tylenchorhynchus dubius (Bütschli, 1873)	Corn field	Eksaarde, Lokeren, Belgium

TABLE 1. *Continued*

Species	Sampling site/Population origin	Location/Source
Filipjev 1936	Riverbank, vegetation dominated by *Crepis capillaries* (L.) Wallr., *Plantago major* L., *Trifolium repens* L. and *Poa annua* L. Light sandy loam soil	Canal Roeselare-Leie, Ingelmunster, Belgium
T. microphasmis Loof, 1960	*Ammophila arenaria* (L.) Link in sand pot	Heteren, The Netherlands[b]
	Grassy riverbank	Moervaart canal, Lokeren, Belgium
T. ventralis (Loof, 1963) Fortuner & Luc, 1987	*Ammophila arenaria* (L.) Link in recipient filled with sand	Heteren, The Netherlands[b]
T. maximus Allen, 1955	Grassy riverbank	Moervaart canal, Lokeren, Belgium
Nagelus obscurus (Allen, 1955) Powers, Baldwin & Bell, 1983	*Phragmites australis* (reed)	Bourgoyen-Ossemeersen, Ghent, Belgium
Amplimerlinius icarus (Wallace & Greet, 1964) Siddiqi, 1976	Lawn in the vicinity of a willow tree (*Salix matsudana* Koidz)	Botanical garden Ghent University, Belgium
	Apple orchard, sandy loam soil	Vliermaal, Belgium
Helicotylenchus cf. *dihystera* (Cobb, 1893) Sher, 1961	Unknown	Khartoum, Sudan[d]
H. pseudorobustus (Steiner, 1914) Golden, 1956	Wetland	Bourgoyen-Ossemeersen, Ghent, Belgium
H. varicaudatus Yuen, 1964	Grassy riverbank, vegetation dominated by *Arrhenatherion elatius* and *Holcus lanatus*	Moervaart canal, Lokeren, Belgium
H. canadensis Waseem, 1961	Riverbank, vegetation dominated by *Crepis capillaries* (L.) Wallr., L., *Plantago major* L., *Trifolium repens* L. and *Poa annua* L., light sandy loam soil	Canal Roeselare-Leie, Ingelmunster, Belgium
Rotylenchus goodeyi Loof & Oostenbrink, 1958	Riverbank, vegetation dominated by *Crepis capillaries* (L.) Wallr., L., *Plantago major* L., *Trifolium repens* L. and *Poa annua* L., light sandy loam soil	Canal Roeselare-Leie, Ingelmunster, Belgium
	Ammophila arenaria (L.) Link in sand pot	Heteren, The Netherlands[b]
	Unknown	Sennar state, Sudan[d]
Rotylenchus uniformis	Ligustrum hedge (*Ligustrum vulgare* L.)	Gentbrugge, Belgium
Scutellonema bradys (Steiner & Le Hew, 1933) Andrássy, 1958	Tropical plants in greenhouse	CABI Bioscience, Egham, UK[e]
Hoplolaimus aegypti Shafee & Koura, 1970	Banana	Hantop, Suda[d]
Rotylenchulus reniformis Linford & Oliveira, 1940	Original host: carnation, Inida	Wageningen, The Netherlands[c]
	Cultured on beet	
Meloinema odesanens Kim, Vovlas, Choi & Lee, 2005	Mountain tree (*Tilia amurensis* Rupr.)	Gwangweon Province, Korea[f]
Meloidogyne incognita (Kofoid and White 1919) Chitwood, 1949	Tomato culture	Wageningen, The Netherlands[c]

Samples obtained from:
[a] E. Pourjam, Tarbiat Modares University, Tehran, Iran
[b] Netherlands Institute of Ecology, Heteren, The Netherlands
[c] P. De Ley, Department of Nematology, University of California, Riverside, USA
[d] G. Elbadri, Crop Protection, Wad Medani, Sudan
[e] G. Karssen, Plant Protection Service, Wageningen, The Netherlands
[f] D. G. Kim, Department of Agricultural Environment, Gyeongbuk Agricultural Technology Administration, Daegu, Korea

trator 10.0 software (Adobe Systems, Mountain View, CA, USA). The morphology was also recorded as video clips that mimic multifocal observation through a light microscope following the Video Capture and Editing procedures developed by De Ley and Bert (2002). The resulting virtual specimens are available on the web at: http://www.nematology.ugent.be/VCE.htm.

The terminology of the reproductive system used here is based on Geraert (1983), who followed Chitwood and Chitwood's (1950) interpretation. A genital branch, as occurs in a in a didelphic or monodelphic genital system, consists of an ovary (= gonad) and gonoduct. The ovary consists of three functional zones, namely, the germinal zone, the growth zone and the ripening zone. The oviduct is the constricted region between ovary and spermatheca. The term uterus is here restricted to the eggshell-formation region (also demarcated as columnar region if distinct uterus cell rows are visible) of the gonoduct; a constriction may be present between the uterus and the uterine sac. The uterine sac follows the uterus and terminates in the vagina, which is connected to the vulva. The classification of the studied organisms is largely based on De Ley and Blaxter (2002), but below family level on "a reappraisal of Tylenchina" (see Maggenti et al., 1987).

Expelled gonoducts of *Meloidogyne incognita* were examined by transmission electron microscopy (TEM). Excised reproductive systems from 30 specimens were transferred to ice-cooled Karnovsky's (1965) fixative. The fixation, embedding, sectioning and TEM procedures are as previously described for in toto specimens (Bert et al., 2003).

RESULTS

A summary of the results is presented in Table 2.

Tylenchidae (Figs. 1–3): The oviduct of members of

TABLE 2. The cellular composition of oviduct (number of cell rows, number of cells per row), spermatheca (cell number, structure) and uterus (number of cell rows, number of cells per row) is listed for each species studied. When data are unknown or uncertain, a question mark (?) is printed.

	Oviduct	Spermatheca		Uterus
	Cell rows × cells per row	Cell number	Cell number offset pouch	Cell rows × cells per row
Tylenchidae Örley, 1880				
Tylenchus davainei	2 × 3–4	12–14 (+2)	5–7	4 × 4
T. elegans	2 × 3	14 (+2)	4	4 × 4
T. arcuatus	2 × 4 (3)	12 (+2)	—	4 × 4
Filenchus vulgaris	2 × 3	14	6–10	4 × 4
F. thornei	2 × 3	14–16	?	4 (irregular) × 4?
F. quartus	2 × 3 (4)	14	—	4 × 4
F. facultativus	2 × 3	14	6–8	4 × 4
Filenchus cf. *facultativus*	2 × 3	14 (16)	7–10	4 × ?
Filenchus cf. *terrestris*	2 × 4	15–16	6–8	4 × 4
Filenchus cf. *orbus*	2 × 3	14	6–8	4? × ?
Coslenchus polonicus	2 × 4	14 (16)	7–10	4 × 7–8
C. andrassyi	2 × 4	14	7–10	4 (irregular) × 5–6
C. cf. *polygyrus*	2 × 4	14	7–10	4 (irregular) × ?
C. costatus	2 × 4	10–12	—	4 × 5 (6)
Aglenchus agricola	2 × 4 (3)	(12)–14–(16)	4–12	4 × 4
Basiria graminophila	2 × 5(6)	16	—	4 (irregular) × 4 (5)
B. duplexa	2 × 5	16	—	4 (irregular) × 4–5
B. gracilis	2 × 5	16	—	4 (irregular) × 4 (5)
Boleodorus thylactus	2 × 5	16	>7	4 ×
Neopsilenchus magnidens	2 × 5	16	—	4 (irregular) × 8–10
Psilenchus aestuarius[a]	2 × 5	18–20	—	4? × 38–55?
Cephalenchus leptus	2 × 5–7	12	—	4 × 7–8
C. hexalineatus	2 × 5–7	12	—	4 × 7–8
Belonolaimidae Whitehead, 1960			Spermatheca structure	
Geocenamus nothus[a]	2 × 4	14	4 lobes	3 × 4–5
G. quadrifer[a]	2 × 4	12	offset	3 × 3–7
G. cf. *nuyseri*[a]	2 × 4	12–14	spherical	3 × 5–7?
G. brevidens[a]	2 × 4	12	spherical	3 × 4–5
G. microdorus[a]	2 × 4	?	2 lobes	3 × 4
Tylenchorhynchus dubius[a]	2 × 4	12 (14)	round-oval	3 × 4–5
T. microphasmis[a]	2 × 4	12–14	oval, 2 compartments	3 × 5
T. ventralis[a]	2 × 4	12–14	oval	3 × 5–6
T. maximus[a]	2 × 4	8–10	oval	3 × 7–9
Nagelus obscurus[a]	2 × 4 (5)	12–14	offset	3 × 4
Amphimelinius icarus[a]	2 × 4	12–14	bell-shaped	3 × ?
Hoplolaiminae Filipjev, 1934				
Helicotylenchus varicaudatus[a]	2 × 4	12	maximally offset	3 × 4
H. canadensis[a]	2 × 4	12	maximally offset	3 × 4
H. pseudorobustus[a]	2 × 4	12 (+2)	offset	3 × 4
H. cf. *dihystera*[a]	2 × 4	12 (+2)	offset	3 × 4?
Rotylenchus uniformis[a]	2 × 4	12 (14)	oval	3 × 4 (5)
R. goodeyi[a]	2 × 4	12 (14)	oval	3 × 4 (3)
Scutellonema bradys[a]	2 × 4	12–14	oval—bell-shaped	3 × ?
Hoplolaimus aegypti[a]	2 × 4	9–12?	unclear	3 × ?
Rotylenchulinae Husain & Khan, 1967				
Rotylenchulus reniformis[a]	2 × 4	12–17?	elongated?	3? × ?
Meloinema Choi & Geraert, 1974				
M. odesanens[a]	2 × 4	12–14	oval	3 long rows
Meloidogyne Göldi, 1892 (see also Bert et al., 2003)				
M. incognita	2 × 4	16 lobe-like cells with interlaced cell boundaries	spherical	3 long rows

[a] didelphic reproductive system

the family Tylenchidae comprises two rows of three to seven cells. The oviduct of *Tylenchus*, *Filenchus*, *Coslenchus* and *Aglenchus* is composed of two rows of three or four cells. In *Basiria*, *Boleodorus*, *Neopsilenchus* and *Psilenchus*, five (exceptionally six) cells per row are present with the most proximal oviduct cells usually being slightly larger; *Cephalenchus* is characterized by a longer and slightly bent oviduct that comprises two rows of five, six or seven cells.

The uterus cells, except for *Psilenchus aestuarius*, are

arranged in four regular rows (= quadricolumella) each with four to ten cells; the nuclei of these cells are in most cases distinctly larger than the spermatheca. Six tightly packed cells between the quadricolumella and uterine sac, forming a sphincter-like structure, were observed for *Tylenchus arcuatus*. The genital branches of the didelphic reproductive system of *Psilenchus aestuarius* are considerably longer. The uterus cells do not form a quadricolumella, but are arranged in irregular rows, each comprising 38 to 55 cells. Also, *Coslenchus andrassyi*, *C.* cf. *polygyrus* and *Neopsilenchus* display a partially irregular arrangement of the uterus cells.

The spermatheca shows several differences in cellular architecture within the Tylenchidae. The spermatheca of *Tylenchus* (Fig. 1A-C) comprises 12 to 14 cells and is axial in *T. arcuatus*, while partially offset in *T. davainei*

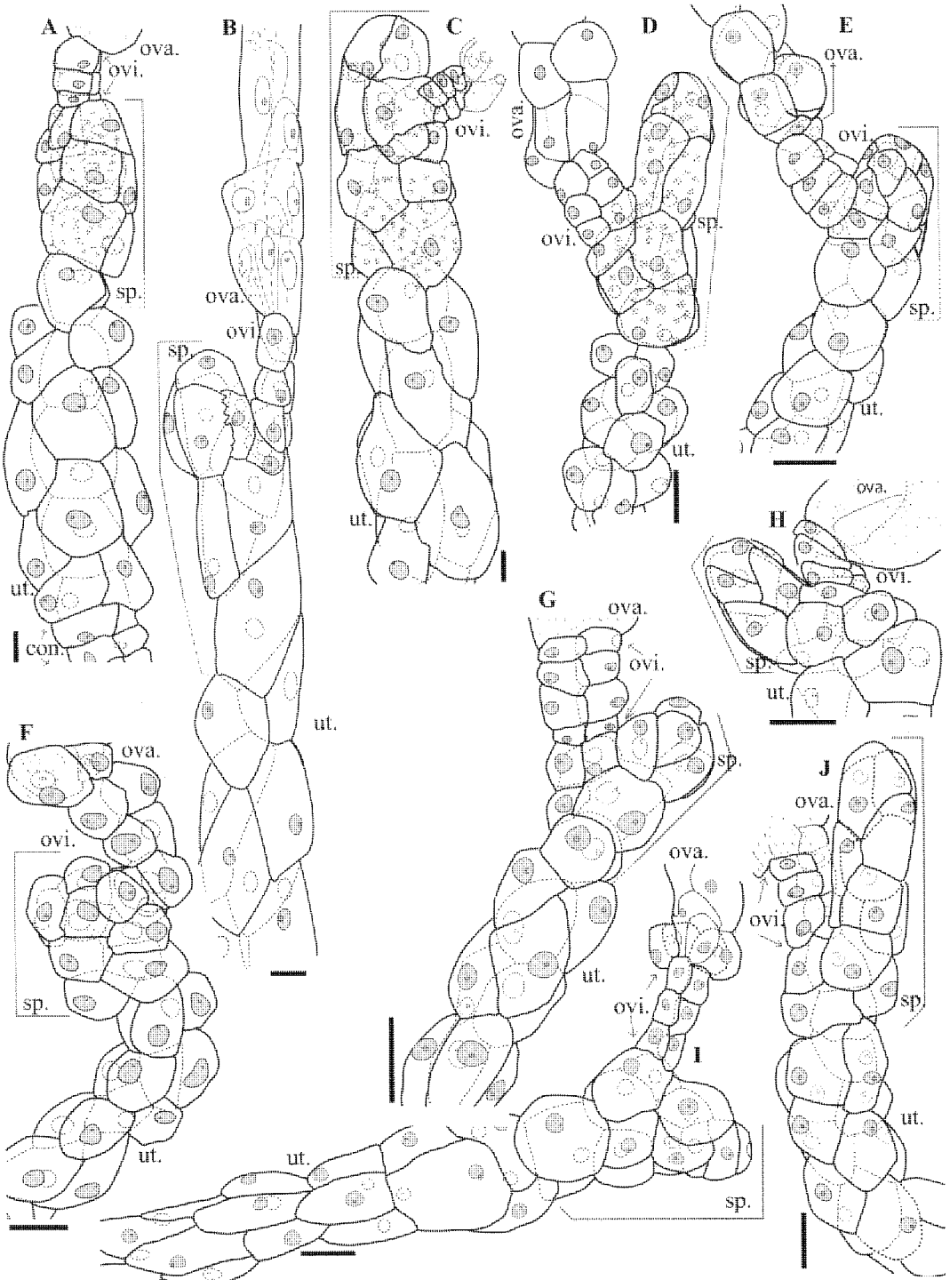

FIG. 1. The cellular architecture of oviduct, spermatheca and uterus of *Tylenchus* spp. *and Filenchus* spp. A) *T. arcuatus*. B) *T. davainei*. C) *T. elegans*. D) *F. vulgaris* from dump site. E) *F. vulgaris* from botanical garden. F) *F. thornei*. G) *F. orbus*. H) *F. facultativus*. I) *F.* cf. *terrestris*. J) *F.* cf. *facultativus*. ova.: proximal end of ovary; ovi.: oviduct; sp.: spermatheca; ut.: uterus; con.: constriction between uterus and uterine sac. Scale bars = 10 µm

and *T. elegans* with the offset pouch composed of five to seven cells and four cells, respectively. The spermatheca is connected to the uterus by two large cells. The spermatheca of *Filenchus* species (Fig. 1D-J) is axial (*F. quartus*), slightly offset (*F. thornei*), with a distinctly offset pouch comprising six to eight cells (*F. facultativus*, *F.* cf. *terrestris*, *F.* cf. *orbus*) or with an oblong offset pouch encompassing seven to 10 cells (*F.* cf. *facultativus*). Two large, rounded cells connect the spermatheca with the uterus in *F. quartus*, *F.* cf. *terrestris* and *F.* cf. *orbus*. *Coslenchus polonicus*, *C. andrassyi* and *C.* cf. *polygyrus* display a similar spermatheca (Fig. 2A-D), comprised of 14 cells (exceptionally 16 cells are observed in *C. polonicus*). The spermathecae of these *Coslenchus* species consist of a distinctly offset pouch, composed of seven to 10 cells, and an axial part, encompassing two large cells that are nearly as long as the offset sac. The majority of the examined *C. andrassyi*

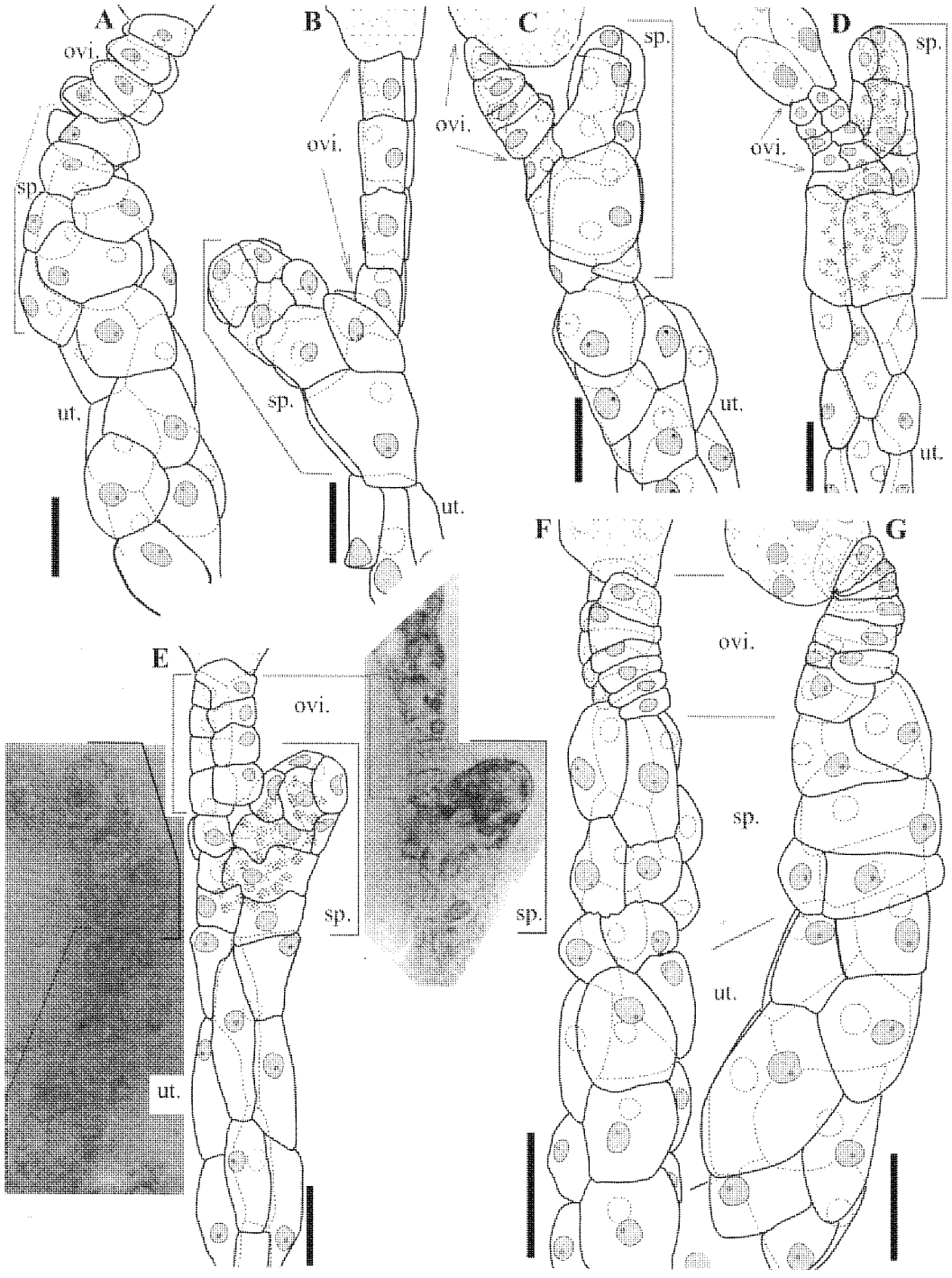

FIG. 2. The cellular architecture of oviduct, spermatheca and distal part uterus of *Coslenchus* spp., *Agelenchus* and *Cephalenchus* spp. A) *Coslenchus costatus*. B) *C.* cf. *polygyrus*. C) *C. andrassyi*. D) *C. polonicus*. F) *Aglenchus agricola*, including LM photographs. F) *Cephalenchus leptus* from apple orchard. G) *C. hexalineatus*. ovi.: oviduct; sp.: spermatheca; ut.: uterus. Scale bars = 10 µm.

specimens display two particularly small cells that connect spermatheca to uterus. *Coslenchus costatus* is characterized by an axial spermatheca that is indistinctly demarcated from adjacent regions of the gonoduct and comprises only 10 to 12 cells. The spermatheca of *Aglenchus agricola* (Fig. 2E) generally comprises 14 cells; though cell numbers between 12 and 16 were also observed, their cell boundaries are often only vaguely visible. The size of the offset pouch (consisting of four to 12 cells) shows a remarkable intraspecific variability in this species. The genus *Cephalenchus* (Fig. 2F,G) is characterized by an axial spermatheca comprised of 12 cells; the spermatheca cells of *C. leptus* are clearly arranged in four rows of three cells, while in *C. hexalineatus* the spermatheca cells are more randomly distributed. The spermatheca-uterus transition is indistinct in *Cephalenchus*, and this is especially the case in *C. leptus*, where the spermatheca and uterus cellular pattern are comparable.

The *Basiria* species studied as well as *Boleodorus thylactus* and *Neopsilenchus magnidens*, (Fig. 3A-F) have a spermatheca comprised of 16 cells. The spermatheca is long and axial in *Basiria* while always clearly offset, but it is variable in shape in *Boleodorus thylactus*. In *Basiria*, the spermatheca is connected to the uterus by two cells. The axial spermatheca of *Neopsilenchus magnidens* (Fig. 3F) shows a remarkable variation in length, ranging from short and oval-shaped to long and cylindrical; the number of cells does not vary with size. In *Psilenchus aestuarius* (Fig. 3G), the spermatheca comprises a long axial sac of 18 to 20 cells; the spermatheca cells are elongated except for two more distal-proximally flattened cells that connect spermatheca to uterus.

Belonolaimidae (Fig. 4): The oviduct and uterus structure is comparable in all examined Belonolaimidae species. The oviduct consists of two rows of four cells; however, in several specimens the oviduct cells are difficult to observe since they are compressed between ovary and spermatheca. The uterus comprises three distinctive rows of large columnar cells. Each row encompasses four to five cells in *Geocenamus nothus, G. brevidens, G. microdorus, Tylenchorhynchus dubius, N. obscurus:* five to seven cells in *G.* cf. *nurserus, G. quadrifer, T. ventralis* and *T. microphasmis*, and seven to nine cells in *T. maximus*.

The spermatheca within the Belonolaimidae is axial to offset, spherical to distinctly lobed in shape, and the number and arrangement of its cells is species specific. The offset spermatheca of *G. quadrifer* (Fig. 4A) is composed of 12 cells, variable in arrangement, with the two distal cells in continuation with the oviduct cell rows. The axial spermatheca of *G.* cf. *nurserus* and *G. brevidens* (Fig. 4B,C) consists of 12 to 14 cells, and cell boundaries are highly meandering in *G.* cf. *nurserus* (Fig. 4C). The spermatheca of *G. nothus* (Fig. 4D) shows four lobes, which are more pronounced in the presence of sperm; two cells, often with unclear cell boundaries, build up each offset lobe. More proximal, six cells constitute the axial part of the spermatheca; the two largest cells are positioned centrally, while two to four smaller cells are connected with the uterus.

The spermatheca of *Tylenchorhynchus dubius* (Fig. 4E,K) usually comprises 12 cells of equal size and in variable position (14 cells observed in a few species); a characteristic bend occurs at the junction of the spermatheca and uterus. The spermatheca of *T. microphasmis* (Fig. 4G) consist of two compartments; six slightly protruding cells form a wider distal component and six to eight slightly smaller cells, of which two cells connect spermatheca with uterus, constitute a proximal component. The spermatheca of *T. ventralis* (Fig. 4H) comprises 12 to 14 cells, with two distal cells connecting to the oviduct and two proximal cells connecting to the uterus; the remaining cells are variable in shape and arrangement. The spermatheca of *T. maximus* (Fig. 4I) consists of only eight to 10 cells with indistinct cell boundaries; two relatively large cells, resembling uterus cells, connect spermatheca to uterus.

The spermatheca of *Nagelus obscurus* (Fig. 4J) is offset and comprises 12 to 14 cells with distinctly crenate cell boundaries; two large cells form the connection to the uterus. The spermatheca outline of *Amplimerlinius icarus* (Fig. 4F) is bell-shaped. The cellular architecture is variable; 12 to 14 cells of inconsistent shape and size have crenate or smooth cell boundaries.

Hoplolaimidae (Fig. 5): The oviduct of the examined Hoplolaiminae species persistently comprises two rows of four cells, which can be difficult to discern since the oviduct is often compacted between ovary and spermatheca. The uterus consists of three cell rows, each row being four to five cells long.

The spermatheca of *Helicotylenchus* comprises 12 cells; the extent to which the spermatheca is offset differs within the species studied. The spermatheca of *H.* cf. *dihystera* and *H. pseudorobustus* (Fig. 5 A,B) (from the latter only a limited number of extruded gonoducts were available) is only partially offset; the spermatheca cell boundaries of *H.* cf. *dihystera* are slightly crenate. *H. varicaudatus and H. canadensis* (Fig. 5 C,D) have a maximally offset spherical spermatheca; the proximal oviduct cells reach the distal uterus cells. *Helicotylenchus pseudorobustus* and *H.* cf. *dihystera* are characterized by a pair of cells, situated between spermatheca and uterus, that are larger than spermatheca cells but smaller than uterus cells.

The spermatheca of the *Rotylenchus* species studied (Fig. 5E,F,H) likewise comprises 12 cells, except 14 cells in one dissection. The arrangement of the spatial spermatheca cell of *R. uniformis* is variable, while in *R. goodeyi* two distal spermatheca cells usually connect with the oviduct, and two proximal spermatheca cells connect with the uterus. Two rows of three smaller cells can form a sphincter-like structure between the tricolumella and uterine sac (observed in *Rotylenchus goodeyi*

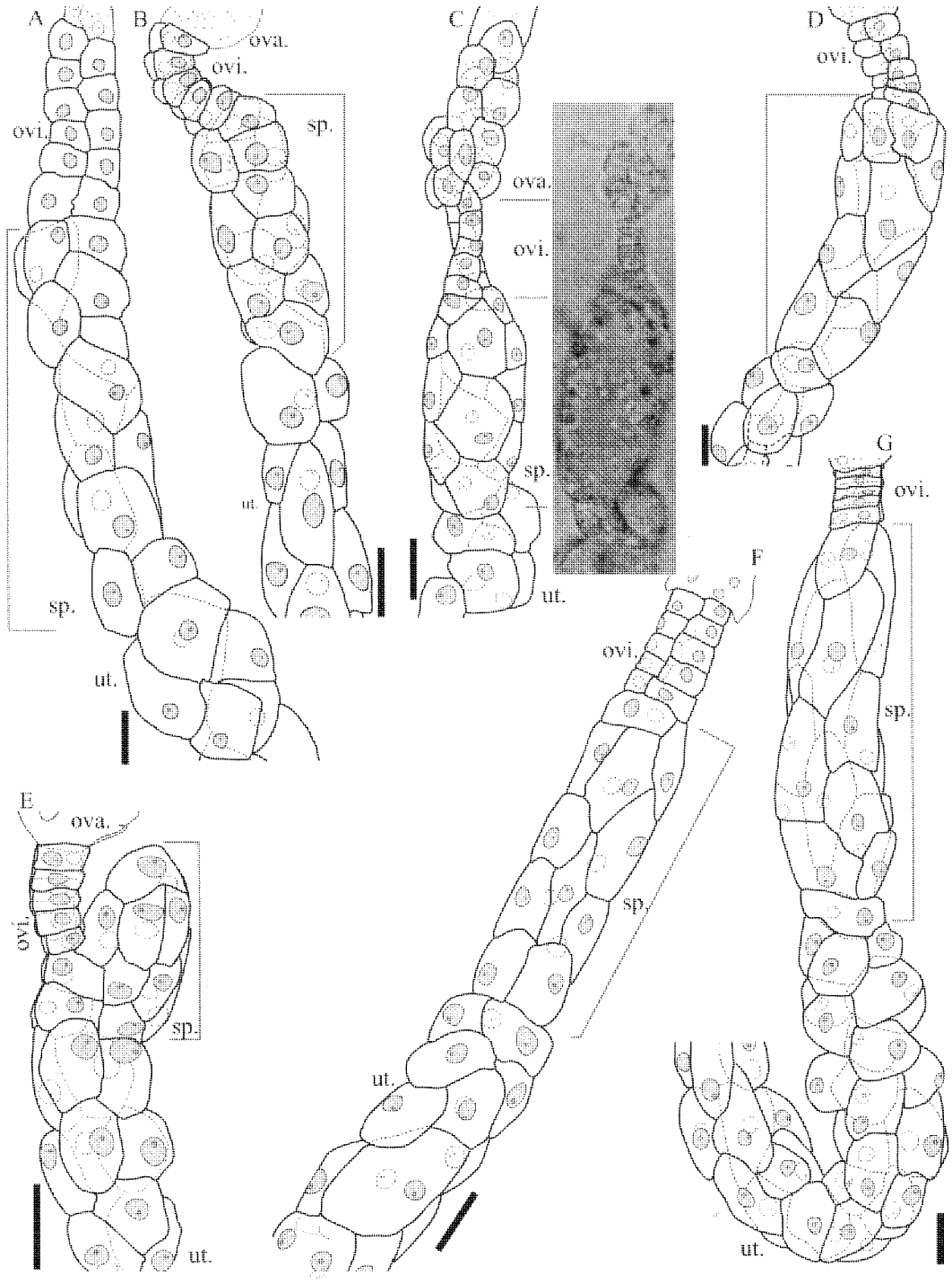

FIG. 3. The cellular architecture of oviduct, spermatheca and distal part uterus of Boleodorinae. A) *Basiria gracilis*. B) *B. graminophila* from dumpsite. C) *B. graminophila* from potato field, including LM photographs. D) *B. duplexa*. E) *Boleodorus thylactus* from botanical garden. F) *Neopsilenchus magnidens*. G) *Psilenchus aestuarius*. ova.: proximal end of ovary; ovi.: oviduct; sp.: spermatheca; ut.: uterus. Scale bars = 10 µm.

(Fig. 5E)). *Scutellonema bradys* (Fig. 5G) has an axial spermatheca comprising 12 to 14 cells with unclear cell boundaries. The spermatheca of *Hoplolaimus aegypti* (Fig. 5H) is weakly differentiated from the gonoduct and consists of nine to 12 cells; the exact number of cells could not be determined since the spermatheca-uterus transition is not clear-cut. The nature of several cells occurring between spermatheca and tricolumella is not obvious for *H. aegypti*; these cells show an uterus-like pattern but are more compact (only a limited number of gonoducts were examined, n = 4).

The oviduct of *Rotylenchulus reniformis*, which represents the Rotylenchulinae in this study, consists of two rows of four cells. The remaining cellular gonoduct architecture could not be unambiguously determined since the cell boundaries appeared indistinctly and only

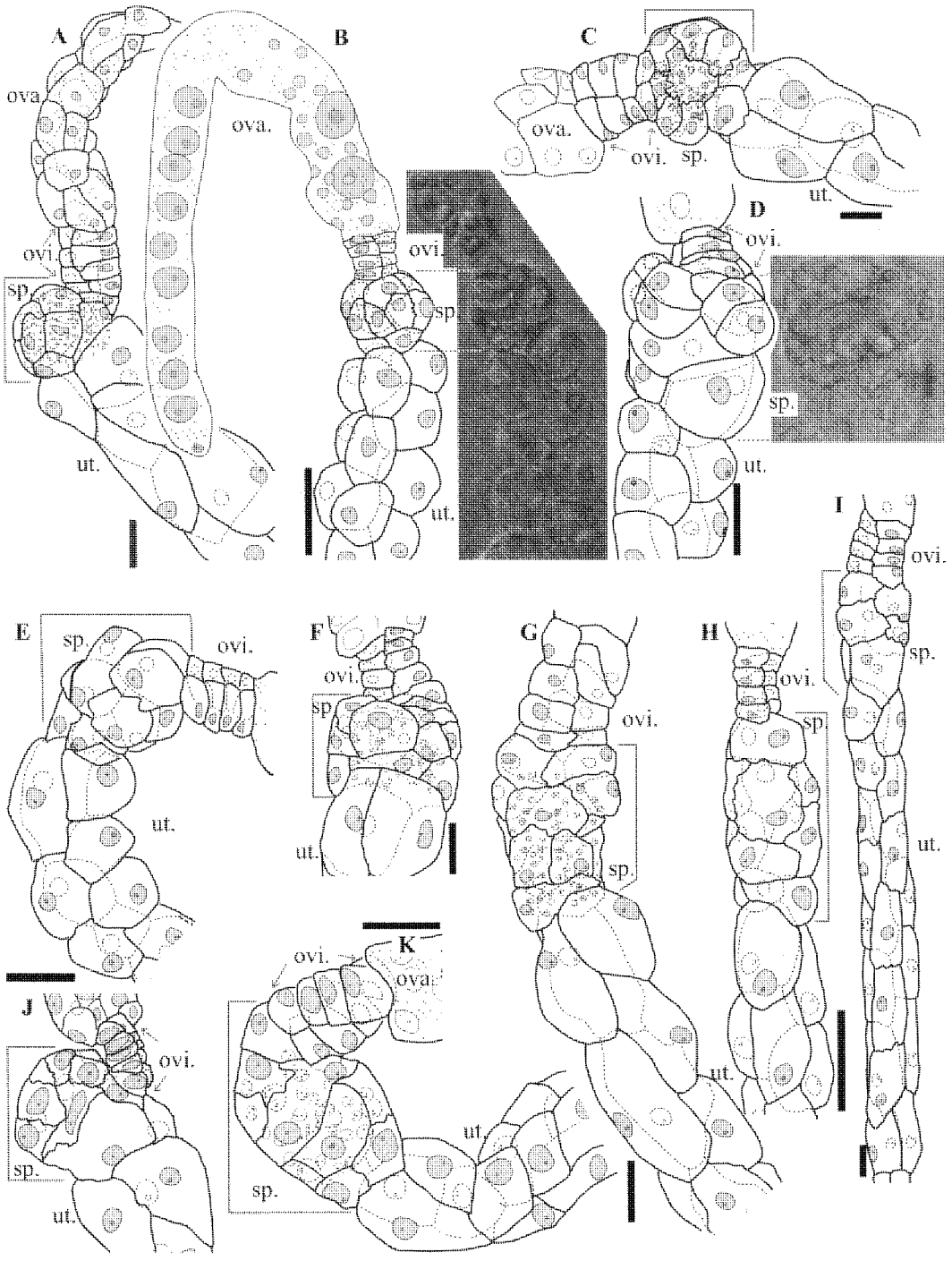

FIG. 4. The cellular architecture of oviduct, spermatheca and distal part uterus of Belonolaimidae. A) *Geocenamus quadrifer*. B) *G. brevidens*, including LM photographs. C) *G.* cf. *nurserus*. D) *G. nothus* from canal bank Lokeren, including LM photographs. E) *Tylenchorhynchus dubius* from canal bank Roeselare-Leie. F) *Amplimerlinius icarus* from apple orchard. G) *T. microphasmis*. H) *T.ventralis*. I) *T. maximus*. J) *Nagelus obscurus*. K) *T. dubius* from corn field. ova.: proximal end of ovary; ovi.: oviduct; sp.: spermatheca; ut.: uterus. Scale bars = 10 µm.

a limited number of gonoducts were successfully extruded. The spermatheca apparently comprises 12 to 17 variably arranged cells. The spermatheca-uterus transition is indistinct, and the uterus cells appear to be arranged in three unclear rows.

Meloinema (Fig. 5I): The gonoduct structure of *Meloinema* is similar to that of the Belonolaimidae and Hoplolaimidae: the oviduct consists of two rows of four cells, an oval spermatheca is comprised of 12 to 14, and uterus cells are arranged in three rows. However, each row does not consist of a limited number of cells, but the exact number of cells in this elongated uterus could not be determined.

Meloidogynidae, ultrastructure of oviduct-spermatheca region of Meloidogyne incognita: Previous LM results have shown that the gonoduct of the genus

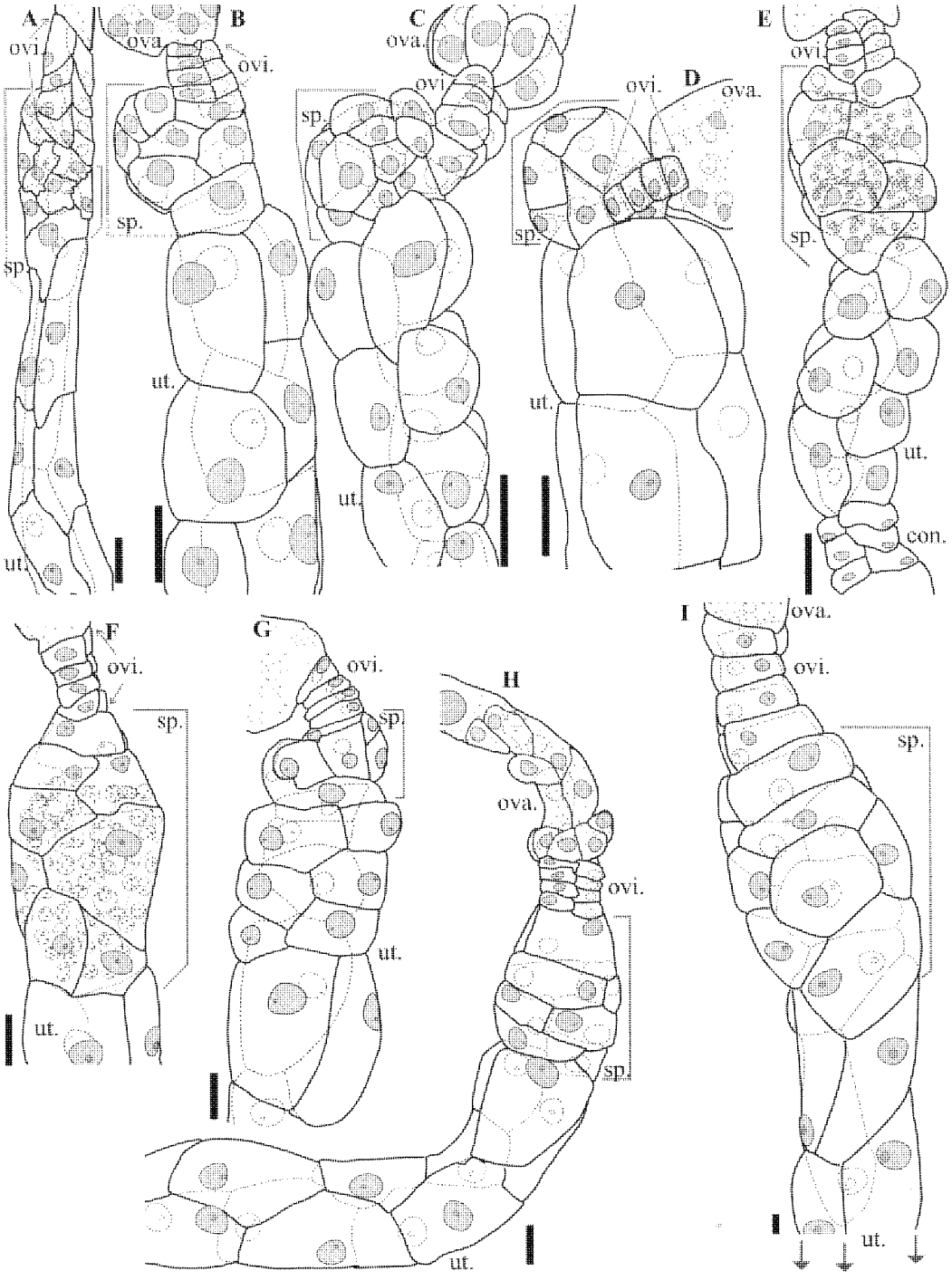

FIG. 5. The cellular architecture of oviduct, spermatheca and distal part uterus of Hoplolaimidae and *Meloinema*. A) *Helicotylenchus* cf. *dihystera*. B) *H. pseudorobustus*. C) *H. varicaudatus*. D) *H. canadensis*. E) *Rotylenchus goodeyi*. F) *Scutellonema bradys*. G) *Hoplolaimus aegypti*. H) *R. uniformis*. I) *Meloinema odesanens*. ova.: proximal end of ovary; ovi.: oviduct; sp.: spermatheca; ut.: uterus; con.: constriction between uterus and uterine sac. Scale bars = 10 μm.

Meloidogyne typically consists of an oviduct that is comprised of two rows of four cells; its spherical spermatheca is composed of characteristic lobe-like protruding cells that have often interlaced cell boundaries; and the uterus cells are arranged in three elongated cell rows (see Bert et al., 2002). As seen by TEM, two facing oviduct cells are connected to each other only for 4 to 5 μm; adhering junctions strengthen the connection (Fig. 6B,C). The lumen of the oviduct is extremely narrow (Fig. 6C). The oviduct cells are filled with lipid droplets, strongly stained lamellar bodies, and rough endoplasmic reticulum. Mitochondria are concentrated around the variably shaped nucleus. The oviduct cell membrane displays several invaginations. The cytoplasm of the spermatheca cells is distally densely filled with rough endoplasmic reticulum, and

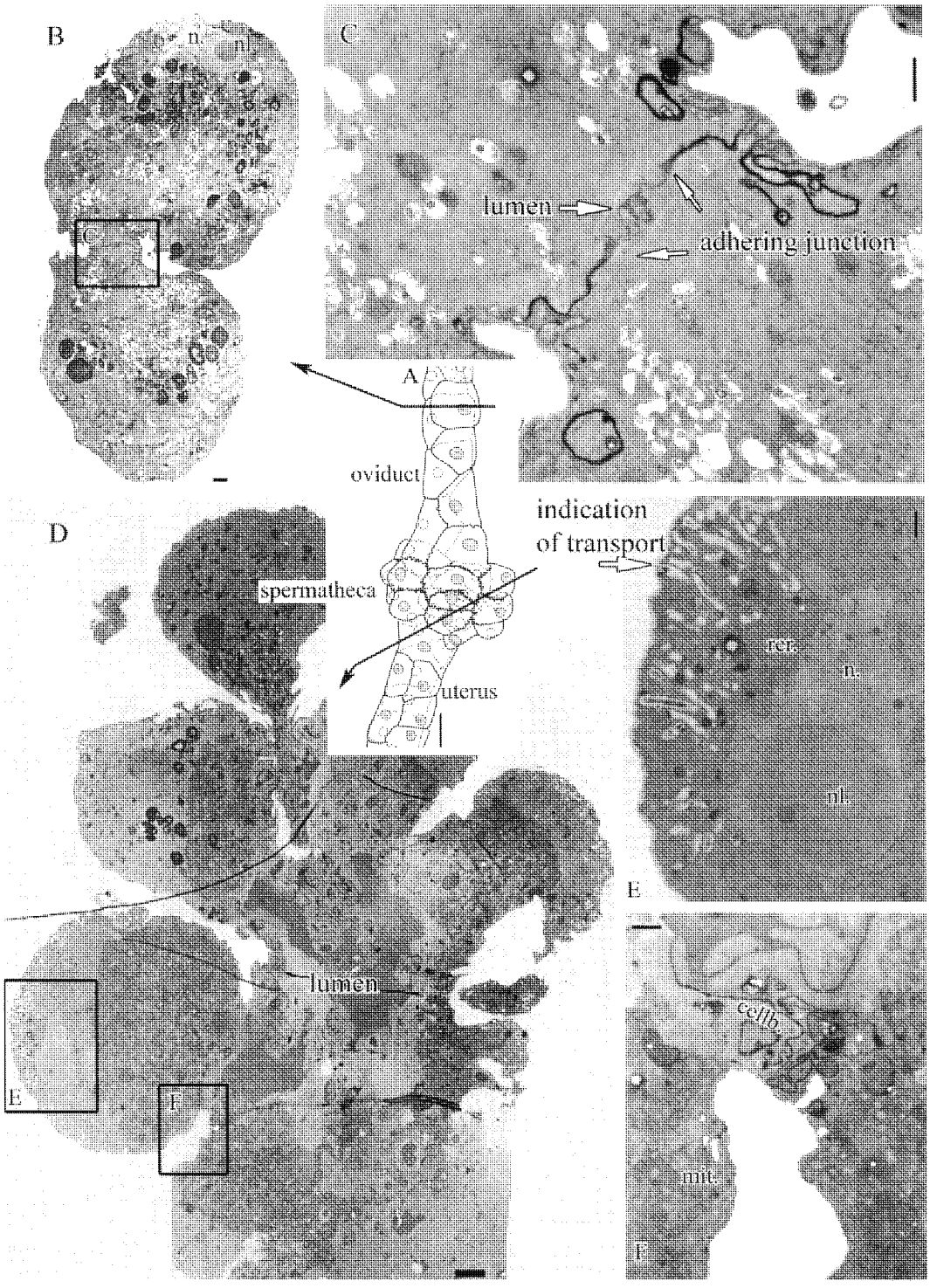

FIG. 6. TEM study oviduct-spermatheca region of *Meloidogyne incognita*. A) Overview drawing based on LM of expelled gonoduct. B) Transversal section through oviduct. C) Detail lumen oviduct. D) Oblique section through spermatheca and distal end of uterus. E) Detail proximal region spermatheca cell with indication of trans-membranous transport. F) Detail base of spermatheca cell lobe. cellb: cell boundaries of two adjacent spermatheca cells; mit: mitochondria; n: nucleus; nl: nucleolus, rer: rough endoplasmic reticulum. (Scale bars: A = 30µm; B = 1µm; C = 500 nm; D = 2 µm; E and F = 500 nm)

vesicular bodies are in connection with the outside of the cell (Fig. 6D,E). At the base of each lobe, the connection between two adjacent cells is sinuous (Fig. 6F). Microtubules connect to the cell membranes in this region (not shown). The spermatheca has a wide lumen, and the spermatheca wall can be less than 1 µm wide at the base of the lobes. The spermatheca nuclei and nucleoli are similar to the oviduct nuclei and nucleoli. The uterine wall is relatively wide, even in the presence of an egg (uterus not shown). The uterus cell

nuclei each contain a large round nucleolus that almost completely fills the nucleus. The uterus cytoplasm is dense, rich in mitochondria and endoplasmic reticulum.

Discussion

Females of the Tylenchidae taxa possess an oviduct with two rows of three to seven cells; a spermatheca may or may not be offset and is comprised of 10 to 20 cells; the uterus cells are arranged in four rows. Based on a limited number of populations studied, the spermatheca cellular morphology, and more particularly the number of cells in the offset part of the spermatheca, can provide an additional tool to characterize species within the genus *Tylenchus*. For example, the spermatheca of *T. arcuatus* is not offset, while *T. elegans* has an offset part of four cells and in *T. davainei* five to seven cells compose the offset part. Unlike the results of Brzeski (1999), who suggested the synonymization of *T. davainei* and *T. elegans*, a minimal but consistent difference in spermatheca cell composition between these species provides an additional argument to maintain the two as valid species. Conversely, the *Filenchus* populations apparently show relatively high intraspecific variability of the gonoduct. *Filenchus* species are particularly difficult to characterize, and the study of additional morphological gonoduct attributes does not solve this problem. Only *F. quartus* appears to be consistently different from the other described *Filenchus* species in having an offset spermatheca pouch. Remarkably, the actual presence of a spermatheca can not always be discerned from in toto material; when a spermatheca appears to be absent or is described as being absent, a distinct spermatheca is always manifest after dissection. This is in disagreement with the results of Chizhov and Berezina (1988a), who noted a negative correlation between tail length and development of spermatheca for their studies of *Filenchus* species.

The cell composition of the gonoduct in *Coslenchus* (with exception of *C. costatus*) and *Aglenchus* (Atylenchinae Skarbilovich, 1959) is comparable with that observed in *Filenchus* and *Tylenchus* (Tylenchinae). Conversely, the oviduct and spermatheca structure in *Basiria, Boleodorus* and *Neopsilenchus* is different by the presence of a slightly helical oviduct consisting of two rows of five cells and a spermatheca comprised of 16 cells. These gonoduct attributes appear characteristic for the Boleoderinae Khan, 1964. Although the oviduct and the spermatheca outlines resemble those of Boleodorinae, *Psilenchus aestuarius* differs in having a paired genital system and a uterus containing more than 35 irregularly organized cells. Considering the didelphic gonoduct arrangement and presence of phasmids on the tail, Ryss (1993), Sturhan and Rahi (1996) and Siddiqi (2000) stated that the placement of *Psilenchus* into a separate, though obviously paraphyletic, taxon (Psilenchidae in Dolichodoroidea, see Siddiqi, 2000) appeared more justified compared with the classification of *Psilenchus* in the Tylenchidae (Geraert and Raski, 1987). According to the current study, the absence of a clear quadricolumella, or tricolumella, does not allow one to assign the position of *Psilenchus* to either Tylenchidae or Dolichodoroidea on the basis of this character alone.

The *Cephalenchus* species studied (Tylodorinae Paramonov, 1967) are distinguished by their elongated oviduct, which comprises five to seven cells in each of the two rows of oviduct cells; this is higher than hitherto reported for the Tylenchoidea. Other Tylodorinae (*Eutylenchus, Cephalenchus, Campbellenchus* and *Tylodorus*) have been characterized by the spatial sequence of an elongate spermatheca followed by a transition zone of several cells, five or six cells in each row of the crustaformeria-part of the uterus, a second transition zone of several cells and a long uterine sac (*in toto* observations by Geraert and Raski, 1987).

Belonolaimidae and Hoplolaimidae show an overall similarity in the spatial arrangement of the gonoduct cells: two rows of four cells constitute the oviduct; the spermatheca comprises eight to 14 (mainly 12) cells; and the uterus is composed of three cell rows (tricolumella). The highest diversity is observed in the number and spatial arrangement of the spermatheca cells, especially within the genera *Geocenamus* and *Tylenchorhynchus*. The spermatheca outline in *Geocenamus* species (based on in toto observations) has previously been used in descriptions. For example, *G. (Merlinius) nothus* and *G. (Merlinius) microdorus* are known to have a bilobed spermatheca when the spermatheca is filled (Brzeski, 1998). However, closer examination after dissection can refine these species delimitations. The spermatheca of *G. nothus* comprises four lobes, while *G. microdorus* comprises only two lobes (independent of the presence or absence of sperm). The spermatheca of *G.* (*Scutylenchus*) *quadrifer* resembles the spermatheca of *H. canadensis* and *H. varicaudatus*, while the spermatheca of *Geocenamus* cf. *nurserus* is distinctive by the highly indented spermatheca cells. The spermatheca of *T. (Bitylenchus) dubius* (12–14 equally sized cells) is clearly different from the spermatheca of *T. (Bitylenchus) maximus* (eight-10 cells, two large cells proximally) and more similar to the spermatheca of *T. (Telotylenchus) ventralis* (12–14 cells, cells rather equally sized). This noticeable spermatheca diversity contradicts Geraert (1981), who reported a constancy of the spermatheca structure at the generic level for the Tylenchomorpha. However, the genus *Geocenamus* is considered here in a broad sense (following Fortuner and Luc, 1987), and the question arises if the gonoduct structure supports the subdivision of *Geocenamus* as advocated by Siddiqi (2000). Yet, the spermatheca diversity observed in our study does not correspond with known subdivisions within the genera *Geocenamus* and

Tylenchorhynchus. However, our data do not permit corroboration of any alternative for current generic definition. Nevertheless, the spatial cellular arrangement of the spermatheca provides valuable information to characterize species within the genera *Geocenamus* and *Tylenchorhynchus*. The spermatheca of *Helicotylenchus* and *Rotylenchus* shows less variation; their 12-celled spermatheca structure appears to be stable, confirming former observations (Geraert, 1981).

The gonoduct structure described in this study for the Belonolaimidae, Hoplolaiminae, Rotylenchulinae and *Meloinema* is analogous to that of the Pratylenchidae (with exception of *Nacobbus aberrans*) and the Heteroderinae. The characteristic elongation of the three uterus cell rows in *Meloinema* is an attribute shared with the Heteroderidae and Meloidogyne (Bert et al., 2002, 2003). Although the genus *Meloinema* is classified in Meloidogynidae, its spermatheca is clearly hoplolaimid-like and does not share the typical *Meloidogyne* characteristics, namely, spherical in shape and with lobe-like protruding cells. Further phylogenetic analyses including molecular data are necessary to clarify the position of *Meloinema*.

Our results obtained for *M. incognita* agree well with the observations of the ultrastructure of the gonoduct in *M. javanica* (McClure and Bird, 1976). We refer to these authors for a more extended discussion about the morphological and functional aspects of the *Meloidogyne* gonoduct. The morphology of the oviduct was described as a remarkably stable structure within the tylenchs (Geraert, 1983). Our expanded LM results do not deviate from this, without exception, with respect to the oviduct being comprised of two rows of four cells. The very narrow lumen between the two rows of tightly packed oviduct cells shows clearly that the oviduct is a constriction between ovary and spermatheca. In other nematodes, e.g., *Xiphinema theresiae* and *X. meridianum* (Van de Velde et al., 1990a, 1990b), the complete absence of an apparent lumen is known. For *X. meridianum*, it is assumed that the oviduct cells are separated and pushed aside when an oocyte passes through, this because two or three nuclei are observed at the same level and the cell membranes of neighboring cells are highly intertwined (Van de Velde et al., 1990a). In *Meloidogyne*, oocytes pass through the oviduct in a clearly different way. Most likely, the oviduct lumen stretches considerably, and adhering junctions prevent the separation of two adjoining oviduct cells during this process. Ultrastructural analysis of the spermatheca substantiates that the *Meloidogyne* spermatheca is distinctive from that in any other currently known nematode genus (Bert et al, 2002), and that the spermatheca of *M. incognita* is an exceedingly complex structure. The outer membranes are distinctly invaginated and surround densely packed rough endoplasmic reticulum. This increased surface area could function in the transfer of metabolites, and thus the spermatheca probably has a more complex role than that of a simple receptacle. TEM of *Meloidogyne* was used in this study as a test case to further evaluate our LM observations of expelled gonoducts. The latter, relatively easy technique preserves the three-dimensional structure well. However, with light microscopy some aspects of interpretation may be speculative because morphological discrimination between the cells of adjacent gonoduct parts can be difficult. On a subcellular level, and with the aid of TEM, these morphological differences are much more evident. Based on our limited TEM data, ultrastructural information does confirm LM observations. Consequently, it seems that the LM-based interpretation of the gonoduct components is justified. The combination of expelled organs and TEM offers valuable possibilities. When a TEM study of a specific organ is required, it is labor saving to perform this study after expelling the organ of interest. More indirectly, TEM studies can be combined with LM studies of expelled organs to retain the spatial overview. This is especially useful if only a limited number of sections are studied by TEM. The combination of TEM on selected sections related to the knowledge of the cellular structure based on expelled organs is a relatively uncomplicated method that combines three-dimensional knowledge with ultrastructural detail.

We conclude that the information associated with the cellular gonoduct structure offers promising results for diagnostic purposes, as well as for analyzing relationships. Therefore, we advocate the description of the gonoduct morphology as a valuable component in the (re) description of (new) nematode species. Further, gonoduct data, together with other morphological data and molecular data, need to be assessed to infer phylogenetic relationships, especially in Tylenchina.

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