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The somatic female gonad of Cephalobidae (Nematoda): cellular architecture and associated function

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Summary – The female reproductive system of the free-living nematode family Cephalobidae is examined by means of differential interference contrast, scanning electron and fluorescent microscopy. The model nematode *Caenorhabditis elegans* and the predatory nematode *Prionchulus punctatus* are also included in this study; the former mainly to test our results with the very detailed knowledge of this model organism, the latter to provide a representative of the more distantly related Enoplea. In this comparative approach, the analysed gonad structures are discussed with respect to their functional and phylogenetic significance. The general cellular composition of the cephalobid gonad – namely an oviduct comprising two rows of four cells, a distinctly offset spermatheca consisting of 8-16 cells, and a uterus composed of distinct cell rows – differs from all known Nematoda except that of the plant-parasitic Tylenchomorpha. Despite the striking evolutionary conservation of the cellular architecture of the cephalobid gonad there is a complex subcellular specialisation, namely a significant and functionally relevant variation in myofilament organisation, both among Cephalobidae and between major groups of nematodes. We demonstrate the presence of microfilaments that vary in pattern among species and that may play an important role in egg propulsion. The phenomenon of *endotokia matricida*, in which eggs do not leave the female body, is found to be associated with a massive rupture of the cytoskeleton in the uterus wall. The complexity of the myofibril structure and the associated potential to propagate oocytes actively cannot be solely explained by differences in phylogenetic history, but is also linked to body diameter. In the larger *Acrobeloides maximus*, the proximal end of the ovary sheath is adorned with 12 distinct longitudinal bands, antibody binding positively for paramyosin, while in the smaller *Cephalobus cubaensis* myofilament organisation is at random.

Keywords – *Acrobeloides*, *Caenorhabditis*, *Cephalobus*, *Chiloplacus*, cytoskeleton, gonoduct, paramyosin, *Prionchulus*, propulsion, *Zeldia*.

The reproductive system of the phylum Nematoda has proved to be exemplary for the study of fundamental problems in cell biology and developmental biology (Hubbard & Greenstein, 2000). It exhibits a variation in form (Chitwood & Chitwood, 1950): within some families the structure is conserved, whereas in others it varies significantly across genera (Geraert, 1981). It is a complex organ composed of both somatic and germ line tissues, the former generally an amalgam of tubes containing oocytes and eggs, often with developing embryos. It is thought that in some nematodes oocytes and eggs are propelled by the kneading action of the nematode body during movement (Doncaster & Seymour, 1973; Seymour & Doncaster, 1973). Geraert (1978) proposed a crucial role for the lateral fields as being necessary for the maintenance of close contact between the body wall and egg so that the somatic muscles can assist in egg laying, a proposition he supported by noting that in older females the elasticity of the lateral fields diminishes so that eggs are no longer propelled. Subsequently, eggs develop and juveniles hatch within the female body, a process known as *endotokia matricida* (Geraert, 1978; Luc *et al*., 1979).

Although the nematode reproductive system includes characters that can be useful for taxonomic purposes, phylogenetic patterns in form and function explaining this variability are unknown. While Geraert (1973, 1976, 1978, 1981, 1983) and Geraert *et al*. (1980a, b) mainly analysed the cellular composition of the reproductive system for several free-living, plant-parasitic and insect-parasitic nematodes, Bert *et al*. (2002, 2003, 2006) focused on the cellular gonoduct composition of plant-parasitic Tylenchomorpha. Typically these plant-parasitic

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nematode families have a mono- or didelphic reproductive system with an oviduct comprising two rows of four cells, a distinct spermatheca comprising 10-20 cells, and a uterus composed of mostly regular cell rows. Very detailed information on gonad development and structure is known for the model organism *Caenorhabditis elegans*, including results from reconstructions of serial electron micrographs (Kimble & Hirsch, 1979; White, 1988) and fluorescent microscopy which reveal the cytoskeleton and myofibril structures (Strome, 1986; Ardizzi & Epstein, 1987). For a review of *C. elegans* morphology, see the *Handbook of Worm Anatomy* on the Wormatlas website (Altun & Hall, 2005). In the last 10 years, *Pristionchus pacificus* has been established as a satellite organism in evolutionary developmental biology, and recently its hermaphrodite gonad has also been studied in detail by electron and fluorescent microscopy (Rudel *et al*., 2005). The *Pristionchus* gonad exhibits numerous differences from *C. elegans*, including general cellular composition, *e.g.*, absence of a tissue (*i.e.*, the spermathecal valve cells) and changes in the cellular and sub-cellular morphology of individual ovary sheath cells.

In this study the reproductive system of representatives of the Cephalobidae are examined by means of differential interference contrast (DIC), scanning electron and fluorescent microscopy. Cephalobidae are bacterial-feeding nematodes that are not closely associated with animal hosts or vectors and include some of the most widespread opportunists, but also numerous specialists of sandy soils and extreme temperatures. Molecular evidence strongly supports an (exclusive) common ancestry for the morphologically disjunct cephalobs and plant-parasitic tylenchs (Blaxter *et al*., 1988; Holterman *et al*., 2006; Meldal *et al*., 2006). As such, the Cephalobidae have been proposed as a good model for plant-parasitic nematode research (Blaxter *et al*., 1988) and subject of structural evolution hypotheses related to parasitism (Baldwin *et al*., 2004). Furthermore, Cephalobidae display some intriguing developmental patterns, *e.g*., aspects of regulative development (Wiegner & Schierenberg, 1998) and, recently, a molecular framework for this group has been provided (Nadler *et al*., 2006). We also include *C. elegans* and the predatory mononchid *Prionchulus punctatus* in this study, the former mainly to test our results with the very detailed knowledge of this model organism, the latter so that we have a representative of the more distantly related Enoplea. The main purpose of this study is to present the first morphological analysis of the cephalobid female gonad, which is based on more sophisticated techniques than merely light microscopy, permitting a comparison to the gonad of other nematodes. The specific analysis of the microfilaments and myofibrils in the gonad offers improved insight into transport of oocytes/embryos, and this is discussed with respect to their functional and phylogenetic significance.

Materials and methods

Nematode strains and culture conditions

Acrobeloides bodenheimeri (Steiner, 1936) Thorne, 1937 (parthenogenetic, PDL32); *A. maximus* (Thorne, 1925) Thorne, 1937 (parthenogenetic with rare males, DF5048); *Cephalobus cubaensis* Steiner, 1935 (parthenogenetic, PS1197); *Chiloplacus* sp. (parthenogenetic, field population Sudan); *Zeldia punctata* (Thorne, 1925) Thorne, 1937 (parthenogenetic, PDL3) (Cephalobidae, Tylenchina) and wild-type *Caenorhabditis elegans* (Maupas, 1899) Dougherty, 1953 (hermaphrodite, N2) (Rhabditina) were cultured on NG agar plates seeded with *Escherichia coli* OP50 (Brenner, 1974). *Prionchulus punctatus* (Cobb, 1917) Andrássy, 1958 (parthenogenetic, GB0043) (Mononchina) was cultured on soil extract agar with *Oscheius* as prey. Soil extract agar consists of 1% agar made with a mixture of 70% water and 30% soil extract, the latter being prepared by boiling rotting leaves for 45 min in water in a microwave oven, filtering the material over a coffee filter and freezing at -20°C before use.

Light microscopy (LM)

Extraction and examination of the female reproductive system was based on the method of Geraert (1973), *i.e.*, bisecting at the vulva region with a small scalpel induced expulsion of gut and reproductive tissue. This was observed directly in temporary mounts with an Olympus BX 51 DIC microscope (Olympus Optical, Tokyo, Japan). This procedure was repeated until at least 20 preparations could be observed for each population. The morphology of the ovary was not studied using this method as it turned out that LM alone is insufficient to reveal the resolved cellular composition of the ovary. Illustrations were prepared using a camera lucida; the drawings were prepared using Illustrator CS 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.

Scanning electron microscopy (SEM)

Three or four nematodes were transferred to freshly prepared 3% paraformaldehyde in Dulbecco's 'A' phosphate-buffered saline (PBS) (Oxoid, Unipath, Basingstoke, UK), pH 7.2, cooled on ice and directly bisected. The gonad did not extrude easily under the influence of the fixative but this method was preferred to minimalise manipulation and resulting damage. Limited success was partly compensated for by repeatedly moving the worm in and out of the fixative leading to more extruded gonads, and by merely using numerous specimens. No attempt was made to separate the extruded gonad from the worm remains: moreover, including the remains made it easier to transfer individual specimens with a needle without touching the fragile gonad structures. These preparations were fixed overnight in the refrigerator and subsequently dehydrated, critically point dried, sputter coated with gold and observed using a Jeol JSM-840 (for details of SEM preparation procedure, see Willems *et al*., 2005).

Staining and antibody binding

Ten to 20 specimens of *C. elegans*, *A. maximus*, *C. cubaensis* and *P. punctatus* were transferred to egg salts (Strome, 1986) (118 mM NaCl, 40 mM KCl, 3.4 mM $CaCl_2$, 3.4 mM $MgCl_2$, 5 mM Hepes, pH 7.2) and bisected leading to expulsion of the gut and gonad. The gonads were transferred in small glass tubes fitting into Eppendorf tubes to minimise loss of gonads due to sticking to the plastic walls of the Eppendorf tube. As a control of staining and antibody binding (described below), *C. elegans* tissues were incubated simultaneously with the antibodies to confirm their activity.

Actin

Actin filaments were both incubated with antibodies and stained with phalloidin. Antibody staining was done as in Barstead and Waterston (1991). Gonads were fixed in ice-cooled, freshly prepared 3% paraformaldehyde, 0.1 M sodium phosphate, pH 7.0, 0.1 mM EDTA, and fixed overnight in the refrigerator. After washing twice in PBS pH 7.0, the embryos were submersed for 10 min in 100% methanol at −20°C. Gonads were washed twice in PBS, twice in PBS + 0.5% Tween-20 (Sigma, St Louis, MO, USA), and once in antibody binding buffer (PBS, pH 7.0, 0.5% Tween-20, 5% dried milk powder (Nestlé, Brussels, Belgium). The gonads were incubated overnight at 5°C in polyclonal rabbit anti-actin. Primary antibodies were removed by four washes in PBS + 0.5% Tween-20. Gonads were subsequently incubated in tetramethylrhodamine B isothiocyanate (TRITC) (Sigma) labelled anti rabbit for 6 h at room temperature. Secondary antibodies were removed by four washes in PBS + 0.5% Tween-20 and one wash in PBS. Embryos were mounted in glycerol and the slide was sealed with clear nail polish.

Phalloidin staining of actin was done as in Strome (1986), using the rapid one-step fixation/permeabilisation/staining procedure. After dissection in egg salts the gonads were transferred to fixative (1.5% paraformaldehyde, 0.1% vol/vol glutaraldehyde in embryonic culture medium: 80 mM NaCl, 20 mM KCl, 10 mM Mg_2Cl, 5 mM Hepes, pH 7.2, 50% foetal calf serum (FCS, Sigma). This fixative contained 80 nM rhodamine- or fluorescein-Phalloidin (Molecular Probes, Eugene, OR, USA) and 0.5 mg ml^{-1} lysolecithin. Unlike Strome (1986), no additional pressure was applied to the tissues, since no staining of the cytoskeleton of the developing embryos was envisaged. Samples were washed four times in PBS and mounted as described above.

Myosin and paramyosin

Fixation and incubation was as for actin staining described above. The following monoclonal antibodies (mAb) were generously made available by H.F. Epstein (Ardizzi & Epstein, 1987): mAb 5-6 anti myosin A, mAb 5-8.1 and 28.2 anti myosin B, mAb 9.2.1 anti myosin C, mAb 5-17.1.1 anti myosin D, mAb 5-9.1.1 and 5-23 anti paramyosin.

Tubulin

After extraction, gonads were immediately transferred to 100% methanol at −20°C for 10 min, washed three times in PBS and once in antibody incubation buffer PBS + 0.5% Tween-20. The specimens were then incubated overnight at 5°C in monoclonal rat anti-tubulin antibody YL 1/2 generously provided by J.V. Kilmartin (Kilmartin *et al*., 1982), washed three times in PBS + 0.5% Tween-20, and incubated for 6 h at room temperature in TRITC-anti-rat IgG (Sigma). Subsequently, the specimens were washed three times in PBS + 0.5% Tween-20, once in PBS and mounted in glycerol as described above.

Double staining

Extraction with 100% methanol alone produces good results for tubulin staining, but does not preserve the components of the actin cytoskeleton (Waddle *et al*.,

1994). To be able to stain elements of the actin and microtubule cytoskeletons simultaneously, gonads were treated according to Waddle *et al*. (1994). Gonads were fixed overnight in a mixture of 75% methanol, 3.7% formaldehyde, 0.005 M PBS and 0.1 mM EDTA, followed by extraction with 100% methanol at −20°C for 10 min. Washing and incubation were performed as described previously for actin antibody staining.

Desmosomes

Dissected gonads were transferred to methanol at −20°C for 5 min followed by acetone at −20°C for 5 min. They were rinsed three times in PBS for 10 min each and transferred to PBS + 0.5% Tween-20. The tissue was incubated overnight at 5°C with monoclonal antibody MH27 (binds adherens junctions; Francis & Waterston, 1991) diluted 1:300 in PBS-Tween-20, 2% dried milk. This was followed by three 5 min rinses in PBS-Tween-20. Dissected tissues were then incubated in FITC-labelled goat anti-mouse IgG (Sigma) at a 1:50 dilution for 1 h at room temperature and rinsed once in PBS-Tween-20 and three times in PBS before being mounted as described.

Nuclei staining

To stain nuclei, the gonads were incubated in 1 μg ml^{-1} 4,6-diamidino-2-phenylindole (DAPI, Sigma) added to one of the washing buffers.

Terminology

The terminology of the reproductive system used here is mainly based on Geraert (1983) who followed the interpretation of Chitwood and Chitwood (1950). A genital branch in a didelphic or monodelphic genital system consists of an ovary (= gonad) and gonoduct. However, herein we use for simplicity the term gonad for ovary plus gonoduct, thus following the *C. elegans* literature in this respect. Three zones in the telogonic ovary are delineated with reference to degree of development of germ cells (germinative, growth, ripening). The oviduct is the constricted region between ovary and spermatheca. In *C. elegans* the oviduct is often considered as the proximal corridor or neck of the spermatheca. The term uterus is here restricted to the eggshell formation region of the gonoduct. The uterine sac follows the uterus and terminates in the vagina, which is connected to the vulva.

Results

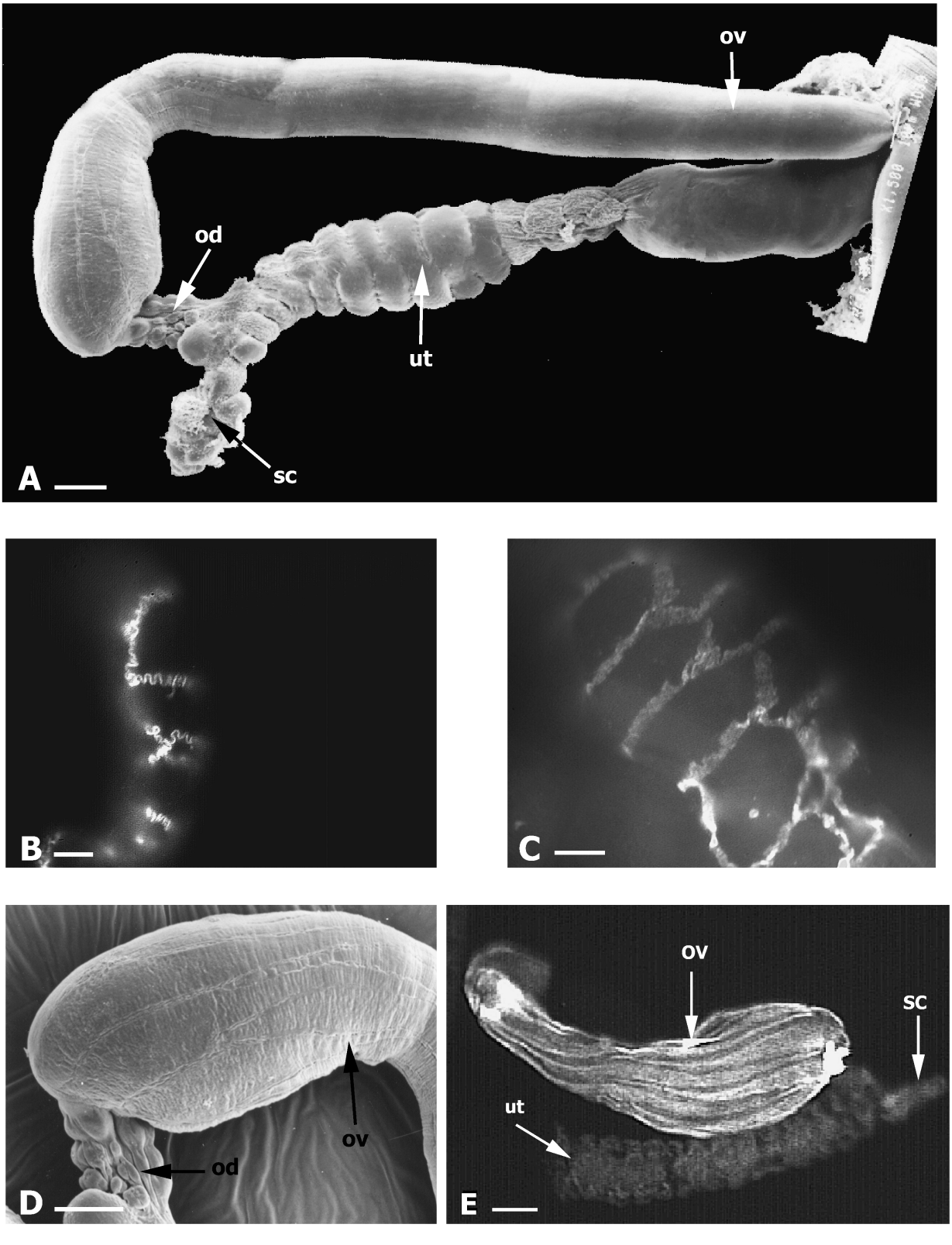
Cross reactivity of the antibodies used

Non-commercial antibodies of myosin and paramyosin were originally raised against *C. elegans* proteins. Cross reactivity of myosin antibodies to the other nematode species was limited, *e.g.*, the mAb 5-8.1 against myosin B bound weakly to the somatic body wall muscle of *A. maximus*, but binding patterns were inconsistent, indicating at least partial non-specific binding. Thus, the corresponding epitopes of myosin are possibly phylogenetically too variable to permit binding in non *Caenorhabditis* species. The mAb against paramyosin bound specifically in all species. Parallel control incubations with *C. elegans* confirmed the specificity and activity of the antibodies as reported (Strome, 1986; Ardizzi & Epstein, 1987).

Ovary

In *A. maximus*, although DAPI staining reveals eight flattened sheath nuclei, SEM shows no external cell boundaries (Fig. 1A), which is consistent with the complete absence of any binding of the MH27 antibody. The distal end of the ovary shows no external differentiation. The proximal end of the sheath is adorned with 12, clearly distinct longitudinal bands (Fig. 1A, D). A first set of six bands starts at a point about half way along the ovarian sheath and ends with a wide base at the junction of the ovary and oviduct (Fig. 1D). A second set of six bands starts closer to the distal end of the sheath, alternating with the first set, and all bands taper conically (Fig. 1D). The pattern of these bands is similar to the staining pattern for paramyosin (Fig. 1E). Longitudinal bands of (rather weak) tubulin and actin binding are also observed (not

Fig. 1. Acrobeloides maximus *gonad. A: General morphology of the female reproductive system as visualised by SEM; B: mAb MH27 binding reveals adherens junctions of the uterine wall cells when uterus is relaxed (no embryo passage), cell borders curl up like an accordion; C: Same as B but when stretched by passage of an embryo; D: SEM of the ovary showing bands on the surface of the basal lamina, these align with the distal-proximal axis of the gonad; E: mAb 5-23 binding reveals paramyosin filaments in ovary sheath cells, their organisation is similar as shown in D. Abbreviations: od = oviduct; ov = ovary; ut = uterus; sc = spermatheca. (Scale bars = 10 μm.)*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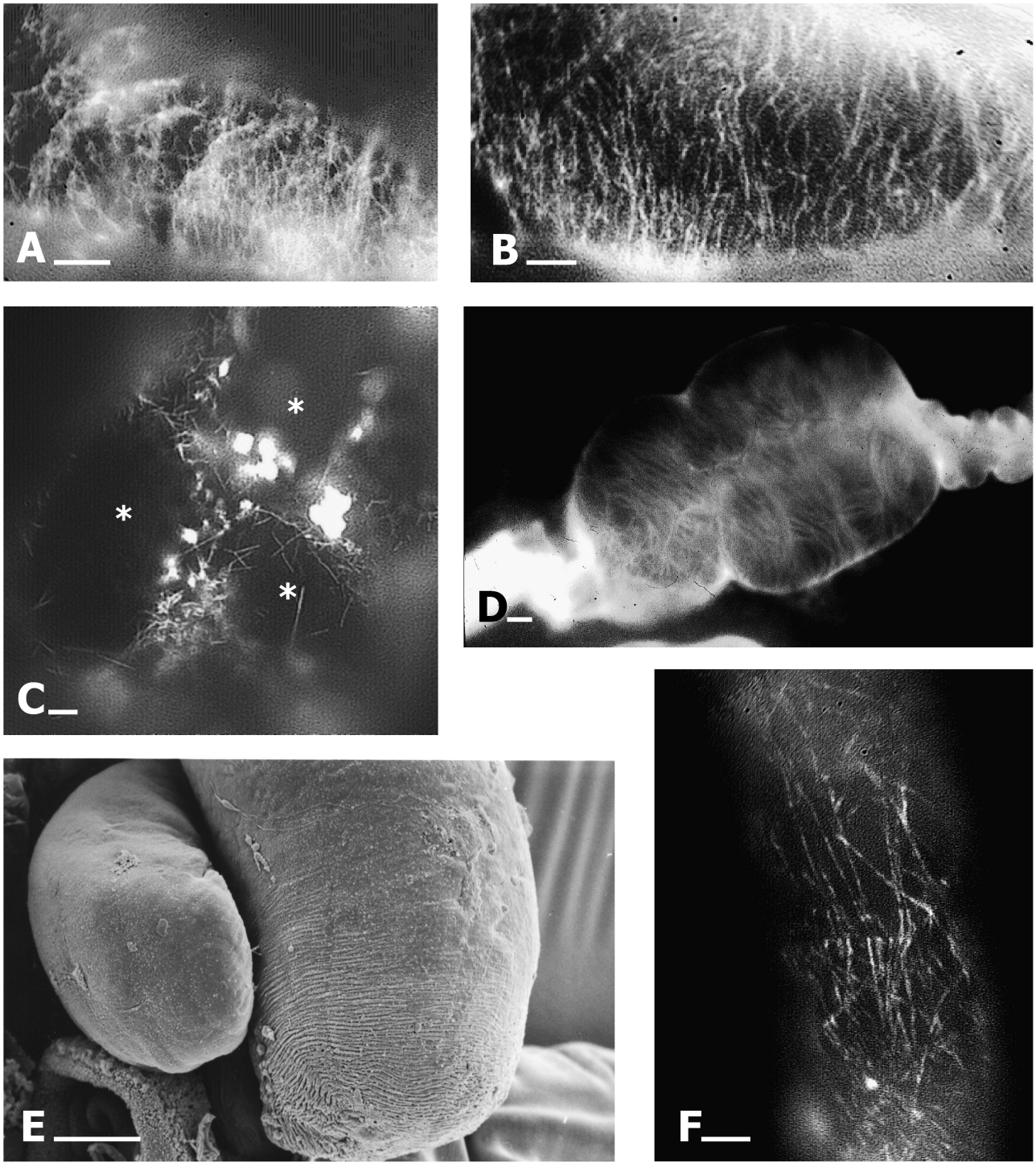


Fig. 2. *Uterus of* Acrobeloides maximus*; ovary of* Prionchulus punctatus *and* Cephalobus cubaenis. *A: mAb YL 1/2 reveals tubulin of the cytoskeleton of uterine wall cells of* A. maximus*, uterus without passing embryo. Tubulin filament distribution without clear pattern; B: Same as A, but during embryo passage. Filaments are stretched circumferentially in the cells enclosing the passing embryo; C: Accumulation of eggshell encased embryos (*) in the uterus of* A. maximus*.* *(Continued on next page.)*

shown). In contrast, paramyosin (Fig. 2F), actin and tubulin binding in *C. cubaensis* exhibit a dispersed pattern in the proximal part of the ovary. The arrangement of myofilaments in *P. punctatus* is completely different. Antibody binding of paramyosin and actin reveals a large number of circumferential filaments in this part of the ovary. Also SEM observations clearly demonstrate the presence of circumferential bands (Fig. 2E). Tubulin binding shows a similar pattern but with a rather weak signal.

Oviduct

The oviduct of all studied Cephalobidae is clearly differentiated from ovary and spermatheca and comprises two rows of four distinct cells (Figs 1D; 3A-E). In *C. elegans* it comprises four pairs of cells, but the transition between oviduct and spermatheca is not as clear-cut as observed by LM (Fig. 3F). The cellular arrangement of oviduct cells is more irregular in *P. punctatus*, the oviduct gradually widens from one cell row at the connection with the ovary to multiple unordered cells at the spermatheca (Fig. 3G). No oocytes were observed in the oviduct. Contrary to paramyosin, actin and tubulin stain strongly in the oviduct in all studied species.

Spermatheca

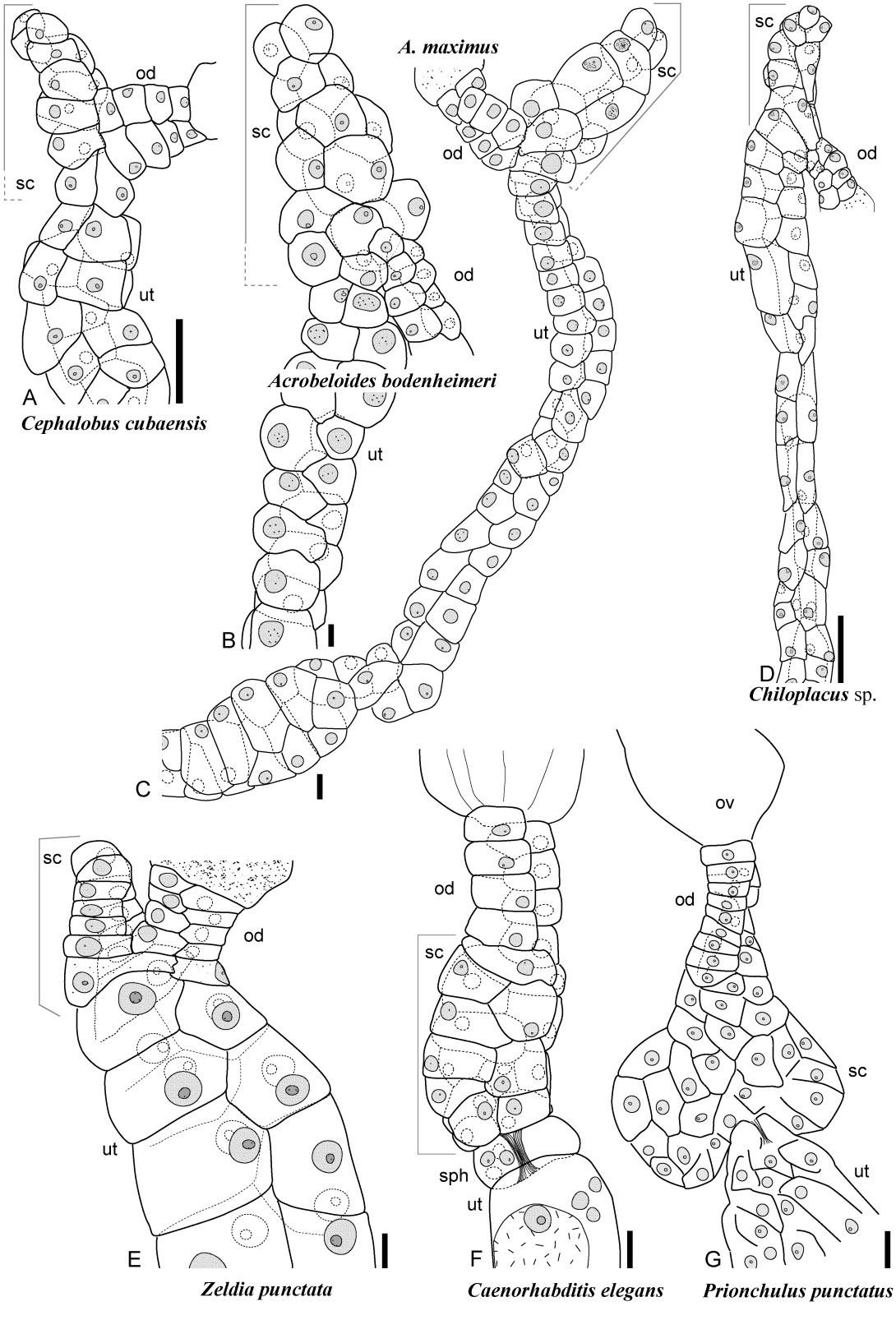
The distinct cephalobid spermatheca is always offset and comprises 12-14 cells (*Acrobeloides maximus*, *A. bodenheimeri*), 12 cells (*Cephalobus cubaensis*), ten cells (*Chiloplacus* sp.) or is arranged in two rows of five cells (*Zeldia punctata*) (Figs 1A; 3A-E). A spermatheca-uterus valve is absent and two or three cells connect these two instead. In *A. maximus* two cells at the oviduct-spermatheca-uterus junction are more or less integrated in the offset spermatheca pouch but are also in tandem with the two uterus rows. In *Z. punctata* two or three flattened cells are present at this junction, which have irregular cell boundaries and nucleoli that are larger than the cells composing the spermathecal pouch but distinctly smaller than the uterus nuclei. In no case does DAPI staining reveal the presence of sperm in either fourth-stage juveniles, young or old adult females of *A. maximus*, nor are embryos observed either in or close to the spermatheca. MH27 Ab binding is strong in the cells closest to the oviduct, but very weak at the top of the spermatheca. In *C. elegans* 18-22 spermathecal cells occur, spermatheca and oviduct appearing to form a single morphological entity. In this species there is a single spermatheca-uterus valve containing four nuclei, with a 'lariat'-like organisation of actin filaments in the middle (Fig. 3F). The spermatheca of *P. punctatus* is pear-shaped, comprising more than 60 irregularly organised cells, and a valve-like structure is present between ovary and uterus. The exact cellular composition of the spermatheca could not, however, be determined (Fig. 3G). Except for the strongly staining actin and tubulin, no other filaments are detected in the spermatheca.

Uterus

The cephalobid uterine wall consists of distinct interdigitated cells arranged in two to four rows. The uterus of *Acrobeloides maximus* comprises two rows, each row more than 24 cells long (Figs 1A; 3C). However, the cellular arrangement shows some variability in that the uterus can be enlarged closer to the vulva (10-20 cell pairs proximal to the spermatheca) with an additional short cell row. The uterus cells of *Zeldia punctata* are arranged in four rows (Fig. 3E). These rows become gradually more irregular closer to the vulva, showing a completely irregular arrangement at about 16-20 cells proximal to the spermatheca. The uterus cells of *C. cubaensis* and *Chiloplacus* sp. are arranged in three rows (Fig. 3A, D). Their exact total number could not be determined. In *C. elegans* and *P. punctatus*, the uterine wall cells are irregularly arranged and often indistinct (Fig. 3F, G).

Antibody binding (MH27) in *A. maximus* reveals that during relaxation (no egg passing through) the cell boundaries between adjacent cells curl up accordion-like (Fig. 1B), whereas during egg passage these membranes become stretched (Fig. 1C). Most tubulin filaments become circumferentially stretched during egg passage (see Fig. 2B *vs* 2A). In old females eggs tend to accumulate in the uterine sac. In specimens where this accumulation is considerable, the tubulin filaments are all ruptured (Fig. 2C). This phenomenon is only observed in older animals and always in the presence of accumulated embryos.

Fig. 2. *(Continued.) The tubulin filaments in the uterine wall are ruptured and appear as individual filaments organised at random; D: Phalloidin staining reveals actin filaments in the uterine wall. Uterus in similar condition as C but actin filaments are not ruptured; E: SEM demonstrates the presence of circumferential bands in the ovary of* P. punctatus*; F: mAb 5-23 binding reveals paramyosin filaments in ovary sheath cells of* C. cubaensis*. Very few filaments are present and organisation is at random. (Scale bars = 10 μm.)*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Actin staining is strong and fibres are densely packed in relaxed uterus cells; individual fibres cannot be distinguished. However, at sites where embryos are accumulated and where the tubulin is ruptured, the actin network is intact and individual fibres can be identified (Fig. 2D). The fact that the actin network remains intact confirms observations by LM which show that the uterus wall is not effectively ruptured at these places. No paramyosin was detected either in the uterus or in the uterine sac.

Discussion

Gross morphology of the cephalobid gonad

Although the genera studied here contain about 95 species, roughly one-third of the known species of the family Cephalobidae, the sampled morphological diversity cannot necessarily be assumed to be representative of the whole family. However, our observations on dissected cephalobid gonads agree well with what can be deduced from LM of *in toto* preparations of other Cephalobidae. An oviduct comprising two rows of four cells, a distinctly offset spermatheca consisting of about 10-16 cells and a uterus composed of distinct cell rows can to a certain extent be inferred from illustrations or observations in the descriptions of the following taxa: *Acrobeles emmatus* Shahina & De Ley, 1997 (see herein); *Cervidellus vexilliger* (de Man, 1880) Thorne, 1937 (see De Ley *et al*., 1994; Boström & De Ley, 1996); *Eucephalus striatus* (Bastian, 1865) Thorne, 1937 (see Anderson & Hooper, 1971); several *Nothacrobeles* species (see Shahina & De Ley, 1997; De Ley *et al*., 1999a; Poiras *et al*., 2002) and *Seleborca* (*Acrobeles*) species (see Rashid *et al.*, 1990). Only minor differences in the cellular composition of the spermatheca and oviduct cells are reported. For example, De Ley *et al.* (1999b) and De Ley *et al*. (1999a) reported only about eight spermatheca cells in *A. camberenensis* (De Ley, Geraert & Coomans, 1990) Siddiqi, De Ley & Khan, 1992 and *Nothacrobeles nanocorpus* De Ley, De Ley, Baldwin, Mundo-Ocampo & Nadler 1999, the spermatheca of *Cervidellus vexilliger* consists of eight smaller cells that form a proximal stem and eight larger swollen cells that form a swollen distal sac (De Ley *et al*., 1994; Boström & De Ley, 1996) and Rashid *et al.* (1990) noticed four or five pairs of cells in the oviduct of *S. welwitschiae* Rashid, Heyns & Coomans 1990. The cephalobs under investigation, supplemented with the above literature data, well cover the phylogenetic diversity within the Cephalobidae as outlined by Nadler *et al*. (2006).

It appears that the general cellular composition of the cephalobid gonad differs from that of other nematodes (see among others: Geraert, 1981; Stock *et al.*, 2002), except that of the plant-parasitic Tylenchomorpha, where the number and composition of cells of oviduct, spermatheca and uterus are similar (see Geraert, 1973, 1976, 1981; Bert *et al.*, 2002, 2003, 2006). However, differences from the tylenchid gonad include a more variable arrangement of uterus cells (in tylenchs usually three or four rows, depending on the family) and strictly offset spermatheca shape (the tylenchid spermatheca can be offset or not, with intermediate states). Molecular evidence supports common ancestry for the morphologically disjunct cephalobs and tylenchs (Blaxter *et al*., 1998; Holterman *et al*., 2006; Meldal *et al*., 2006) and shared morphological character states include certain detailed aspects of the feeding system. The position and number of muscle cells of the basal bulb of *Basiria gracilis*, a putative relatively primitive tylenchid nematode (Subbotin *et al*., 2006), is identical to that of cephalobs (Baldwin *et al*., 2001) and the tylenchid stomatostylet is hypothesised to be homologous with the cephalobid stoma (Baldwin *et al*., 2004). However, despite obvious resemblances between the cephalobid and tylenchid gonoduct, the phylogenetic significance of this relationship requires a thorough phylogenetic analysis on character polarity to achieve more conclusive gonad evolution hypotheses.

Remarks on the gross morphology of *Prionchulus* and *Caenorhabditis*

The general morphology of the *P. punctatus* gonad is in accordance with what is known for the Enoplea (Geraert, 1981), except that the presence of only a single cell row in the oviduct is not substantiated. Our findings for *C. elegans* correspond with elaborate former descriptions of the gonad of this model organism (Kimble & Hirsch, 1979; White, 1988; McCarter *et al*., 1997). However, we ob-

Fig. 3. *Line drawing of the cellular composition of oviduct, spermatheca and distal part of uterus of representatives of Cephalobidae,* Caenorhabditis elegans *and* Prionchulus punctatus. *A:* Cephalobus cubaensis*; B:* Acrobeloides bodenheimeri*; C:* A. maximus*; D:* Chiloplacus *sp.; E:* Zeldia punctata*; F:* Caenorhabditis elegans*; G:* Prionchulus punctatus*. Abbreviations: od = oviduct; ov = ovary; ut = uterus; sc = spermatheca; sph = sphincter. (Scale bars = 10 μm.)*

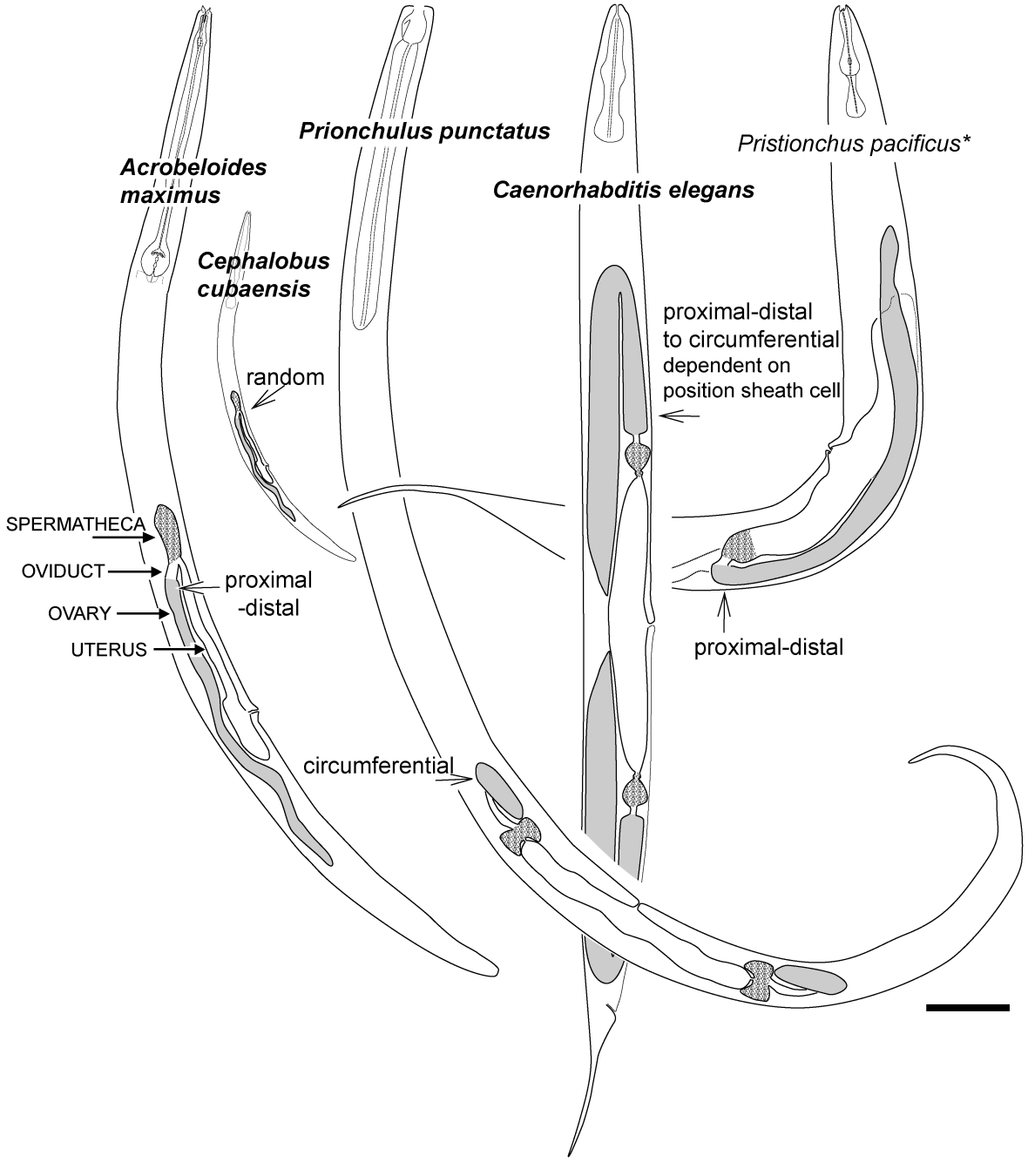

Fig. 4. *Schematic overview of the nematodes under investigation (* with exception of* Pristionchus pacificus*, see Rudel* et al.*, 2005) and their gonad structure. Nematodes are represented in respect to their relative size. Text refers to the myofilament organisation in the ovarial sheath cells. (Scale bar = 100 μm.)*

served slight variations in numbers of spermatheca cells, from 18-20, as opposed to the 16 found by Kimble and Hirsch (1979) based on cell lineages and the 22 found by White (1988) based on TEM. We assume that the variation we observed reflects practical difficulties in pinpointing the transition from oviduct to spermatheca rather than ac-

tual variation. However, the remarkable differences in the observed number of cells in either microscopic or cell lineage analyses cannot be explained for the moment. Nevertheless, our relatively uncomplicated method, LM of expelled organs, is able to reveal an important part of the general cellular morphology of the gonad. It is remarkable that in an organism like *C. elegans*, where virtually all morphological techniques have been applied, this technique has not been established as a simple explanatory method. We advocate the combination of direct light microscopic visualisation of expelled organs, as described above, in combination with more advanced techniques in order to retain a spatial overview of the organ under study.

Myofibril organisation

Despite the striking evolutionary conservation of the cellular architecture of the cephalobid gonad, there is a complex subcellular specialisation, namely a significant and functionally relevant variation in myofilament organisation both among Cephalobidae and between major groups of nematodes. The arrangement of myofilament bundles in the ovary in *Acrobeloides maximus* is distinctly different from that in *Cephalobus cubaensis*. In the former distinct bands align with the proximal-distal axis of the gonad, while in the latter they show a random organisation. Well organised myofibrils are likely to have a more important contractile function in pushing the embryos through the oviduct. A contractile system of myofilaments within the ovary sheath cells has already been described for *C. elegans* (Strome, 1986; Ardizzi & Epstein, 1987; McCarter *et al*., 1997) and *Pristionchus pacificus* (Rudel *et al*., 2005). In the latter, distinct myofilament bands are aligned with the proximal distal axis, unlike in *C. elegans* but similar to *A. maximus*. The number of sheath nuclei (eight) in *P. pacificus* is also the same as for *A. maximus*. The arrangement of myofibrils in circumferential bands, as observed in the more distantly related *Prionchulus punctatus*, is another possible way to actively push oocytes through the oviduct.

The simplest explanation for striking differences in myofibril organisation within the phylum Nematoda would be differences in phylogenetic history analogous to what is known for the gross cellular composition of the gonad (Geraert, 1981; this study). However, for the closely related larger *A. maximus* and smaller *C. cubaensis* (Fig. 4), body diameter could also be a crucial factor. Since the kneading action of the body muscles is supposed to be vital for oocyte transport (Doncaster & Seymour, 1973; Seymour & Doncaster, 1973), close body contact and active movement are necessary. Unlike *C. elegans*, cephalobid nematodes are generally less active than rhabditids, tending to remain at the same spot on the agar dish for long periods of time yet nevertheless continuing to lay eggs. At this stage we doubt whether the narrow ovary in larger and wider species has sufficient continuous contact with surrounding tissues to provide the kneading action. Hence, a sufficiently organised myofilament system is certainly needed for wider nematodes.

Once the embryos enter the proximal part of the ovary, they are actively propelled through the oviduct by the massive amount of paramyosin present. This may explain why embryos have never been observed actually passing through the oviduct. Once in the uterus, they are propelled very much like emptying a sausage by squeezing out the contents. The rearrangement of the cytoskeleton in longitudinal bands puts passive pressure on the embryo and forces it onwards into the uterine sac. The observation in older females of several embryos heaped together, one on top of the other, in the uterine sac and close to the vulva, is most likely the origin of the phenomenon of *endotokia matricida*. Anti-tubulin binding clearly indicates massive rupture of the cytoskeleton at that position, indicating that embryo transport through the vulva is inhibited. This finding would seem to contradict Geraert's (1978) suggestion that *endotokia matricida* is the result of loss of flexibility of the lateral fields, since in wider species there is bound to be less contact between body wall and reproductive system from the outset.

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