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REGULAR ARTICLE

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The distribution of F-actin during the development of *Diphyllobothrium dendriticum* (Cestoda)

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Abstract The distribution of actin filaments in all developmental stages of the tapeworm *Diphyllobothrium dendriticum* was studied. It is the first investigation of the placement of microfilaments during the development of a flatworm, and the results show that actin filaments, in all developmental stages, can be found in the subtegument and the flame cells. Muscle fibers possibly corresponding to the longitudinal, transversal, and dorsoventral muscles of the adult and plerocercoid were already detected in the procercoid. Concerning the adult worm, a new set of longitudinal fibers in the peripheral parts of the adult proglottid was found. The ducts of the protonephridial system and the vitellarias were seen to be surrounded by longitudinally oriented actin filaments, while the uterine ducts and the vagina were encircled by microfilaments. Prominent layers of circular muscle fibers surrounded the cirrus and the seminal vesicle, and radial fibers were also detected. Areas faintly stained with TRITC-phalloidin were found in the developing germ cells, the cells of the genital anlage, vitelline cells, the tegument, and the main nerve cords. None of these structures were autofluorescent, which is also true concerning the intensively labeled oncospheral hooks.

Key words F-actin · Microfilament · Phalloidin · Procercoid · Oncosphere · *Diphyllobothrium dendriticum* (Platyhelminthes)

Introduction

The adult *Diphyllobothrium dendriticum* tapeworm is an obligatory endoparasite, which inhabits the intestine of its final host, the gull. Tapeworm eggs pass out with the feces and embryonate in water, where coracidium larvae (the first larval stage) hatch. When a free coracidium is ingested by the first intermediate host, a freshwater copepod, it develops into the second larval stage, the procercoid. Freed in the intestine of the second intermediate host, a fish, the procercoid penetrates the intestinal wall and reaches the coelom, gradually developing into the third larval stage, the plerocercoid. When a fish bearing a plerocercoid is devoured by the definite host, the life cycle is completed (Bylund 1969). *D. dendriticum* undergoes very radical changes, e.g., concerning body temperature, when moving from one host to another.

Actin is an abundant globular protein, which can polymerize into long F-actin filaments. It is involved in several important cellular processes, such as muscle contraction, cytokinesis (Pollard and Weihing 1974), and molecular transport (Stitt et al. 1992; Matthews et al. 1994). Phallotoxins bind to F-actin (Wieland 1977) and can consequently be used when studying the distribution of microfilaments.

In flatworms, very little is known about the distribution of actin filaments. Only concerning the trematode *Fasciola hepatica* (Stitt et al. 1991, 1992) and the turbellarian *Macrostomum hystricinum marinum* (Rieger et al. 1994) have studies examining the localization of F-actin been conducted. No information concerning the changes in distribution of F-actin during the life cycle of flatworms is, however, available. The interest in flatworms lies in the fact that these animals are thought to have separated very early in the metazoan evolution (Adoutte and Philippe 1993; see Wahlberg and Johson 1997 for actin-based phylogeny). The expression of actin genes has been examined in plerocercoid and adult *D. dendriticum* tapeworms, and the results show that the flatworm actins can also be divided into cytoplasmic (cestoda-II, -III) and muscle-specific (cestoda-I) actin isoforms (Wahlberg 1997). In the present study, the localization of filamentous actin in all *D. dendriticum* developmental stages was studied using TRITC-conjugated phalloidin.

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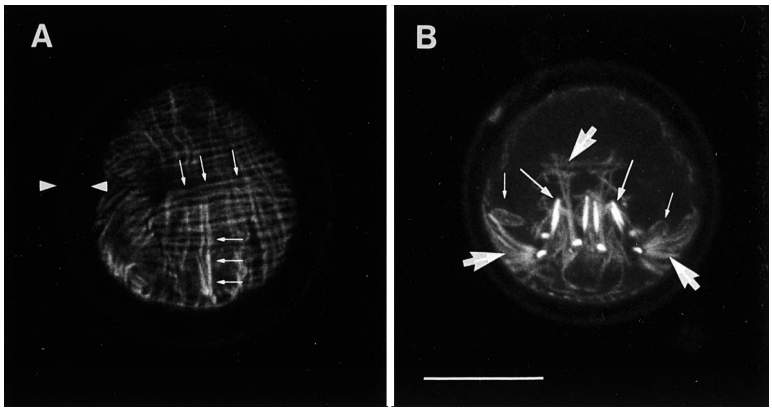


Fig. 1A, B *D. dendriticum* coracidium composed of the oncosphere and the surrounding embryophore (*arrowheads*). Confocal laser scanning-microscopic picture of the distribution of F-actin in **A** peripheral parts of the oncosphere (*arrows* indicate actin filaments in two orientations), and **B** central parts of the oncosphere (*small arrows* flame cells, *medium size arrows* hooks, *big arrows* hook muscles). *Scale bar* 20 μm

Materials and methods

Tapeworm material

D. dendriticum plerocercoids were obtained from whitefish (*Coregonus lavaretus*) and reared to adults in golden hamsters (*Mesocricetus auratus*). After 10–14 days, the adult tapeworms were regained and eggs were collected from gravid uterine coils. Hatching of the eggs was synchronized by incubating them for 8 days in aerated tap water at room temperature in absolute darkness. On exposure to light, all eggs hatched simultaneously and the coracidia were collected. Six weeks after infecting copepods (*Cyclops strenuus*) with newly hatched coracidia, procercoids were excised.

Phalloidin staining

Whole-mounts. Coracidia and procercoids were fixed in 4% formaldehyde (made fresh from paraformaldehyde) in 1×PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4), pH 7.5, at +4°C for 30 and 80 min, respectively. They were permeabilized in PTW (1×PBS, 0.1% Tween20) at room temperature for 15 min and finally stained with 3.8×10^{-7} M TRITC-phalloidin in PTW and 1% bovine serum albumin (BSA) for 20 min at +4°C. BSA was included in order to decrease possible background. Each treatment was followed by washes in 1×PBS.

Tissue sections. Adult and plerocercoid specimens of *D. dendriticum* were fixed in 4% formaldehyde in 1×PBS at +4°C for 4 and 2 h, respectively, and incubated overnight in 5% sucrose in 1×PBS at +4°C. Before embedding in Tissue-Tek O.C.T compound, the tissue was infiltrated with buffered 30% sucrose. Cryostat sections (15 μm) were collected on gelatine-coated slides and fixed for 10 min in 3% paraformaldehyde in 1×PBS, pH 7.5, at +4°C. The sections were stained with 3.8×10^{-7} M TRITC-phalloidin, mainly according to Eriksson et al. (1989). Whole-mounts and sections were mounted with glycerol-PBS and examined by using Leitz microscope and Leica confocal laser scanning microscope.

Controls

To evaluate nonspecific TRITC-phalloidin staining, an excess of unconjugated phalloidin (1.3×10^{-4} M) was included in the staining reaction. Control samples incubated with only TRITC-conjugated antisera (10 μg/ml) were also used. Autofluorescence was checked for by omitting TRITC-phalloidin from the reaction.

Results

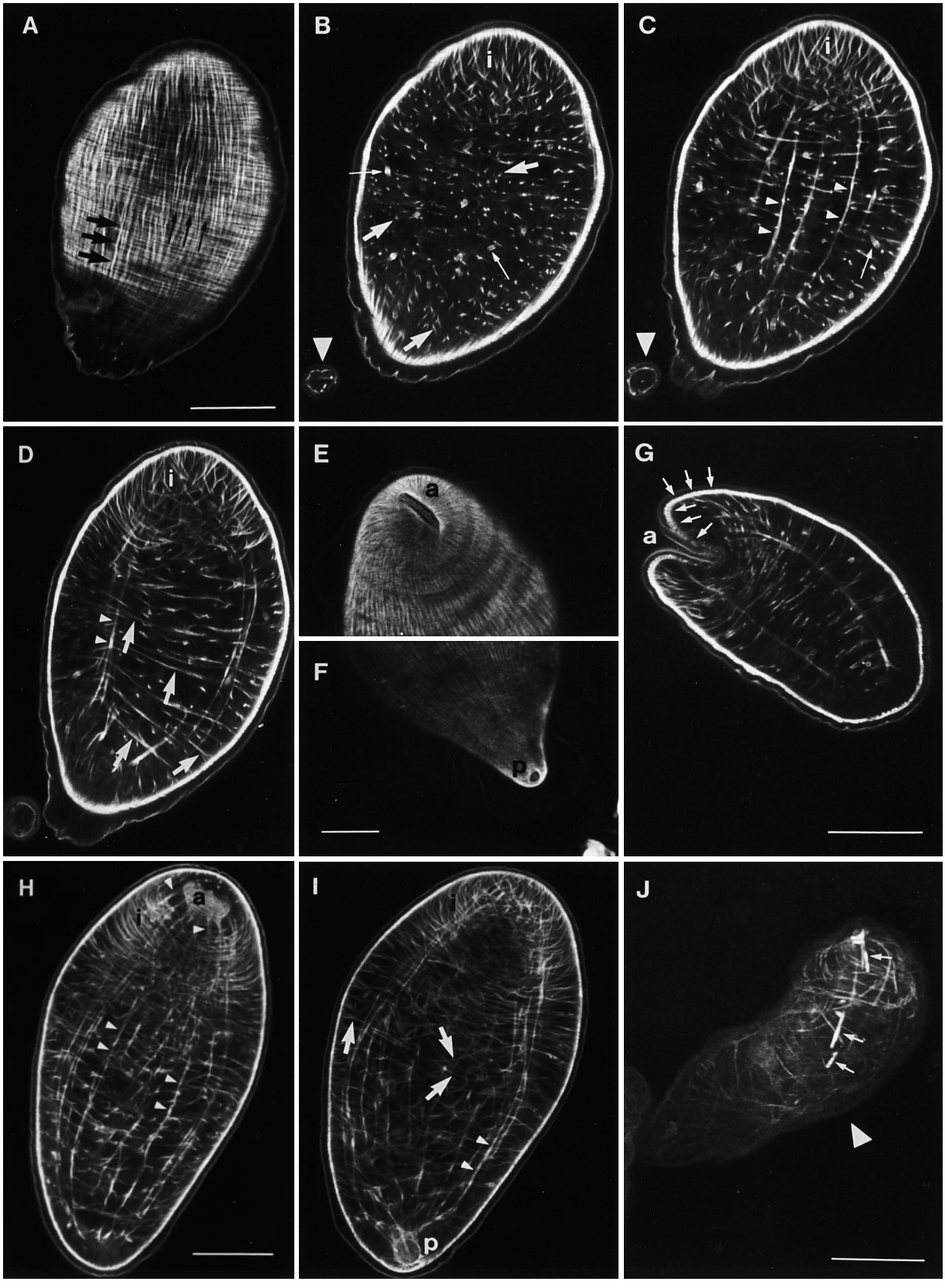
Filamentous actin could readily be distinguished in all developmental stages of *D. dendriticum* using TRITC-phalloidin. Excess unconjugated phalloidin in the staining reaction markedly reduced the staining overall, but did not totally abolish the fluorescence. Tissue treated with TRITC-antisera or specimen omitted of probe showed no staining.

The coracidium

The *D. dendriticum* coracidium is composed of the larva itself (the oncosphere) and the surrounding embryophore (Grammeltvedt 1973) (Fig. 1A,B). After labeling with TRITC-phalloidin, fibers crossing each other at right angles in the peripheral parts of the oncosphere were stained (Fig. 1A), as were the oncospheral hooks and the hook muscles (Fig. 1B). The staining of the hooks was very intensive. After omission of TRITC-phalloidin from the staining reaction or staining with only TRITC-labeled secondary antibody, the hooks showed no fluorescence, which excludes the possibility of autofluorescence. Blocking with 1000× non-conjugated phalloidin before and during the TRITC-phalloidin staining reaction decreased the intensive staining of the hooks. This would indicate a specific binding of phalloidin to the hook material.

A very faint "cloud" of fluorescence surrounding the embryophore was detected (not shown), as well as staining of the two flame cells, about 7 μm in size (Fig. 1B).

Fig. 2A–J Procercoid of *D. dendriticum*. Confocal laser scanning-microscopic pictures of the localization of filamentous actin in sections of the larva. **A–D** Sections of procercoid showing: **A** circular (*small arrows*) and longitudinal (*big arrows*) subtegumental muscles; **B** possible dorsoventral muscle fibers (*big arrows*) and flame cells (*small arrows*) of the outer parenchyma, *arrowhead* indicates the cercomer; **C** longitudinal actin fibers surrounding the inner parenchyma (*small arrowheads*), flame cell (*arrow*), cercomer (*big arrowhead*); **D** longitudinal (*arrowheads*) and possible transversal fibers (*arrows*). *Scale bar* for **A–D** 50 μm. **E** Anterior and **F** posterior invaginations; *scale bar* 50 μm. **G** Anterior invagination with subtegumental muscles surrounding the pit (*arrows*); *scale bar* 50 μm. **H–I** Procercoid in different orientations showing longitudinal muscles (*arrowheads*) extending from the **H** anterior and the **I** posterior invagination, possible dorsoventral and transversal muscles are indicated by *arrows*; *scale bar* 50 μm. **J** Cercomer, hooks are indicated by *arrows*; *scale bar* 20 μm. *a* Anterior invagination, *i* muscles surrounding the anterior invagination, *p* posterior invagination ▶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Details on the distribution of F-actin in flame cells are found in the chapter describing the plerocercoid and adult protonephridial system.

The procercoid

Actin filaments crossing each other at right angles were detected in the peripheral parts of the *D. dendriticum* procercoid. The filaments were outer circular and inner longitudinal subtegumental muscle fibers (Fig. 2A), which were also found to surround the invaginations situated in the anterior and posterior ends of the larva (Fig. 2E–G). In addition, muscle fibers starting from these invaginations were seen to run longitudinally from one end to the other, surrounding the inner parenchyma in a radially symmetric manner (Fig. 2C,D and H,I). From the area of the subtegumental muscles, actin filaments in two orientations, crossing each other at right angles, were seen to reach through the worm (Fig. 2B–D and H,I). These fibers seem to branch in the outer parenchyma. In the anterior end of the larva, additional actin fibers extended from the subtegument, surrounding the anterior invagination (Fig. 2B–D and H,I). Also, flame cells (approximately 9 μm in size), situated in the cortical region only, were stained with TRITC-phalloidin (Fig. 2B,C), (see protonephridial system of plerocercoids and adult worms). In the cercomer, the posterior appendage of the procercoid, peripheral actin fibers and the hooks were labeled (Fig. 2J, see also 2B–D).

The plerocercoid and adult *D. dendriticum* tapeworm

The distribution of F-actin in the plerocercoid was very similar to that of the neck region of the adult worm. The TRITC-phalloidin staining of the plerocercoid scolex, nerve cords, tegument, and protonephridial system was found to be identical to the distribution of microfilaments in corresponding parts of the adult worm.

Scolex. Scolex, the organ of attachment found in plerocercoids and adult worms, was seen to be composed of circular and longitudinal subtegumental muscles, as well as of longitudinal, transversal, and dorsoventral muscles of the parenchyma. An additional network of radial muscles extending from the region of the subtegumental muscles was detected (Fig. 3A).

Body muscles. Outer circular and inner longitudinal muscle fibers of the subtegument were detected in the plerocercoid and the adult worm. In the parenchyma, layers of outer longitudinal and inner transversal muscles were seen, as well as dorsoventral muscle fibers extending through the whole worm excluding the tegument (Fig. 3B–E). A new set of longitudinal muscles was observed in the outer parenchyma of the adult *D. dendriticum* (Fig. 3D,E). These were situated in the peripheral part of the proglottid, and they were not detected in the plerocercoid.

Tegument. The tegument was unstained except for thin filaments of actin running through it (Fig. 3F). Staining of the most peripheral part of the tegument was non-specific.

Nerve cords. The main nerve cords in the inner parenchyma of plerocercoid and adult tapeworms were faintly stained with TRITC-phalloidin (Fig. 3B,E), fluorescing dots and short fibers being detected throughout the cord (Fig. 3G).

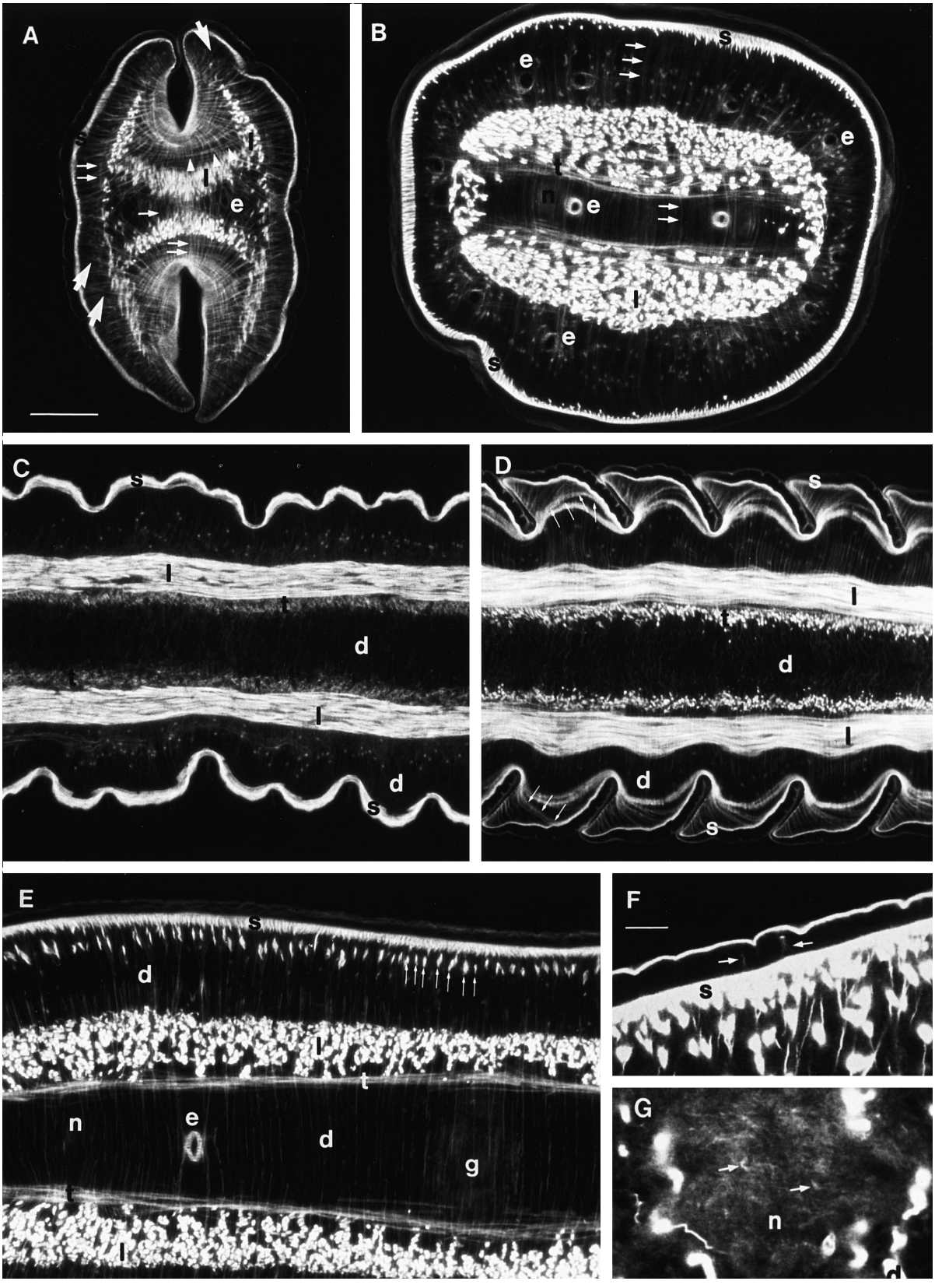
The protonephridial system. Parts of the excretory system of adult and plerocercoid *D. dendriticum* showed fluorescence after staining with TRITC-phalloidin. The main excretory ducts of the inner parenchyma were surrounded by longitudinal actin fibers located close to each other (Fig. 4A). Peripheral ducts of the outer parenchyma, especially in the anterior part of the worm, were also surrounded by longitudinal actin fibers, but these did not occur as densely as around the main ducts (Fig. 4B). The primary capillary duct extending from the flame cells became faintly stained as well (Fig. 4C). Also, the main excretory ducts of scolex were found to be surrounded by longitudinal muscles.

The flame cells (about 11 μm) were faintly stained in the area of the external ribs, and a more intensively labeled ring-like structure surrounding the flame close to its base was seen (Fig. 4C). These cells were found exclusively in the outer parenchyma, mostly oriented with the flame facing inward. They seem to be somewhat fewer in the adult worm than in the plerocercoid.

The reproductive organs. Except for the genital anlage, which is found in adults and in big specimens of the plerocercoid larvae, reproductive organs are present only in the adult tapeworm. The reproductive organs found to contain F-actin in this study were the female vitellaria, ovary, receptaculum seminalis, uterus, and vagina, as well as the male testes, seminal vesicle, and cirrus. A schematic drawing of the reproductive organs of a proglottid is shown in Fig. 5A and B.

The genital anlage. Cells migrating to the center of the proglottid in the neck region form a cluster called the genital anlage, which starts the development of most of the

Fig. 3A–G Localization of actin filaments in *D. dendriticum* plerocercoid and adult. Cross section of: **A** adult scolex, transversal muscle fibers (*arrowheads*), dorsoventral fibers (*small arrows*), radial fibers (*big arrows*); **B** anterior region close to the scolex of plerocercoid, dorsoventral fibers (*arrows*). Sagittal section of **C** plerocercoid and **D** adult, *arrows* indicate peripheral longitudinal muscle fibers. **E** Cross section of adult immature region, *arrows* indicate peripheral longitudinal fibers. *Scale bars* for **A–E** 100 μm. Confocal laser scanning-microscopic picture of **F** tegument, *arrows* indicate F-actin bridges; *scale bar* 20 μm; and **G** main nerve cord, *arrows* indicate actin filaments; *scale bar* 20 μm. *d* Dorsoventral muscle fibers, *e* excretory duct, *g* genital anlage, *l* longitudinal muscle fibers, *n* nerve cord, *s* subtegumental muscle fibers, *t* transversal muscle fibers ▶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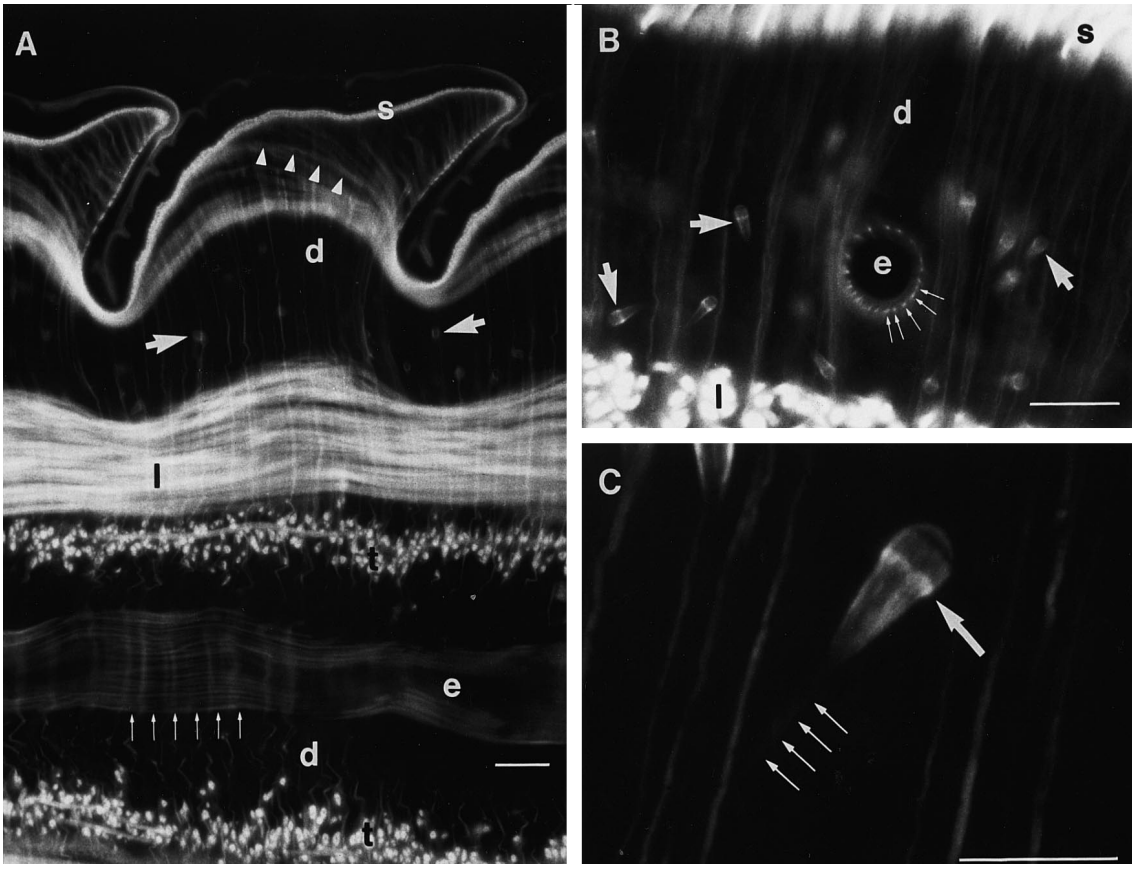


Fig. 4A–C Distribution of F-actin in the protonephridial system of *D. dendriticum*. **A** Sagittal section of adult showing longitudinal muscles surrounding the main excretory duct (*small arrows*) and flame cells (*big arrows*); longitudinal fibers in the peripheral part of the proglottid are indicated with *arrowheads*; *scale bar* 25 µm. **B** Longitudinal fibers surrounding the parenchymal excretory duct (*small arrows*) and flame cells (*big arrows*) of plerocercoid; *scale bar* 25 µm. **C** Confocal laser scanning picture of capillary duct (*small arrows*) and flame cell with intensively labeled ring-like structure (*big arrow*); *scale bar* 10 µm. *d* Dorsoventral muscle fibers, *e* excretory duct, *l* longitudinal muscle fibers, *s* subtegumental muscle fibers, *t* transversal muscle fibers

reproductive organs. When labeled with TRITC-phalloidin, the cortical cytoplasm of these cells became faintly stained (Fig. 6A).

Vitellaria. In the vitellaria, a faint staining of the shell protein material was detected (Fig. 6B,C). The shell of eggs present in the worm seemed unspecifically stained, since neither excess of unconjugated phalloidin in the staining reaction, nor additional dilution of the TRITC-phalloidin, reduced the fluorescence (not shown). However, since the shell protein material of the vitellarias only was faintly stained, the reduction of this fluorescence,

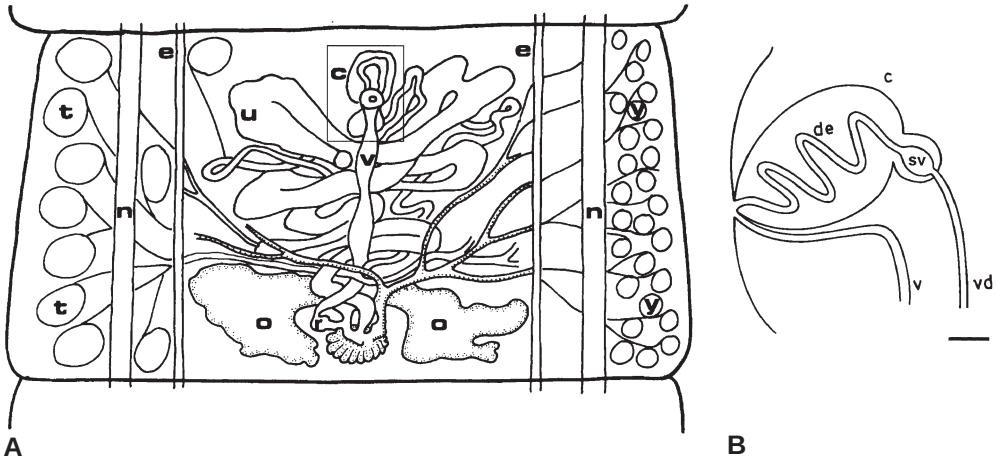
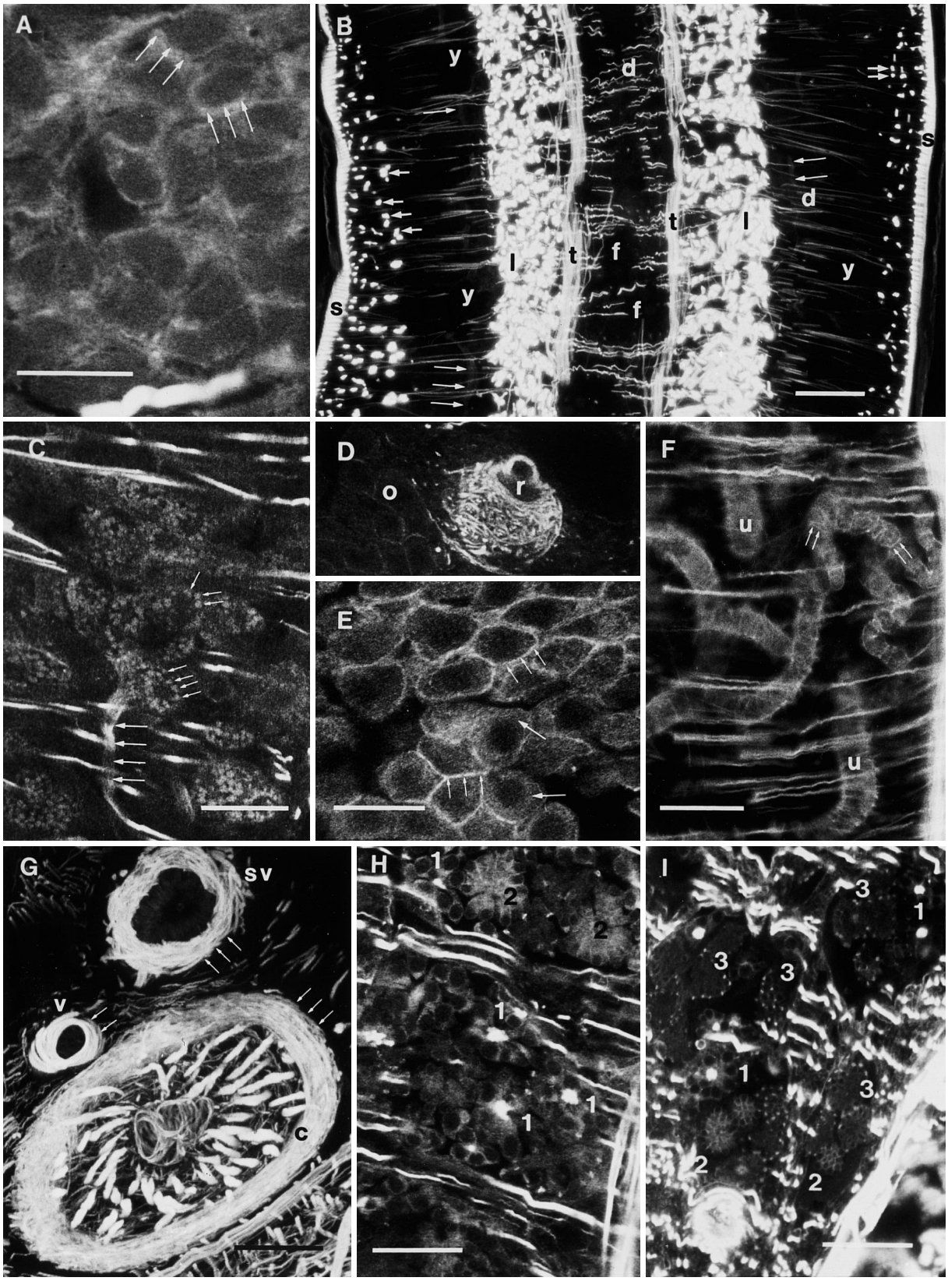


Fig. 5 **A** Schematic drawing of the *D. dendriticum* reproductive system (modified from Gustafsson 1985); **B** magnification of detail (see rectangle in **A**) containing seminal vesicle (*sv*), cirrus (*c*) and vagina (*v*) (modified from Andersen 1971). *Scale bar* in **B** approximately 50 µm. *de* Ductus ejaculatorius, *e* main excretory duct, *n* main nerve cord, *o* ovary, *r* receptaculum seminale, *t* testes follicles, *u* uterine ducts, *vd* vas deferens, *y* vitelline cells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in correlation to the overall reduction of fluorescence, was difficult to determine. This is also the case concerning the other faintly labeled structures. No labeling of the vitelline cells was detected after staining with TRITC-conjugated antisera.

The ducts running from the vitellaria to a collecting duct were visualized with TRITC-phalloidin, due to presence of longitudinal actin filaments in the duct walls (Fig. 6B,C).

Ovary. The ovary consists of two lobes connected by a narrow bridge (Andersen 1971). It is the site for the early onset of oogenesis where primary oocytes are formed (Rybicka 1966). F-actin was detected in the oocyte epithelium, displayed as a sheet of fluorescence along the borders of the oocytes. Also the cytoplasm of the oocyte seemed to be faintly stained (Fig. 6D,E).

Receptaculum seminale. The receptaculum seminale is a sac for storage of spermatozoa before fertilization of the ova (Andersen 1971; Mehlhorn 1988). With TRITC-phalloidin staining, the wall of this organ was found to consist of mainly circular fibers arranged like a ball of yarn (Fig. 6D).

Uterus and vagina. The most prominent organ of the female reproductive system is the uterus, often almost completely filling the gravid proglottids. After staining with TRITC-phalloidin, circular actin filaments were detected in the uterine walls (Fig. 6F). Also, the vagina was encircled by actin filaments (Fig. 6G). This layer of fibers was thicker than the circular fibers of the uterus wall. Additionally, very faint radial fibers of F-actin were detected inside the circular muscles of the vagina (Fig. 6G).

Testes. In non-mature parts of adult *D. dendriticum* tapeworms (no eggs present), the testes follicles primarily consist of groups of large cells, possibly representing rosettes of primary spermatocytes. After labeling with TRITC-phalloidin, the cytoplasm of these cells became faintly stained, and the central cytophore showed a very strong fluorescence (Fig. 6H, rosette 1). An indication of actin filaments surrounding testes follicles was seen in non-mature developmental stages of *D. dendriticum* (not shown).

In testes of mature proglottids (eggs present), only a small portion of the rosettes were of the presumed primary spermatocyte type (Fig. 6I, rosette 1). The major part comprised smaller cells, possibly representing spermatocytes/spermatid cells of later developmental stages. The cells of one type of rosette showed a faint cytoplasmic staining, as well as labeling of the cortical region (Fig. 6I, rosette 2). The other type of rosette contained cells with barely visible cytoplasmic staining, but more intensively labeled spots distal to the cytophore (Fig. 6I, rosette 3). Especially in the mature proglottid, the testes follicles partly disrupt the fiber structure of the inner parenchyma (Fig. 6B,I).

Seminal vesicle. A large number of sperm is stored in the seminal vesicle (Mehlhorn 1988). The peripheral wall of this organ turned out to be highly muscular, consisting of a thick layer of mainly circular fibers strongly stained with TRITC-phalloidin. From this muscular wall, very thin radial fibers containing F-actin were found to extend inward towards the seminal vesicle, which in its inner wall had an additional layer of thin circular actin fibers (Fig. 6G).

Cirrus. The male copulatory organ, cirrus, was seen to consist of peripheral layers of mainly circular muscle fibers from which thick radial muscles extended towards the ductus ejaculatorius. The ductus ejaculatorius was additionally surrounded by circular actin fibers. The part consisting of thick radial muscles also contained thinner F-actin filaments of different orientation (Fig. 6G).

◀ **Fig. 6A–I** Distribution of F-actin in reproductive organs of *D. dendriticum*. **A** Genital anlage, staining indicated with *arrows*. **B** Overview of cross section of mature adult worm showing vitellaria and testes, vitelline ducts (*long arrows*), peripheral longitudinal fibers (*short arrows*). **C** Vitellaria with vitelline cells containing shell protein material (*small arrows*) and vitelline duct (*big arrows*). **D** Receptaculum seminale and ovary. **E** Primary oocytes of the ovary, *small arrows* indicate cortical staining, *big arrows* indicate cytoplasmic staining. **F** The uterine ducts, circular actin filaments (*arrows*). **G** Reproductive organs peripherally surrounded by circular actin fibers (*arrows*). Testes follicles of **H** immature and **I** mature *D. dendriticum*, *numbers* indicate rosettes of cells of different developmental stages. *Scale bar* in **A** 10 µm, **B** 100 µm, **C,D** (=**C**), **F, G, I** 50 µm, **E, H** 20 µm. Figures **A, C, D, E** and **G–I** are confocal laser scanning pictures. *c* Cirrus, *d* dorsoventral muscle fibers, *f* testes follicle, *l* longitudinal muscle fibers, *o* ovary, *r* receptaculum seminale, *s* subtegumental muscle fibers, *sv* seminal vesicle, *t* transversal muscle fibers, *u* uterine ducts, *v* vagina, *y* vitelline cells, *1, 2, 3* different stages of sperm development (*1* rosette of primary spermatocyte)

Discussion

The coracidium

The *D. dendriticum* coracidium (oncosphere) has six hooks arranged in three pairs (Bylund 1975). These are operated by two types of hook muscles, those attached to the collar and those to the base of the hook (Rybicka 1966). These muscles, and especially the hooks themselves, were stained with TRITC-phalloidin (Fig. 1B). Controls indicate that the staining of the hook material is specific, although amino-acid analysis as well as microscopic and histochemical examinations of cestode hooks indicate that they contain keratin-like proteins as major components (see Swiderski 1973; Pearson et al. 1985). The spines of *F. hepatica* also show a very strong signal after staining with a monoclonal anti-actin antibody (Stitt et al. 1992), although Pearson et al. (1985) concluded that they are not composed of actin.

Actin fibers crossing each other at right angles are detected surrounding the oncosphere (Fig. 1A). Since the hooks mark the posterior end of the coracidium (Hyman 1951) (see Fig. 1B), these peripheral filaments represent circularly and longitudinally oriented muscle fibers.

Grammeltvedt (1973) detected oncospheral muscles beneath the embryophore inside the basal lamina of the *D. dendriticum* coracidium, but no specific examination of the muscle fibers was made.

The faint "cloud" of fluorescence surrounding the embryophore could indicate actin fibers of the cilia surrounding the coracidium.

The procercoid

The distribution of the procercoid muscles/actin fibers has been very poorly examined. In the present study, possible progenitors for the transversal and dorsoventral muscles of the plerocercoid were found extending from the subtegumental muscles through the procercoid. Also, longitudinal fibers were detected in this developmental stage, as well as fibers surrounding the anterior invagination (Fig. 2B–D and H,I).

The plerocercoid and adult worm

Thorough studies on the distribution of muscles in *D. dendriticum* have only been made concerning the plerocercoid scolex (Andersen 1975) and parenchyma (von Bonsdorff et al. 1971). The scolex is the holdfast with which the tapeworm attaches to the gut, and the localization of actin filaments in this region correlated well with the distribution of muscles reported by Andersen (1975). Radial muscle fibers, extending from the whole surface area (subtegument), were seen with TRITC-phalloidin staining (Fig. 3A).

The localization of longitudinal, transversal, and dorsoventral muscles of the plerocercoid parenchyma detected in this study coincides with the outcome of the work of von Bonsdorff et al. (1971). The subtegumental muscles were not included in the study of von Bonsdorff et al. (1971), but they are generally detected in parasitic flatworms (Mehlhorn 1988). Concerning *D. dendriticum*, subtegumental muscles were observed in plerocercoids by Lindroos (1983), but no detailed description was made. The longitudinal muscle fibers in the peripheral parts of the adult proglottids have not been described earlier (Figs. 3D,E and 4A). In expression studies, cestoda-I and to some extent cestoda-II actin genes were found to be transcribed in all types of body muscle cells (Wahlberg 1997). This indicates that muscle fibers of *D. dendriticum* contain cestoda-I and possibly also cestoda-II actin filaments.

The current study shows faint TRITC-phalloidin staining of F-actin bridges running through the tegument of plerocercoid and adult *D. dendriticum*. These bridges might be part of a mechanism for the transport of molecules in and out of the worm (Fig. 3F) (see also Stitt et al. 1992), and they could originate from the cestoda-III actin mRNA found in the tegument cell bodies (Wahlberg 1997).

The TRITC-phalloidin staining of the genital organs was found to vary to a great extent. The current results indicate a faint labeling of the shell-protein material of the vitelline cells in the outer parenchyma (Fig. 6C). No autofluorescence was detected. When stained with a polyclonal anti-actin antibody, the shell protein material of the vitellarias of *F. hepatica* was also labeled (Stitt et al. 1992).

Cortical parts of the oocytes of the *D. dendriticum* ovary were found to be stained with TRITC-phalloidin (Fig. 6E). A similar distribution of F-actin was seen in the previtellogenic stages of the cockroach ovary (Zhang and Kunkel 1992) and during mid to late previtellogenesis of *Rhodnius prolixus* (Insecta) oocytes (McPherson and Huebner 1993). In *D. dendriticum*, a faint staining of the cytoplasm was also seen. Cytoskeletal proteins are important components of the oocyte. Actin filaments are responsible for formation of the fertilization core, the contractile force needed for cleavage, and for the formation of the polar body (see McPherson and Huebner 1993). However, F-actin seems to also play an important role in the development of male germ cells.

The rosette of large cells (primary spermatocytes), mostly detected in the testes of the immature region of *D. dendriticum*, showed TRITC-phalloidin staining in the cytoplasm and the central cytophore (Fig. 6H,I). A similar pattern of F-actin was found in the testes of *F. hepatica*, although the cytoplasmic staining seemed more concentrated to the outer margins of the cells (Stitt et al. 1991). The testes follicles of *F. hepatica* were found to be surrounded by actin filaments (Stitt et al. 1991), an indication of which was also seen concerning testes follicles in immature regions of *D. dendriticum*.

Cestoda-II actin genes were found to be expressed in cells of the genital anlage, testes, and vitellaria, as well as in flame cells and cells surrounding the main nerve cords and excretory ducts (Wahlberg 1997). This gives an indication of the presence of cestoda-II actin filaments in these areas.

General conclusions

Subtegumental muscle fibers were detected in all the developmental stages of *D. dendriticum* (Figs. 1A, 2A, 3B,E). In the procercoid, plerocercoid, and the adult worm the subtegumental muscles were found to consist of outer circular and inner longitudinal fibers. This is apparently also the case in the coracidium, although the order of the fibers at this stage remained somewhat unclear. The role of the subtegumental muscles could be involvement in peripheral (tegumental) mobility. The longitudinal muscles detected in the peripheral part of the proglottid of the adult worm possibly originated from the longitudinal subtegumental muscles (Fig. 3D,E).

The excretory system of cestodes is of the protonephridic type (Mehlhorn 1988) with the flame cell as its basic component. According to the results of this study, the flame cells contain F-actin in a ring-like structure surrounding the flame close to its base, and it is also faintly stained with TRITC-phalloidin overall in the area of the external ribs (Fig. 4C). No previous studies concerning the F-actin content of flame cells have to my knowledge been conducted.

In procercoids, the flame cells are located exclusively in the outer parenchyma, peripherally from the longitudinal muscles. This is also the case in the plerocercoid and the adult worm, which was observed by von Bonsdorff et al. (1971) as well. The size of the flame cells varies from about 7 µm in the coracidium and 9 µm in the procercoid, to about 11 µm in the plerocercoid and the adult worm.

The flame cells lead to primary (capillary) ducts, which join with others to form larger longitudinal ducts, making up a peripheral network of excretory ducts. The left and the right longitudinal main excretory ducts connected to each other with thinner ducts, are found in the inner parenchyma (Lindroos and Gardberg 1982). According to the present study, the main excretory ducts of the inner parenchyma, and peripheral ducts particularly in the anterior part of the worm, are surrounded by longitudinal muscle fibers (Fig. 4A,B). These results differ from those of Lindroos (1983), according to which the large protonephridial ducts partly are surrounded by dorsoventral muscles, while a distinct muscle layer is lacking.

Several of the ducts of *D. dendriticum* were found to be surrounded by muscle fibers. The walls of the excretory and vitelline ducts contained longitudinal fibers (Figs. 4A,B and 6C), while the walls of the uterine ducts and the vagina were encircled by circular muscles (Fig. 6F,G). The male reproductive organs, cirrus and the seminal vesicle, were surrounded by several layers of muscles, the most prominent consisting of peripheral, mainly circular muscle fibers (Fig. 6G). Also, the receptaculum seminale was surrounded by circular muscles (Fig. 6D).

This work, in combination with in situ hybridizations (Wahlberg 1997), indicates that the muscle fibers of *D. dendriticum* are composed of cestoda-I and possibly cestoda-II actin filaments. Cestoda-II actins seem to be important proteins in developing and fully developed genital organs (anlage, testes, and vitellaria), as well as in the excretory and nervous system. The tegumental F-actin bridges possibly consist of cestoda-III actin protein.

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