## 3.3. ULTRASTRUCTURE DE LA SPERMIOGENESE ET DU SPERMATOZOÏDE DE LA DOUVE DU FOIE *FASCIOLA GIGANTICA* COBBOLD, 1856 (DIGENEA, FASCIOLIDAE), PARASITE DU BETAIL AU SENEGAL

**Résumé :**

Cet article porte sur l'étude ultrastructurale de la spermiogenèse et du spermatozoïde de *Fasciola gigantica* au microscope électronique à transmission.

Chez *F. gigantica*, la spermiogenèse commence par la formation d'une zone de différentiation formée de deux centrioles associés à des racines striées et séparés par un corps intercentriolaire. Chaque centriole donne naissance à un flagelle. La fusion proximo-distale de ces flagelles avec l'expansion cytoplasmique médiane aboutit à la formation du spermatozoïde. Chez *F. gigantica*, la spermiogenèse est caractérisée par la formation d'une expansion cytoplasmique dorso-latérale, d'une ornementation externe de la membrane cytoplasmique et des corps en forme d'épine. Ces trois structures sont également observées au niveau de la partie antérieure du spermatozoïde. Nous décrivons, pour la première fois, la présence simultanée de l'expansion cytoplasmique dorso-latérale, de l'ornementation externe de la membrane cytoplasmique et des corps en forme d'épine dans le spermatozoïde d'un trématode.

**Mots clés :**

Ultrastructure, spermiogenèse, spermatozoïde, *Fasciola gigantica*, Trematoda, Digenea, Fasciolidae.

# ULTRASTRUCTURE OF SPERMIOGENESIS AND THE SPERMATOZOON OF THE LIVER FLUKE *FASCIOLA GIGANTICA* COBBOLD, 1856 (DIGENEA, FASCIOLIDAE), A PARASITE OF CATTLE IN SENEGAL

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**ABSTRACT:** The present paper describes the ultrastructure of spermiogenesis and the spermatozoon of *F. gigantica* as revealed by transmission electron microscopy. Spermiogenesis in *F. gigantica* begins with the formation of a differentiation zone containing 2 centrioles with associated striated roots and an intercentriolar body between them. Each centriole develops a flagellum. Proximodistal fusion of these flagella with the median cytoplasmic extension gives rise to the spermatozoon. Spermiogenesis in *F. gigantica* is characterized by the formation of a dorsolateral cytoplasmic expansion, an external ornamentation of the cell membrane and spine-like bodies. These 3 structures were also observed in the anterior part of the spermatozoon. Our study describes for the first time the simultaneous presence of dorsolateral cytoplasmic expansion, external ornamentation of the plasma membrane, and spine-like bodies in the spermatozoon of a trematode.

*Fasciola* Linnaeus, 1758 includes 2 species, *Fasciola hepatica* Linnaeus, 1758 and *Fasciola gigantica* Cobbold, 1856. These liver flukes infect both domestic and wild animals, as well as humans. The definitive hosts of the *Fasciola* spp. are generally cattle, goats, rabbits, and deer, with humans accidentally infected as a result of ingestion of the metacercariae encysted on vegetables. Fascioliasis is a major disease that is generally caused by *F. hepatica* in temperate regions and by *F. gigantica* in the tropics. It causes significant economic losses, estimated at US $2 to 3 million annually (Boray,

1985). Tropical fascioliasis caused by infection with *F. gigantica* is regarded as one of the most significant single helminth infections of ruminants in Asia and Africa. Estimates of the prevalence of *F. gigantica* in ruminants range up to 80-100 % in some countries, e.g., 50 % in Mali, 65 % in Nigeria, 11-88 % in Egypt, 62 % in Chad, and up to 97 % in West Africa (Spithill et al., 1999). In Africa, *F. gigantica* has been reported in 16 species of wild herbivores (Boray, 1985). Human fascioliasis due to *F. gigantica* is an occasional disease in tropical countries of the former USSR, Asia, and Africa, e.g., *F. gigantica* is reported in 2.4 % of human fecal samples in Malawi. Several authors suggest that as a result of erroneous diagnosis, human infections may be more common than indicated by the occasional reports. Nevertheless, a recent review of human fascioliasis suggests that human disease results mainly from *F. hepatica* infections, with 2.4 million people infected and a further 180 million at risk. Human cases of *F. gigantica* have been reported in the southwest of Africa, Egypt, the Samarkand region of the former USSR, Thailand, and Germany (Spithill et al., 1999).

In *Fasciola,* ultrastructural studies of spermatogenesis have been conducted only for *F. hepatica* (Gresson and Perry, 1961; Stitt and Fairweather, 1990, 1992; Stitt et al., 1991). Moreover, the available ultrastructural data for *F. gigantica* are restricted to scanning electron microscopy of the tegument (Dangprasert et al., 2001, Meaney et al., 2002). In the present work, we describe the ultrastructural features of spermiogenesis and the mature spermatozoon of *F. gigantica*.

**MATERIALS AND METHODS**

Live specimens of *F. gigantica* were collected from the liver of *Bos indicus* in the SERAS abattoir in Dakar (Senegal). Adult digeneans were initially kept in a 0.9 % NaCl solution. Different portions of these specimens were then dissected and fixed in cold (4 C) 2.5 % (W/V) glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for 1 hr, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4 C) 1 % (W/V) osmium tetroxide in the same buffer for 1 hr, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in an ascending series of ethanol and propylene oxide, embedded in Spurr resin, and polymerized at 60 C for 48 hr. Ultrathin sections (60 to 90 nm) at different levels in the body (testes, vas eferens, and vas deferens) were cut using a Reichert-Jung Ultracut E ultramicrotome. They were then placed on 200-mesh copper grids and double-stained with uranyl acetate and lead citrate following Reynolds (1963).

The Thiéry (1967) technique was applied for the location of glycogen. Gold grids were treated in periodic acid, thiocarbohydrazide, and silver proteinate (PA-TCH-SP) as follows: 30 min in 10 % (W/V) PA, distilled water rinse, 24 hr in TCH, rinse in acetic acid solution, and distilled water, 30 min in 1 % (W/V) SP in the dark, and distilled water rinse.

The copper and gold grids were examined using 2 Hitachi H-600 transmission electron microscopes (TEMs) operating at an accelerating voltage of 75 kV at the "Serveis Cientificotècnics" of the University of Barcelona (Barcelona, Spain) and at the University of Corse (Corte, France).

## RESULTS

### Spermiogenesis

The beginning of spermiogenesis of *F. gigantica* is marked in each spermatid by the formation of a differentiation zone (Fig.1). In addition to the nucleus and mitochondria, this area contains 2 centrioles with associated striated roots that have an elongated triangular form (longer than 2,600 nm). An intercentriolar body is observed between the centrioles (Figs. 1-3). The differentiation zone is also surrounded by a submembranous layer of cortical microtubules. Each centriole develops a flagellum that grows externally to the emerging median cytoplasmic process. At the beginning of their development, the 2 flagella grow orthogonal to the median cytoplasmic process (Fig.1). Subsequently, they undergo a 90º rotation to an orientation parallel to the median cytoplasmic extension with which they then fuse (Figs. 2-8). The rotation and proximodistal fusion of the flagella are asynchronous, i.e., 1 flagellum fuses before the other (Fig. 6). The fusion of the second flagellum with the median cytoplasmic extension was accompanied by the formation of a so-called dorsolateral cytoplasmic expansion in the proximal part of the spermatid (Fig. 6). Attachment zones indicating the fusion of the flagella to the median cytoplasmic extension were observed before the proximodistal fusion of the free flagella (Fig. 5). The fusion of the flagella with the median cytoplasmic process determines the appearance of 2 sets of cortical microtubules, one dorsal and the other ventral (Figs. 4, 5). The nucleus and mitochondria migrate toward the median cytoplasmic process before the proximodistal fusion of the flagella (Fig. 5). However, a longitudinal section of the differentiation zone revealed that the migration of the nucleus takes place after flagellar rotation (Fig. 4) and before the migration of the mitochondria. Additionally, during this stage, the nucleus reaches distal areas of the median cytoplasmic extension (Fig. 2, 3). We also

observed mitochondrial migration toward the spermatid body in the final stage of spermiogenesis (Figs. 8, 9). Striated rootlets were observed in the proximal part of the spermatid body (Figs. 8, 9). Finally, the ring of arched membranes is constricted and the young spermatozoon detaches from the residual cytoplasm. A cross-section of the young spermatozoon (Fig. 10) reveals a spine-like body and external ornamentation of the cell membrane.

**Spermatozoon**

Ultrastructural features revealed in several longitudinal and cross-sections of the mature spermatozoon of *F. gigantica* have allowed us to distinguish 5 regions (I-V) from the anterior to posterior extremities.

Region I (Figs. 11-14, 25I) corresponds to the anterior region of the spermatozoon. It contains 2 axonemes of the 9 + '1' pattern typical of Trepaxonemata, as well as cortical microtubules. Cross-sections of the anterior extremity of the spermatozoon show a single axoneme (Fig. 11). A second axoneme and a submembranous layer of cortical microtubules soon appear (Figs. 12-14). Cortical microtubules are only absent in a small area where attachment points are observed. However, cross-sections in the posterior extremity of this region show the presence of a dorsolateral expansion of cytoplasm with electron-dense material and external ornamentation of the cell membrane (Figs. 13, 14). Cortical microtubules completely line the periphery of the gamete including the dorsolateral cytoplasmic expansion. These microtubules are regularly spaced except in the dorsolateral expansion, where they are closer together (almost touching each other) on the ventral face (Fig. 14). External ornamentation of the cell membrane also lines the periphery of the sperm, except in the ventral part of the dorsolateral cytoplasmic expansion where the more closely-packed microtubules are found (Fig. 13, 14).

Region II (Figs. 15-20, 25II). In addition to the structures observed in the posterior extremity of region I, region II contains 1 mitochondrion and spine-like bodies with a periodicity of about 1 μm (Figs. 16-19). Particular characteristics of this region include the progressive disappearance of the external ornamentation of the cell membrane and the dorsolateral cytoplasmic expansion (Figs. 16, 17), the disposition of cortical microtubules in 2 bundles (Figs. 17, 18), and the appearance of a significant quantity of glycogen (Figs. 18, 19). Before its disappearance, the dorsolateral expansion displaces toward more dorsal areas (Figs. 13, 14, 16). Consequently, a cross-section of

the spermatozoon in the posterior part of this region shows only 2 axonemes, 1 mitochondrion, and a significant quantity of β-glycogen (Fig. 18).

Region III (Figs. 20, 25III). In addition to the 2 axonemes, the nucleus and a mitochondrion are found to be simultaneously present in this region. Cortical microtubules on the ventral side of the spermatozoon are displaced toward the lateral side. The diameter of the spermatozoon is largest in this region and a reduced quantity of glycogen is present.

Region IV (Figs. 21, 25IV) is characterized by the absence of the mitochondrion, the disappearance of glycogen, and the presence of 1 of the axonemes at its posterior end.

Region V (Figs. 21-23 and 25V) corresponds to the posterior extremity of the spermatozoon. It contains only 1 axoneme and the nucleus (Fig. 21). Later, the axoneme becomes disorganized, the central core disappears, and peripheral doublets lose their arms, become disorganized, and then break apart into singlets and disappear (Fig. 22). The end part of this region contains only the nucleus with a broader diameter (Fig 23).

**DISCUSSION**

In *F. gigantica*, spermiogenesis follows the general pattern described in digeneans (Burton, 1972; Rees, 1979; Justine and Mattei 1982a; Erwin and Halton, 1983; Stitt and Fairweather, 1990; Cifrián et al*.,* 1993; Iomini and Justine, 1997; Miquel et al*.,* 2000; Baptista-Farias et al., 2001; Ndiaye et al., 2002). Thus, 2 free flagella arise from the differentiation zone perpendicular to a median cytoplasmic process. Thereafter, they undergo a rotation of 90º, become parallel to the median cytoplasmic extension and fuse with it. Migration of nucleus and mitochondria is also observed before the proximodistal fusion of the 2 flagella with the median cytoplasmic process. Nevertheless, spermiogenesis of *F. gigantica* is distinguished from the other digeneans by the formation of spine-like bodies and a dorsolateral cytoplasmic expansion. The last character appears after the incorporation of the second flagellum into the spermatid body. To our knowledge, these structures have never been described previously in spermiogenesis of digeneans. In the final stage of spermiogenesis, we observed striated rootlets at a deep level of the anterior part of the spermatid before strangulation of the arched membranes, but these were not observed in the spermatozoon. In fact, these structures, as showed in the micrographs, make a progressive displacement toward the ring of arched membranes. Our observations, therefore, support the hypothesis proposed by most authors that striated rootlets remain in the residual cytoplasm and later disappear through a process of depolymerization (Burton, 1972; Rees, 1979; Justine and

Mattei, 1982a; Erwin and Halton, 1983; Stitt and Fairweather,1990; Miquel et al., 2000; and Ndiaye et al., 2002).

The mature spermatozoon of *F. gigantica* exhibits the usual structures found in the great majority of digeneans so far, i.e., 2 axonemes with the 9 + '1' pattern typical of Trepaxonemata (Ehlers, 1984), mitochondrion, nucleus, and parallel cortical microtubules (Burton, 1972; Jamieson and Daddow, 1982; Miquel et al., 2000; Ndiaye et al., 2002). Taking an approach similar to that adopted in previous studies (Sato et al., 1967; Justine and Mattei, 1982a; Iomini and Justine, 1997), we designated the ventral and dorsal sides of the spermatozoon according to the arbitrary convention established by these authors. In the anterior part of the spermatozoon, the disposition of cortical microtubules in region I and the presence of the structures formed during the final stages of spermiogenesis, i.e., the external ornamentation of the cell membrane, the dorsolateral expansion of cytoplasm and the spine-like bodies, supported the view that the differentiation zone is incorporated in the mature spermatozoon. Similar observations have been made in *Haematoloechus medioplexus* (Justine and Mattei, 1982a), *Echinostoma caproni* (Iomini and Justine, 1997), *Gonapodasmius* sp. (Justine and Mattei, 1982b), *Notocotylus neyrai* (Ndiaye et al., in press), and *Scaphiostomum palaearcticum* (Ndiaye et al., 2002). However, some ultrastructural particularities can also be observed. The simultaneous presence of a dorsolateral cytoplasmic expansion, extramembranar ornamentation and spine-like bodies is described for the first time (see Table I). The dorsolateral expansion of cytoplasm has previously been described only in the spermatozoon of *E. caproni* (Iomini and Justine, 1997), a species that belongs to the same order as *F. gigantica* (Echinostomida La Rue, 1957). In *F. hepatica*, Stitt and Fairweather (1990) neither described the dorsolateral cytoplasmic expansion, nor the extramembranar ornamentation or spine-like bodies. Thus, to date, the dorsolateral cytoplasmic expansion associated with extramembranar ornamentation has been described only in *E. caproni* (Iomini and Justine, 1997). On the other hand, external ornamentation of the cell membrane has been described in 10 species of digeneans (see Table I). Finally, the simultaneous presence of external ornamentation of cell membrane and spine-like body has been described only in 2 species of digeneans, i.e., *Notocotylus neyrai* (Ndiaye et al., in press) and *Opecoeloides furcatus* (Miquel et al., 2000).

Cross-sections along the spermatozoon from the anterior to the posterior end reveal that the number of cortical microtubules decreases gradually until they completely disappear. This variation in their number may suggest that the microtubules

differ in length (Sato et al., 1967; Robinson and Halton, 1982; Stitt and Fairweather, 1990; Cifrián et al., 1993).

In *F. gigantica*, we observed only a single mitochondrion in the anterior part of the spermatozoon. This mitochondrion developed through the migration of numerous mitochondria from the differentiation zone. They fused end-to-end in the spermatid body and formed a long cylindrical body adjacent to the nucleus. The same phenomenon has been described in *Corrigia vitta* (Robinson and Halton, 1982), *Neochasmus* sp. (Jamieson and Daddow, 1982), *E. caproni* (Iomini and Justine, 1997), *Opecoeloides furcatus* (Miquel et al., 2000), and *S. palaearcticum* (Ndiaye et al., 2002).

Our study confirms most of the observations described by Iomini and Justine (1997) for the spermatozoon of *E. caproni*, a species belonging to the same order as *F. gigantica*. Nevertheless, differences between the observations made for *F. hepatica* by Stitt and Fairweather (1990) with respect to our preliminary observations in the same species, point to the need to reinvestigate the ultrastructure of *F. hepatica* sperm. In addition, it is our view that the dorsolateral cytoplasmic expansion, the spine-like bodies, and the external ornamentation of the cell membrane described in *F. gigantica,* should be useful characteristics for phylogenetic purposes.

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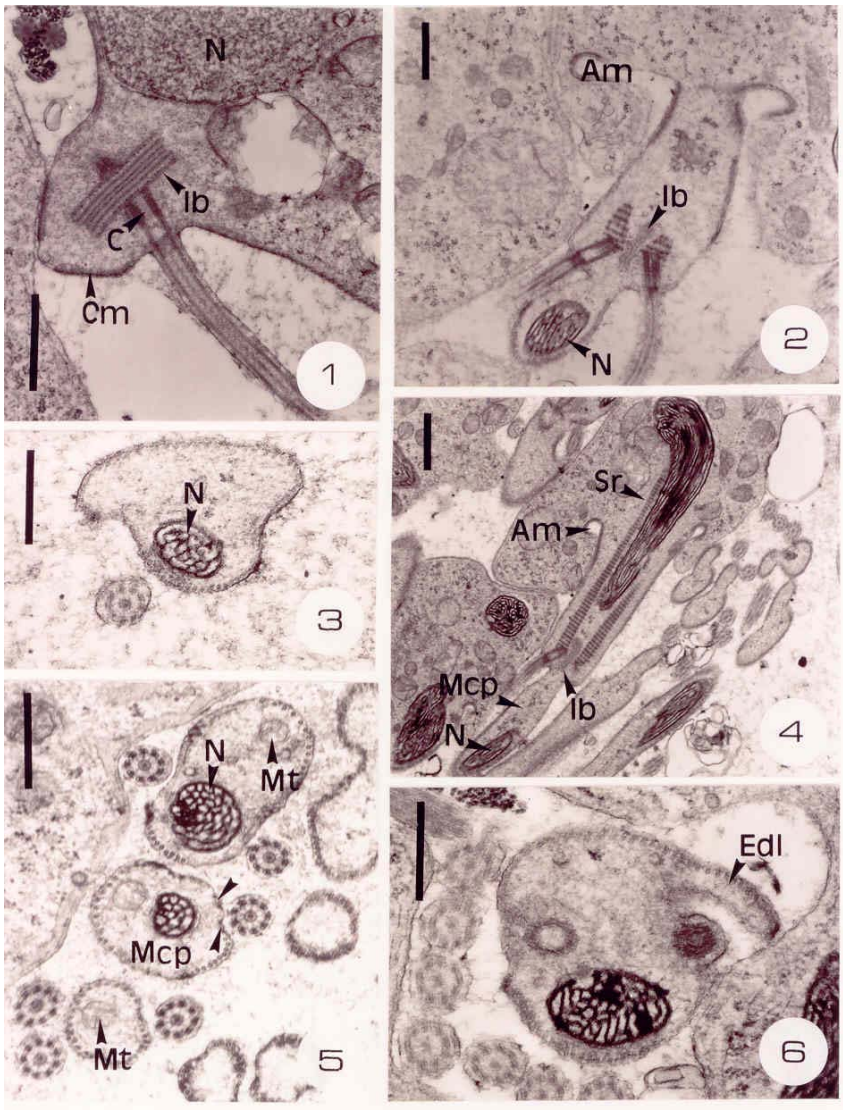
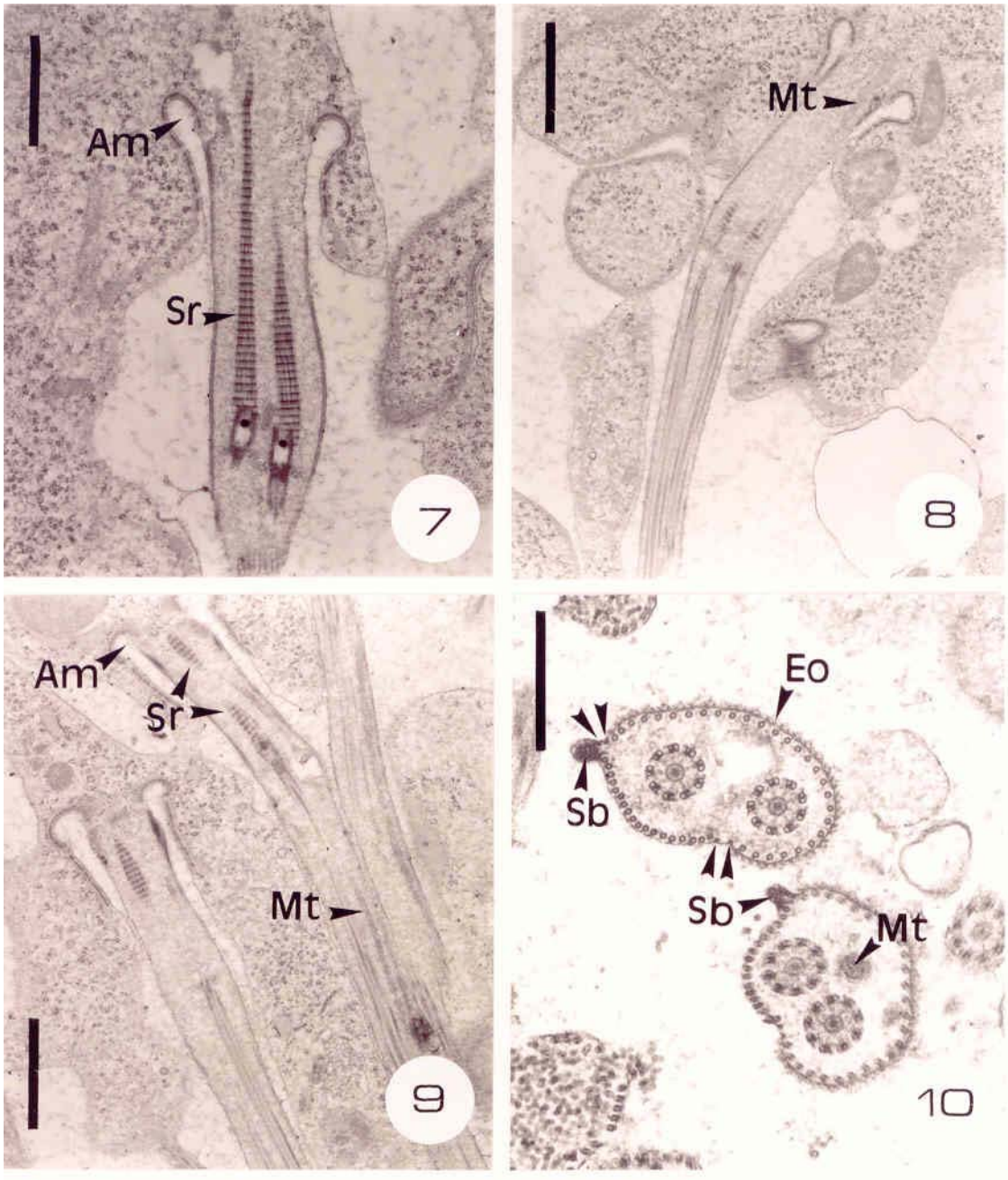
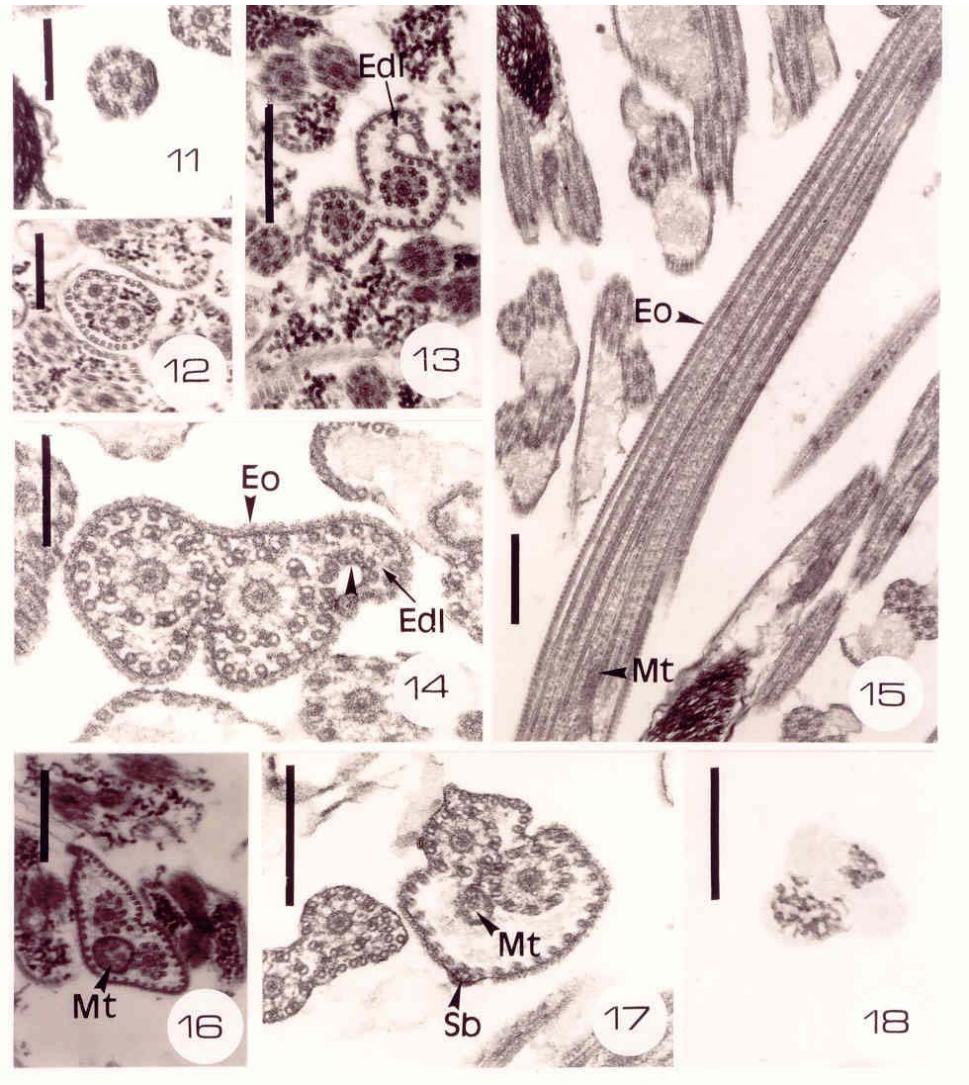


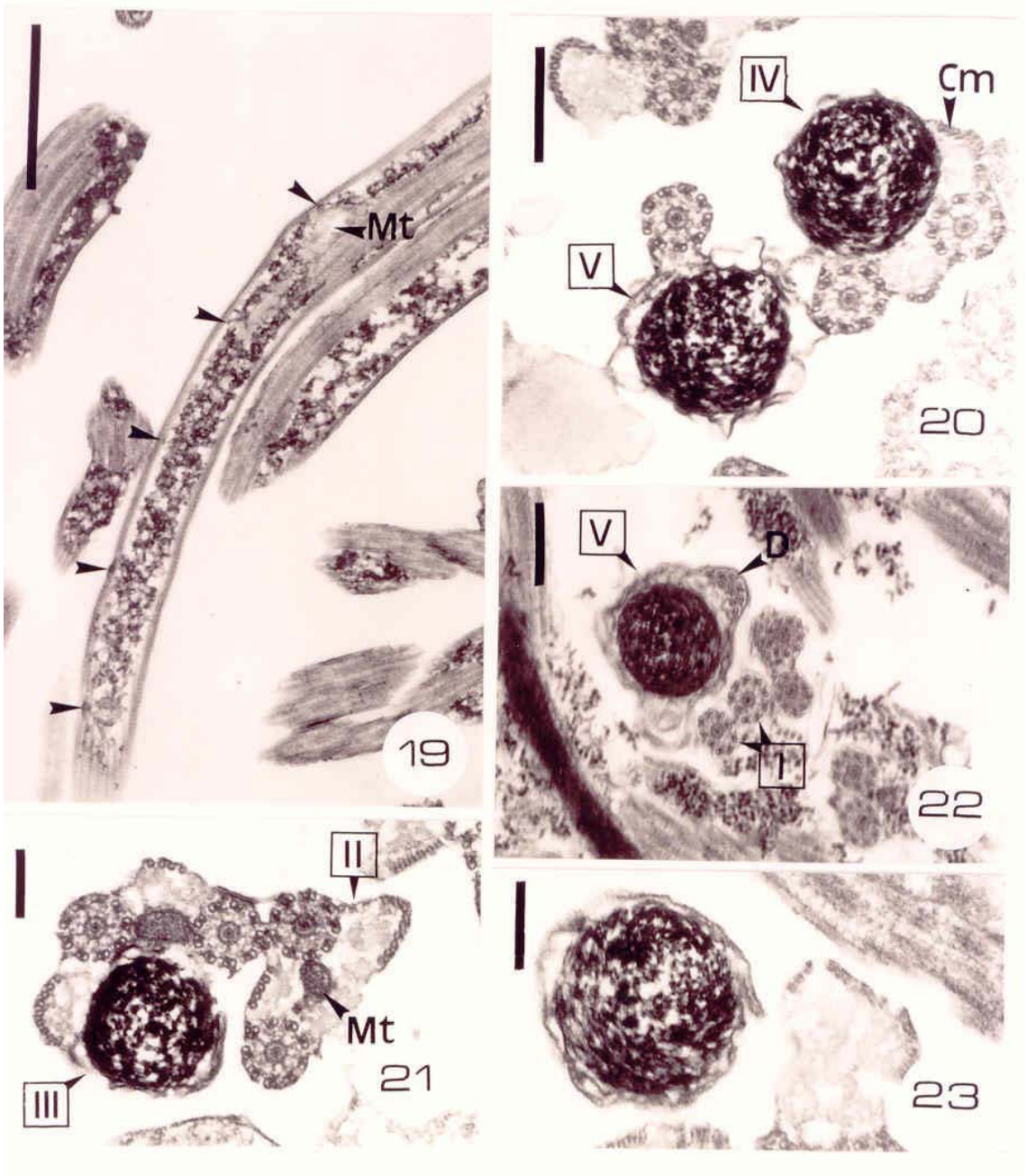

*FIGURES 1-6. Spermiogenesis in Fasciola gigantica. (1) Initial stage of spermiogenesis showing a differentiation zone with the formation of a free flagellum. C, centriole; Cm, cortical microtubules; Ib, intercentriolar body; N, nucleus. Scale bar = 1 μm. (2) Longitudinal section of a differentiation zone showing the asynchronic flagellar rotation and the migration of the nucleus toward the median cytoplasmic process before the fusion of the 3 processes. Am, arched membranes; F, flagellum; Ib, intercentriolar body; N, nucleus. Scale bar = 1 μm. (3) Longitudinal section of a differentiation zone. Note the migration of nucleus to the median cytoplasmic process and the long striated roots. Am, arched membranes; Ib, intercentriolar body; Mcp, median cytoplasmic process; N, nucleus; Sr, striated roots. Scale bar = 1 μm. (4) Cross-section of a spermatid showing the presence of the nucleus in the median cytoplasmic process before the proximodistal fusion of flagella. N, nucleus. Scale bar = 0.5 μm. (5) Several cross-sections showing the three processes (2 flagella and median cytoplasmic process) at different levels. Arrowheads indicate attachment zones. Mcp, median cytoplasmic process; Mt, mitochondrion; N, nucleus. Scale bar = 0.5 μm. (6) Cross-section of a spermatid showing the formation of the dorsolateral cytoplasmic expansion. Ax, axoneme; Edl, dorsolateral cytoplasmic expansion; N, nucleus. Scale bar = 0.4 μm.*




*FIGURES 7-10. Spermiogenesis in Fasciola gigantica. (7) Differentiation zone showing the long striated rootlets. Am, arched membranes; Sr, striated rootlets. Scale bar = 1 μm. (8) Final stage of spermiogenesis showing the migration of the mitochondrion after the proximodistal fusion of flagella. Ax, axoneme; Mt, mitochondrion. Scale bar = 2 μm. (9) Another image of the final stage of spermiogenesis showing the displacement of the striated roots toward the ring of arched membranes and the residual cytoplasm. Am, arched membranes; Ax, axoneme; Mt, mitochondrion; Sr, striated rootlets. Scale bar = 1.5 μm. (10) Two cross-sections of spermatids showing the spine-like bodies and the external ornamentation of the cell membrane. Arrowheads indicate attachment zones. Ax, axoneme; Eo, external ornamentation of the cell membrane; Mt, mitochondrion; Sb, spine-like bodies. Scale bar = 0.5 μm.*




*FIGURES 11-18. Mature spermatozoon of Fasciola gigantica. (11) Cross-section of the anterior extremity of sperm showing 1 axoneme surrounded by the plasma membrane. Note the lacking of cortical microtubules at this level. Scale bar = 0.2 µm. (12) Cross-section of region I showing 2 axonemes and numerous cortical microtubules. Ax, axoneme; Cm, cortical microtubules. Scale bar = 0.2 µm. (13) Cross-section of region I showing the dorsal displacement of the dorsolateral expansion of cytoplasm. Ax, axoneme; Edl, dorsolateral expansion of cytoplasm. Scale bar = 0.5 µm. (14) Detail of the initial portion of region I at the level of the. Arrowhead indicates the absence of external ornamentation of the cell membrane on the ventral face of the dorsolateral cytoplasmic expansion. Ax, axoneme; Edl, dorsolateral expansion of cytoplasm; Eo, external ornamentation of the cell membrane. Scale bar = 0.2 µm. (15) Longitudinal section of the spermatozoon in region II showing the mitochondrion. Eo, external ornamentation of the cell membrane; Mt, mitochondrion. Scale bar = 0.5 µm. (16) Cross-section of region II showing the disappearance of the dorsolateral cytoplasmic expansion after its dorsal displacement. Ax, axoneme; Mt, mitochondrion. Scale bar = 0.5 µm. (17) Cross-section of region II showing the spine-like body. Ax, axoneme; Mt, mitochondrion; Sb, spine-like body. Scale bar = 0.4 µm. (18) Cross-section of region II showing glycogen following application of the Thiéry technique. G, glycogen. Scale bar = 0.5 µm.*




*FIGURES 19-23. Mature spermatozoon of Fasciola gigantica. (19) Longitudinal section of region II showing the periodicity of the spine-like bodies (arrowheads). Mt, mitochondrion. Scale bar = 0.5 μm. (20) Cross-sections of regions II and III showing the presence of mitochondrion, and nucleus and mitochondrion, respectively. Ax, axoneme; Mt, mitochondrion; N, nucleus. Scale bar = 0.2 μm. (21) Cross-sections of regions IV and V showing the nucleus with 2 and 1 axoneme, respectively. Ax, axoneme; Cm, cortical microtubules; N, nucleus. Scale bar = 0.3 μm. (22) Cross-sections of regions I and V. Note the disorganization of the second axoneme. Ax, axoneme; D, doublets; N, nucleus. Scale bar = 0.4 μm. (23) Cross-section of terminal region of sperm showing a nucleus. N, nucleus. Scale bar = 0.2 μm.*

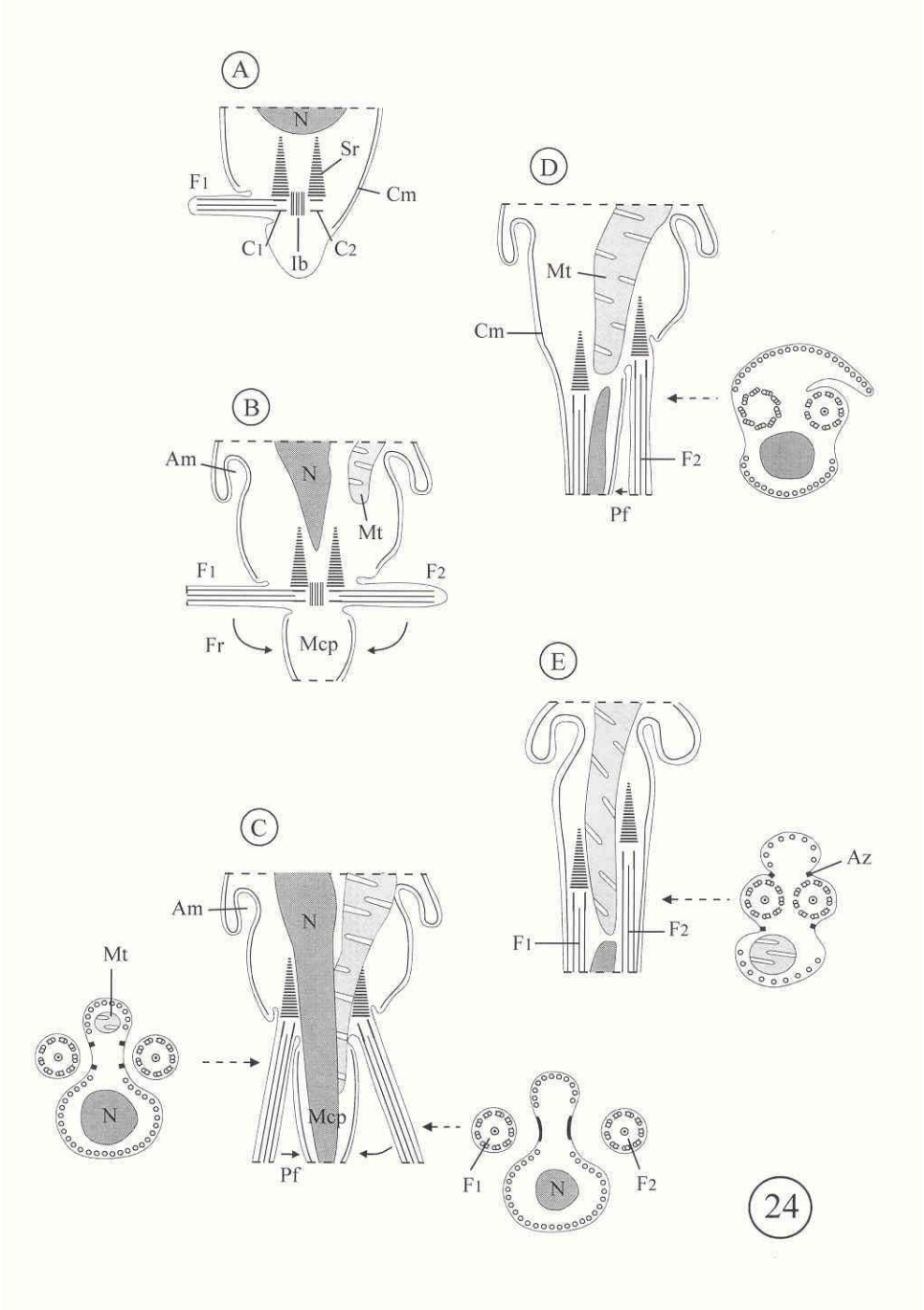



*FIGURE 24A-E. Diagram showing the main stages of spermiogenesis in Fasciola gigantica. Am, arched membranes; Az, attachment zones; C1, centriole 1; C2, centriole 2; Cm, cortical microtubules; F1, flagellum 1; F2, flagellum 2; Fr, flagellar rotation; Ib, intercentriolar body; Mcp, median cytoplasmic process; Mt, mitochondrion; N, nucleus; Pf, proximodistal fusion; Sr, striated rootlets.*

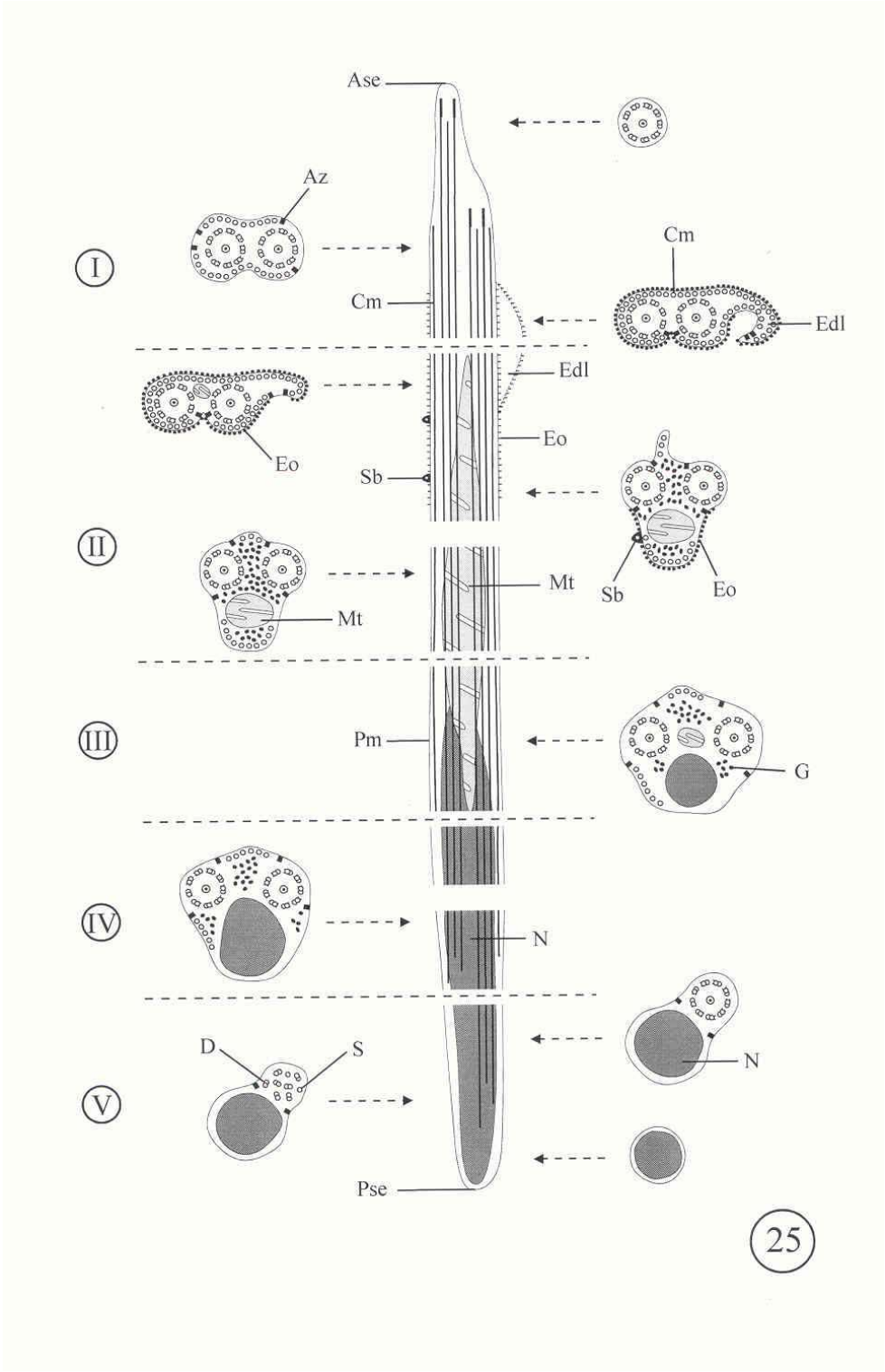



*FIGURE 25I-V. Diagram showing the ultrastructural organization of the mature spermatozoon of Fasciola gigantica. To make the diagram clearer the granules of glycogen are not show in the longitudinal section. Ase, anterior spermatozoon extremity; Az, attachment zones; Cm, cortical microtubules; D, doublets; Edl, dorsolateral expansion of cytoplasm; Eo, external ornamentation of the cell membrane; G, granules of glycogen; Mt, mitochondrion; N, nucleus; Pm, plasma membrane; Pse, posterior spermatozoon extremity; S, singlets; Sb, spine-like bodies.*

**Table I. Species of Digenea in which the ultrastructure of the spermatozoon has been studied.**

Edl, dorsolateral cytoplasmic expansion; Eo, external ornamentation of the cell membrane; Sb, spine-like body; +/- indicates the presence/absence of considered character; *species that requires a reinvestigation.

| Families and species of Digenea | Edl | Eo | Sb | References |
|---|---|---|---|---|
| **BRACHYLAIMIDAE** | | | | |
| *Brachylaimus aequans* | - | - | - | Zdarska et al. (1991) |
| *Scaphiostomum palearcticum* | - | + | - | Ndiaye et al. (2002) |
| **BUCEPHALIDAE** | | | | |
| *Bucephaloides gracilescens* | - | + | - | Erwin and Halton (1983) |
| *Pseudorhipidocotyle elpichthys* | - | + | - | Tang et al. (1998) |
| **CRYPTOGONIMIDAE** | | | | |
| *Neochasmus sp.* | - | + | - | Jamieson and Daddow (1982) |
| **DICROCOELIIDAE** | | | | |
| *Dicrocoelium dendriticum* | - | - | - | Morseth (1969), Cifrián et al. (1993) |
| | - | - | - | Tang (1996), Tang and Li (1996) |
| *Dicrocoelium chinensis* | - | - | - | Robinson and Halton (1982) |
| *Corrigia vitta* | | | | |
| **DIDYMOZOIDAE** | | | | |
| *Didymozoon* | - | - | - | Justine and Mattei (1983, 1984a) |
| *Gonapodasmius* | - | + | - | Justine and Mattei (1982b,1984b) |
| *Didymocystis wedli* | - | - | - | Pamplona-Basilio et al. (2001) |
| **DIPLOSTOMATIDAE** | | | | |
| *Pharyngostomoides procyonis* | - | - | - | Grant et al. (1976) |
| **ECHINOSTOMATIDAE** | | | | |
| *Echinostoma caproni* | + | + | - | Iomini and Justine (1997) |

| | | | | |
|---|---|---|---|---|
| **FASCIOLIDAE** | | | | |
| **Fasciola hepatica*** | - | - | - | **Stitt and Fairweather (1990)** |
| ***Fasciola gigantita*** | + | + | + | **Present paper** |
| **FELLODISTOMIDAE** | | | | |
| **Proctoeces maculatus** | - | + | - | **Justine (1995)** |
| **HAEMATOLOECHIDAE** | | | | |
| **Haematoloechus medioplexus** | - | + | - | **Justine and Mattei (1982a), Justine (1995)** |
| **HAPLOPORIDAE** | | | | |
| ***Saccocoelioides* godoyi** | - | - | - | **Baptista-Farias et al. (2001)** |
| **HETEROPHYIDAE** | | | | |
| ***Cryptocotyle lingua*** | - | - | - | **Rees (1979)** |
| **LECITHODENDRIIDAE** | | | | |
| **Postorchigenes gymnesicus** | - | + | - | **Gracenea et al. (1997)** |
| ***Ganeo tigrinum*** | - | - | - | **Sharma and Rai (1995)** |
| **MESOCOELIDAE** | | | | |
| **Mesocoelium monas** | - | - | - | **Iomini et al. (1997)** |
| **MICROPHALLIDAE** | | | | |
| **Maritrema linguilla** | - | - | - | **Hendow and James (1988)** |
| **NOTOCOTYLIDAE** | | | | |
| **Notocotylus neyrai** | - | + | + | **Ndiaye et al. (in press)** |
| **OPECOELIDAE** | | | | |
| **Opecoeloides furcatus** | - | + | + | **Miquel et al. (2000)** |
| **OPISTHORCHIIDAE** | | | | |
| **Aphalloides coelomicola** | - | + | - | **Justine (1995)** |
| **PARAGONIMIDAE** | | | | |
| **Paragonimus miyazakii** | - | - | - | **Sato et *al.* (1967)** |
| **Paragonimus pulmonalis** | - | - | - | **Fujino and Ishii (1982)** |
| | - | - | - | **Fujino *et al.* (1977), Orido (1988)** |
| ***Paragonimus westermani*** | - | + | - | **Hirai and Tada (1991)** |
| ***Paragonimus ohirai*** | | | | |

| | | | | |
|---|---|---|---|---|
| **PARAMPHISTOMIDAE** | | | | |
| **Ceylonocotyle scoliocoelium** | **-** | **-** | **-** | **Li and Wang (1997)** |
| **SCHISTOSOMATIDAE** | | | | |
| ***Schistosoma curassoni*** | **-** | **-** | **-** | **Justine et al. (1993)** |
| ***Schistosoma rodhaini*** | **-** | **-** | **-** | **Justine et al. (1993)** |
| ***Schistosoma intercalatum*** | **-** | **-** | **-** | **Justine et al. (1993)** |
| ***Schistosoma bovis*** | **-** | **-** | **-** | **Justine et al. (1993)** |
| **Schistosoma mansoni** | **-** | **-** | **-** | **Kitajima et al. (1976), Justine and Mattei (1981), Justine et al.** |
| ***Schistosoma margrebowiei*** | **-** | **-** | **-** | **(1993)** |
| ***Schistosoma mattheei*** | **-** | **-** | **-** | **Justine and Mattei (1981), Justine et al. (1993)** |
| **Schistosoma japonicum** | **-** | **-** | **-** | **Swiderski and Tsinonis (1986)** |
| | | | | **Justine and Mattei (1981), Yang et al. (1998)** |