

## *Fasciola hepatica*: surfaces involved in movement of miracidia and cercariae

C.E. Bennett\*

School of Biological Sciences, Southampton University, Southampton,  
SO16 7PX, UK

### Abstract

Rapid freezing and substitution with fixative prior to scanning electron microscopy was used to demonstrate the pattern of beat and recovery of the cilia of free swimming miracidia of *Fasciola hepatica*. There were stages of dexioplectic metachronal co-ordination and the power stroke was approximately 15° anticlockwise from the anterior–posterior axis. Around the circumference of the body of the miracidia there were approximately 12 metachronal waves of power and recovery. Free-swimming cercariae were recorded by time-lapse photography and, after conventional fixation, by scanning electron microscopy. Cercarial tail-beats were to the posterior of the body in the lateral plane at a rate of 8 Hz. The tail has paired lateral ridges positioned to act as leading edges. There is an array of 32 sensory papillae on the mid-ventral surface of the tail. The tegument of the most distal part of the tail is described: it is free of sensory endings and the surface shows a spiral pattern.

### Introduction

*Fasciola hepatica* is a useful model for the understanding of digenean parasites (Bennett, 2001) and both *F. hepatica* and *F. gigantica* are infections of humans and animals (sheep and cattle) (Boray, 1985). Neuro-cytochemical studies of *Fasciola* have provided evidence of invertebrate-specific neurotransmitters (Brownlee *et al.*, 1994; Brownlee & Fairweather, 1996) and as a result there is interest in the normal mechanisms of movement of these parasites. In all stages of the life cycle, e.g. in the newly excysted juvenile (Bennett & Threadgold, 1973) there is a basket of circular and longitudinal muscle which acts on the enclosed mesenchyme and organs that provide a hydrostatic skeleton.

Various aspects of the structure and movement of the free-living larval stages of *F. hepatica* have been described previously. Miracidia are propelled by cilia and swim in a spiralling movement in the direction of the apical papilla (Wilson, 1969; Wilson & Denison, 1970). The cilia of *F. hepatica* miracidia are found on 21 epidermal cells arranged in rows: 6 cells are in the most anterior row, with 6, 3, 4 and 2 respectively in subsequent rows (Mattes, 1949). The configuration of rootlets of the cilia

(Wilson, 1969; Southgate, 1970) is comparable with that of other Platyhelminthes (Dorey, 1965) with main rootlets directed at right angles to the insertion of each cilium. Rootlets are arranged in a symmetrical circumferential pattern with spacing of approximately 2 µm (Wilson, 1969). In turbellarians, cilia beat synchronously in the same plane, with conspicuous metachronal wavefronts parallel to the meridians (Dorey, 1965). Knight-Jones (1954) described the beating of ciliated epithelia of Turbellaria such as *Dugesia tigrina* and *Crenobia alpina* as in antiplectic metachronism.

This study aims to demonstrate and interpret the beating of both the cilia of the miracidium and the tail of the cercaria of *F. hepatica*. The cercarial body and tail of *F. hepatica* are covered with a tegument, which is also found on other life-cycle stages of *F. hepatica* (Threadgold, 1963; Bennett, 1975a,b). While the surface morphology of the gymnocephalus cercaria of *F. hepatica* has been described from scanning electron microscopy (Køie *et al.*, 1977) there have been no detailed descriptions of its movement. Those cercariae whose movement has been described include *Parorchis acanthus* (Rees, 1932), *Himasthala secunda* and *Cryptocotyle lingua*, (Chapman & Wilson, 1973; Chapman, 1974), and *Transversotrema* (Bundy, 1981). There are lateral fins on the tails of some cercariae, e.g. *Clonorchis sinensis* (Fujino *et al.*, 1979).

The tail musculature of *H. secunda* and *C. lingua* has

\*Fax: +44 (0)2380 594319  
E-mail: ceb@soton.ac.uk

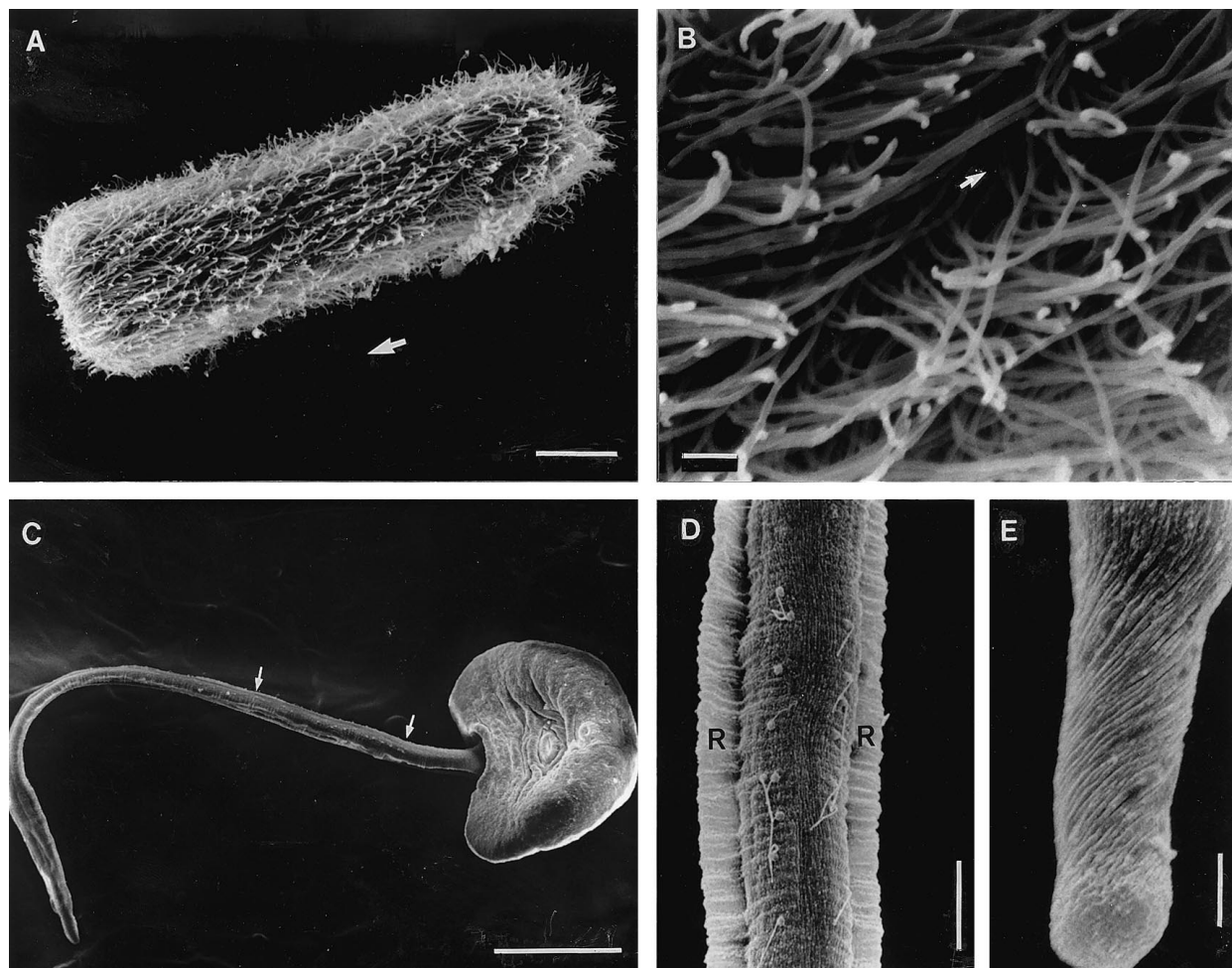


Fig. 1. Scanning electron micrographs of *Fasciola hepatica* miracidia (A and B) and cercariae (C, D and E). A. Miracidium fixed while swimming in direction of the arrow. B. High power region (from A) with cilia fixed in phases of dextroplectic metachronism. The direction of the power stroke of the metachronal wave is arrowed. C. Cercaria: the tail has paired lateral ridges and a region between the arrows with many sensory endings. D. Cercarial tail: (similar to region between arrows in C) with lateral ridges (R) and 8  $\mu$ m cilia on the ventral surface. E. The terminal portion of the cercarial tail with a spiral surface pattern to the tegument.

been examined by transmission electron microscopy (TEM) and is arranged in blocks, two being dorso-lateral and two ventro-lateral (Chapman, 1973). A similar arrangement of muscle blocks is to be found in the furcocercaria of *Schistosoma mansoni* (Nuttman, 1974). The rate of tail beating of cercariae of *H. secunda*, *P. acanthus* and *S. mansoni* has been estimated (Bundy, 1981) as 10.6 Hz, 2.9 Hz and 22.1 Hz respectively. Serotonin (5-HT) is an excitatory transmitter in the body wall musculature of *F. hepatica* (Tembe *et al.*, 1993). In the tail of the cercaria of *Sanguinicola inermis* (McMichael-Phillips *et al.*, 1996) cell bodies associated with the ventral and dorsal longitudinal nerve cords demonstrated either FMRFamide and S1 or 5-HT-like immunoreactivity. In the cercarial tail of *S. inermis* only 5-HT extended into the posterior tail furcae and there is other evidence that the serotonergic and peptidergic components of the nervous system are distinct. The unusual pattern of banding of the musculature, peculiar to the Platyhelminthes, is

thought to be a specific adaptation to rapid tail movement (Reger, 1976).

Rapidly moving cilia of protozoa have been best preserved for scanning electron microscopy (SEM) by a process of rapid freezing followed by fixation as thawing proceeds (substitution) (Barlow & Sleight, 1978). It was proposed to use this method to determine the angle and type of metachronal beating of the cilia of *F. hepatica* miracidia.

Short sensory papillae (1–3  $\mu$ m) are found on the body of the cercaria of *F. hepatica* (Køie *et al.*, 1977) as well as on newly-excysted juveniles around the oral sucker, and scattered over the body surface between the spines (Bennett, 1975a). Relatively long (8  $\mu$ m) ciliary sensory endings are commonly present on the cercarial body and tails of many species of digeneans, e.g. *Metagonimus* (Fujino & Ishii, 1976). Sensory endings of this size have been described previously on the tail of *F. hepatica* cercariae (Køie *et al.*, 1977) along with other prominent

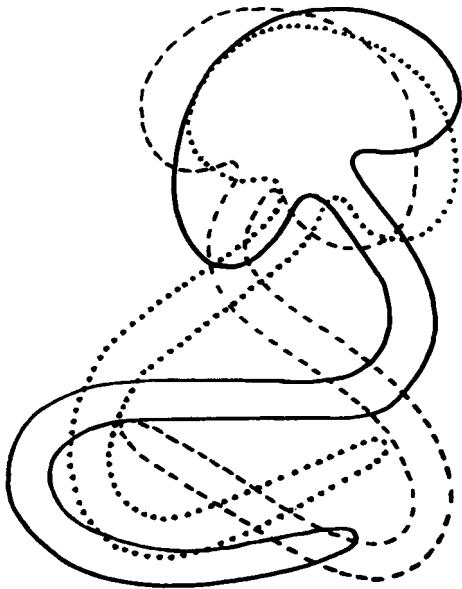


Fig. 2. Phases of movement of *Fasciola hepatica* cercariae. Alternate time-lapse video-fields (intervals of 0.04 sec). One complete cycle = 6 video-fields = 0.12 sec. Scale bar = 50  $\mu\text{m}$ . —, First video-field; - - -, third video-field; ..., fifth video-field.

features: the dorso-lateral folds on either side of the tail. Additional aims of this study, therefore, were to relate the tail structure and position of sensory endings to the behaviour of the cercaria and movement of the tail as viewed by time lapse video-photography.

## Materials and methods

### Miracidia

#### Production

Eggs of *F. hepatica* from experimentally infected animals were supplied by the Veterinary Laboratories Agency (VLA), Weybridge, UK. They were embryonated at 22°C for 14 days (Al-Habbib, 1974) and miracidia were hatched from the eggs after exposure to light in filtered, oxygenated aquarium water.

#### Preparation for SEM

Several hundred, freshly hatched and free-swimming miracidia were frozen by plunging groups of specimens in 3  $\mu\text{l}$  drops of water into liquid isopentane. Substitution was carried out over 30–40 h with a solution comprising 40% ethylene glycol and 50% methanol saturated with  $\text{CaCl}_2$  and  $\text{HgCl}_2$ , and 10% acrolein (Barlow & Sleight, 1978). Critical point drying was carried out (Bartlett & Burstyn, 1975) in a specially constructed basket (Newell & Wrath, 1975). Specimens were sputter-coated with gold (Bennett, 1975a,b).

#### Scanning electron micrograph analysis

The number of metachronal waves per miracidium was estimated from photomicrographs of 50 freeze-substituted miracidia that had been fixed in the typical

cylindrical 'free-swimming' miracidial body shape (Erasmus, 1972). The angle of beating of the cilia was measured with respect to the anterior–posterior axis.

### Cercariae

#### Production

Miracidia of *F. hepatica* were used to infect *Lymnaea truncatula* at a rate of 5 per snail and after 40 days cercariae were stimulated to emerge by 'cold shock', by reducing the temperature of infected snails to 12°C for 30–45 min (Kendall & McCullough, 1951).

#### Preparation for SEM

Free-swimming cercariae were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer and, after dehydration and critical point drying, sputter-coated as above. Scanning electron microscopy was used to interpret the surface features and sensory structures of the tail.

#### Preparation for time-lapse photography

Cercariae were placed in a 4 mm horizontal cuvette under a phase contrast microscope and viewed at  $\times 400$ . Movement of cercariae was recorded by time lapse on VHS equipment, which allowed viewing of video-fields at intervals of 0.02 sec.

## Results

Rapidly fixed *F. hepatica* miracidia had a body shape which was consistent with rapid swimming, i.e. there was a body extension of approximately 110  $\mu\text{m} \times 35 \mu\text{m}$ , (Erasmus, 1972). Cilia were arrested in typical positions of a dexioplectic metachronal beat with the orientation of the ciliary power stroke at 15° ( $\pm$ S.E. 5°) from the longitudinal body axis (fig. 1A). There were approximately 12 metachronal waves around the circumference of each miracidium, (six visible from each lateral view of a single miracidium). Some cilia were slightly damaged at their tips in the freeze-substitution processing (fig. 1B).

Time-lapse images of *F. hepatica* cercarial swimming reveal that the cercarial tail beats broadly in the lateral plane with one cycle of beating per 0.12 sec (8 Hz) (fig. 2). When viewed from the dorsal or ventral aspect in real time the most posterior two-thirds of the tail provide an illusion of a figure of eight centred on an axis horizontally below the anterior–posterior line of the body and tail. As a result there is a side-to-side oscillation of the body of the cercaria centred on the ventral sucker in the middle of the body.

Standard fixation for SEM resulted in tails fixed in a range of configurations similar to those exhibited by time-lapse photography described above (fig. 2). The tail is approximately 500  $\mu\text{m}$  in length and is covered with an invaginated tegument and both anterior–posterior and transverse corrugations (fig. 1E). A pair of prominent lateral ridges or fins extended along either side of the tail (fig. 1C and E). A consistent feature of all cercarial tails was the spiral pattern of the tegument on the most distal portion (35–40  $\mu\text{m}$ ) (fig. 1D). Ciliated sensory endings were arrayed only on the mid-ventral surface of the tail in 16 pairs, 13 pairs with long (approximately 8  $\mu\text{m}$ ) cilia, the most distal three pairs having shorter (1–2  $\mu\text{m}$ ) cilia.

## Discussion

The spiral swimming pattern of the miracidia of *F. hepatica* (Wilson & Denison, 1970) is explained by the images of angled beating of the cilia recorded here. Miracidia fixed by rapid freeze-substitution successfully preserved cilia in positions of power stroke and sideways recovery typical of dextroplectic co-ordination (Knight-Jones, 1954). This pattern of beating has not previously been described in the Platyhelminthes where antiplectic beating was ascribed to the Turbellaria (Knight-Jones, 1954). The ciliary power stroke is at approximately 15° to the main axis of the body (fig. 1A). *Fasciola hepatica*, therefore, swims by power strokes of its cilia directed approximately along the sinistral ciliary rows. The sinistral line of cilia of a platyhelminth (Dorey, 1965) is at approximately 30° to the anterior–posterior line. A fully extended miracidium of *F. hepatica*, in swimming posture, is approximately 170 µm × 20 µm (Erasmus, 1972). This is 1.5–2.0× its relaxed length. Extension and reduction of body length by adjustments of the hydrostatic skeleton may effect both a change of angle of the sinistral line of ciliary rootlets and the direction of the power stroke as indicated by local body shape. The more pronounced spiralling seen in ageing miracidia which are shorter and wider is consistent with this idea. Corkscrewing, as imparted by the rifling of gun-barrels, is associated with stability in flight and the 15° angled beat of the cilia demonstrated here may have been selected for to this end.

In many other species the beating of the cercarial tail is across or over the body. In the case of *F. hepatica*, however, the beating is at a distance from the body with the effective strokes being in the lateral plane. Dorsolateral folds or ridges are present on each side of the tail of *F. hepatica* cercariae (Køie *et al.*, 1977) and are in a similar position to the more prominent fins seen on the tail of *C. sinensis* (Fujino *et al.*, 1979). These structures were consistently found in this study but were not sufficiently large to be described as fins and are therefore referred to here as lateral ridges. Since the beating of the tail is in the direction of these ridges, they may provide an effective leading edge. No sensory papillae are present in the most posterior region of the tail where the tegument is spirally twisted. There is no explanation for this spiralled tail-end in the literature, leaving one to speculate for example that it may play a part in emergence of the cercaria from the redia and snail host.

The beating of the tail of *F. hepatica* cercariae differs from that of other cercariae recorded to date. In *P. acanthus*, for example, the tail and body counter-balance each other while beating as a single unit in one plane (Rees, 1932). It also differs somewhat from the tail-beating of *H. secunda* and *C. lingua*, which both coil the tail behind the body in the cycle of beating. There is, however, some similarity to *C. lingua*, which with its longer tail tends towards a figure of eight pattern of beating (Chapman & Wilson, 1973). There are other similarities with the beating of the furcate cercarial tail of *T. patialense* with respect to the beat and recovery strokes and approximate orientation to the body (Bundy, 1981). In *F. hepatica* the beating of the tail is lateral, being in the same plane as the dorso-ventrally flattened body.

Additionally, the oscillation of the body is such that there is minimal movement of the ventral sucker. Both of these features may be useful adaptations to attachment to a flat substrate to encyst. Bulbous sensory endings with enclosed cilia located around the ventral sucker in the cercaria/infective stage (Bennett, 1975a) are well placed to detect this contact.

The large 8 µm unciliated papillae of *F. hepatica* are found in similar positions to those on the tail of *S. mansoni* cercariae (Gordon *et al.*, 1934; Sakamoto & Ishii, 1978). Ventral papillae on the tail may provide feedback about the movement of the cercarial tail in the lateral plane or, on contact, inhibit tail beating on location of a substrate for encystment.

## Acknowledgements

Dr David Barlow, of Southampton University, for advice on the freeze substitution method, and Mr Gordon Graham, of the VLA, for supply of eggs and metacercarial cysts of *F. hepatica*.

## References

- Al-Habbib, W.M.S. (1974) The effect of constant and changing temperatures on the development of the larval stages of *Fasciola hepatica* (L.). PhD thesis, University of Dublin.
- Barlow, D.I. & Sleight, M.A. (1978) Freeze substitution for preservation of ciliated surfaces for scanning electron microscopy. *Journal of Microscopy* **115**, 81–95.
- Bartlett, A. & Burstyn, N.P. (1975) A review of the physics of critical point drying. *Proceedings of the 8th SEM Symposium*. IIT Research Institute of Chicago. p. 305.
- Bennett, C.E. (1975a) Surface features, sensory structures and movement of the newly excysted juvenile of *Fasciola hepatica* L. *Journal of Parasitology* **61**, 886–891.
- Bennett, C.E. (1975b) Scanning electron microscopy of *Fasciola hepatica* L. during growth and maturation in the mouse. *Journal of Parasitology* **61**, 892–898.
- Bennett, C.E. (2001) Parasites of domestic livestock: sheep and *Fasciola hepatica*. pp. 53–62 in Halton, D.W., Behnke, J.M. & Marshall, I. (Eds) *Practical exercises in parasitology*. Cambridge University Press.
- Bennett, C.E. & Threadgold, L.T. (1973) Electron microscope studies of *Fasciola hepatica* XIII. Fine structure of the newly excysted juvenile. *Experimental Parasitology* **34**, 85–99.
- Boray, J.C. (1985) Flukes of domestic animals. pp. 179–218 in Gaafar, S.M., Howard, W.E. & Marsh, E. (Eds) *Parasites, pests and predators*. Elsevier.
- Brownlee, D.J.A. & Fairweather, I. (1996) Immunocytochemical localization of glutamate-like immunoreactivity within the nervous system of the cestode *Mesocostoides corti* and the trematode *Fasciola hepatica*. *Parasitology Research* **82**, 423–427.
- Brownlee, D.J.A., Brennan, G.P., Halton, D.W., Fairweather, I. & Shaw, C. (1994) Ultrastructural localization of FMRFamide-immunoreactivity and pancreatic polypeptide-immunoreactivity within the central nervous system of the liver fluke, *Fasciola hepatica* (Trematoda, Digenea). *Parasitology Research* **80**, 117–124.

- Bundy, D.A.P.** (1981) Swimming behaviour of the cercaria of *Transversotrema patialense*. *Parasitology* **82**, 319–334.
- Chapman, H.D.** (1973) The functional organisation and fine structure of the tail of the cercaria of *Cryptocotyle lingua* and *Himasthla secunda*. *Parasitology* **66**, 487–497.
- Chapman, H.D.** (1974) The behaviour of the cercaria of *Cryptocotyle lingua*. *Zeitschrift für Parasitenkunde* **44**, 211–226.
- Chapman, H.D. & Wilson, R.A.** (1973) The propulsion of the cercaria of *Himasthla secunda* (Nicoll) and *Cryptocotyle lingua*. *Parasitology* **67**, 1–15.
- Dorey, A.E.** (1965) The organisation and replacement of the epidermis in acelous turbellarians. *Quarterly Journal of Microscopical Science* **106**, 147–172.
- Erasmus, D.A.** (1972) *The biology of trematodes*. London, Edward Arnold Ltd.
- Fujino, T. & Ishii, Y.** (1976) Studies on the cercariae of the genus *Metagonimus* with the scanning electron microscope (Trematoda: Heterophyidae). *Japanese Journal of Parasitology* **25**, 175–185.
- Fujino, T., Ishii, Y. & Choi, D.W.** (1979) The ultrastructural characterisation of the tegument of *Clonorchis sinensis* (Cobbold, 1875) cercaria. *Zeitschrift für Parasitenkunde* **60**, 65–76.
- Gordon, R.M., Davey, T.H. & Peaston, H.** (1934) The transmission of human bilharziasis in Sierra Leone with an account of the life cycle of the schistosomes concerned (*S. mansoni* and *S. haematobium*). *Annals of Tropical Medicine and Parasitology* **28**, 323–418.
- Kendall, S.B. & McCullough, F.S.** (1951) The emergence of the cercariae of *Fasciola hepatica* in *Lymnaea truncatula*. *Journal of Helminthology* **25**, 77–92.
- Knight-Jones, E.W.** (1954) Relations between metachronism and direction of ciliary beat in Metazoa. *Quarterly Journal of Microscopical Science* **95**, 503–521.
- Koie, M., Nansen, P. & Christensen, N.Ø.** (1977) Stereoscan studies of rediae, cercariae, cysts, excysted metacercariae and migratory stages of *Fasciola hepatica*. *Zeitschrift für Parasitenkunde* **54**, 289–297.
- Mattes, O.** (1949) Wirtsfindung, invasionsvorgang und wirtsspezifität beim *Fasciola* miracidium. *Zeitschrift für Parasitenkunde* **14**, 320–363.
- McMichael-Phillips, D.F., Lewis, J.W. & Thorndyke, M.C.** (1996) The distribution of neuroactive substances within the cercaria of *Sanguinicola inermis*. *Journal of Helminthology* **70**, 309–317.
- Newell, D. & Wrath, S.** (1975) A container for processing small volumes of cell suspensions for critical point drying. *Journal of Microscopy* **104**, 321.
- Nuttman, C.J.** (1974) The fine structure and organisation of the tail musculature of the cercaria of *Schistosoma mansoni*. *Parasitology* **68**, 147–154.
- Rees, F.G.** (1932) An investigation into the occurrence, structure and life histories of the trematode parasites of *Lymnaea (truncatula, pereger, palustris, stagnalis)*. *Proceedings of the Zoological Society of London* **1**, 1–32.
- Rees, G.** (1971) Locomotion of the cercaria of *Parorchis acanthus*, (Nicoll) and the ultrastructure of the tail. *Parasitology* **62**, 489–503.
- Reger, J.F.** (1976) Studies on the fine structure of cercarial tail muscle of *Schistosoma* sp. (Trematoda). *Journal of Ultrastructure Research* **57**, 77–86.
- Sakamoto, K. & Ishii, Y.** (1978) Scanning electron microscope observations on miracidium, cercaria and cercarial papillar patterns of *Schistosoma japonicum*. *Journal of Parasitology* **64**, 59–68.
- Southgate, V.R.** (1970) Observations on the epidermis of the miracidium and on the formation of the tegument of the sporocyst of *Fasciola hepatica*. *Parasitology* **61**, 177–190.
- Tembe, E.A., Holden-Dye, L., Smith, S.W.G., Jacques, P.A.M. & Walker, R.J.** (1993) Pharmacological profile of the 5-hydroxytryptamine receptor of *Fasciola hepatica* body wall muscle. *Parasitology* **106**, 67–73.
- Threadgold, L.T.** (1963) The tegument and associated structure of *Fasciola hepatica*. *Quarterly Journal of Microscopical Science* **104**, 505–512.
- Wilson, R.A.** (1969) Fine structure of the tegument of the miracidium of *Fasciola hepatica*. *Parasitology* **59**, 461–467.
- Wilson, R.A. & Denison, J.** (1970) Studies on the activity of the miracidium of the common liver fluke *Fasciola hepatica*. *Comparative Biochemistry* **32**, 301–313.

(Accepted 15 May 2000)

© CAB International, 2001