Ultrastructure of the tegument of the metacercaria of *Timoniella imbutiforme*

H.E.M. El-Darsh and P.J. Whitfield*

Infection and Immunity Research Group, Division of Life Sciences, King's College London, Campden Hill Road, London, W8 7AH

Abstract

The spinous body tegument of the metacercaria of *Timoniella imbutiforme* (Molin, 1859) is described in detail and found to comprise an outer tegumental syncytium connected to subjacently situated subtegumentary 'cells'. There are four types of secretory bodies in the outer syncytial layer as well as serrated overlapping spines and mitochondria. The subtegumentary 'cells' are characterized by the presence of four secretory body types as well as giant bodies which may be involved in the elaboration of the secretory bodies or spine material. The normal configuration of the somatic muscles of *T. imbutiforme* show that the muscular machinery necessary for activity once the larval stage becomes excysted is already in place. The sensory endings are found to be of the uniciliate type occurring in groups of up to eight in raised domes distributed over the body. Secretory gland cells are numerous and possess long ducts connected to the basal lamina of the outer syncytial layer via septate desmosomes.

Introduction

Since the early description of the tegument of the adult digenean *Fasciola hepatica* by Threadgold (1963a,b), the ultrastructure of the tegument of numerous other adult digeneans has been examined (Burton, 1966; Erasmus, 1967; Threadgold, 1968; Hockley, 1973; Robinson & Halton, 1983). Essentially, the digenean tegument has been found to comprise an anucleate apical cytoplasmic syncytium covering the external body of the fluke, and connected by cytoplasmic connectives to subjacently situated nucleated subtegumentary 'cells' which are in fact nucleated regions of a continuous syncytium (see cited authors above). The apical cytoplasm possesses secretory bodies, mitochondria, vacuoles, and frequently spines. The subtegumentary cells are similar to other synthetic cells, in that they possess Golgi complexes, free ribosomes, rough and smooth endoplasmic reticulum, in addition to the nucleus and mitochondria. At relevant stages during a synthetic cycle they also possess abundant secretory bodies. Closely associated with the tegument are several other elements which contribute towards the functional roles of the tegument, but might otherwise be considered as separate systems, or components of other systems (Hockley, 1973; Køie, 1973; Dunn *et al.*, 1992; Mattison *et al.*, 1994) including the muscular system, the sensory endings of the peripheral nervous system and secretory gland cells.

The tegument of juvenile stages (the cercaria and metacercaria), representing several of the main digenean families, has also received some attention (Køie, 1971; Hockley, 1973; Bennett & Threadgold, 1973; Rees, 1974; LeFlore & Bass, 1983; Apinhasmit *et al.*, 1994; Mattison *et al.*, 1994). To date, however, no reports on metacercarial tegumental ultrastructure for the family *Acanthostomatidae* have been produced, although the functional morphology of the circumoral spines of *Timoniella imbutiforme* has been described by McDowall & James (1988).

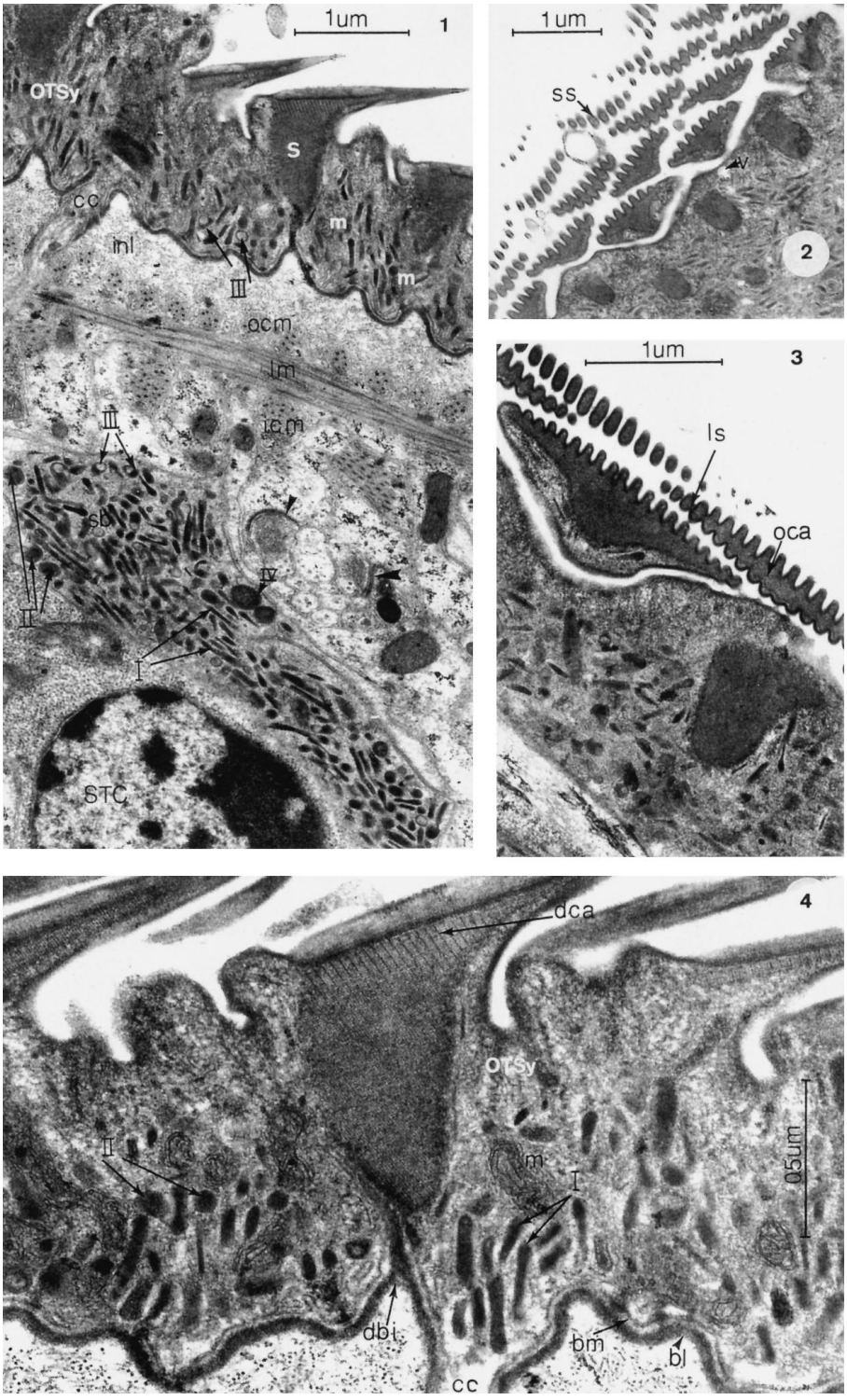
As an acanthostomatid and a parasite recently reported for the first time in British flounders (see El-Darsh & Whitfield, 1999), *T. imbutiforme* was ultrastructurally examined in the present study. The aim was to provide a comprehensive ultrastructural account of the spinous body tegument and closely associated systems.

Materials and methods

Infected flounder, *Platichthys flesus* L. were collected from the filter screens of Lots Road Power Station on the River Thames, at Chelsea, Central London (NER PQ 246

* Author for correspondence:
Fax : 0171 333 4500
E-mail: phil.whitfield@kcl.ac.uk

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press



Figs 1–4. Transmission electron micrographs of the metacercaria of *Timoniella imbutiforme*. 1. Outer tegumental syncytium and the subtegumentary cells. Arrow heads indicate neuromuscular junctions. 2. Small body spines. Note the number of serrations and layers of these spines compared to the larger spines. 3. Large body spines. 4. Internal crystalline organization of body spine. Basal lamina (bl), basal membrane (bm), cytoplasmic connectives (cc), dense basal invaginations (dbi), dense crystalline arrays (dca), inner circular muscle layer (icm), interstitial layer (inl), longitudinal muscle layer (lm), large spine (ls), mitochondria (m), outer circular muscle layer (ocm), oblique crystalline arrays (oca), outer tegumental syncytium (OTSy), subtegumentary cell (STC), spine (s), small spine (ss), Type I secretory body (I), Type II secretory body (II), Type III secretory body (III), Type IV secretory body (IV), vacuoles (v).

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press

770). Fish collected in this way provided metacercarial cysts of *Timoniella imbutiforme* (Molin, 1859) for electron microscopical examinations.

Fish were killed by a blow to the head and metacercarial cysts were removed from the fins and body musculature and placed in 7‰ saline in which the metacercariae were removed from their cysts using fine watchmaker's forceps and a micro-dissecting needle.

Metacercariae were transferred to fresh 7‰ saline, and rinsed several times before fixation in 2.5% gluteraldehyde buffered to pH 7.2 with 0.1 M phosphate buffer. Specimens were fixed for 1 week at 0°C, before they were washed several times in the same phosphate buffer and then left to stand in the same buffer for 12 h. Specimens were then post-fixed in 1% osmium tetroxide (in 0.1 M phosphate buffer at pH 7.2) for 1 h, until they turned black. The specimens were washed in the same buffer, before initiating dehydration in 30% alcohol. Subsequent dehydration steps through 70%, 90% ×2, 100% ×3, were carried out for 10 min in each step, before transferring to two changes of propylene oxide each of 30 min. The specimens were then infiltrated in diluted resin and propylene oxide in an initial ratio of 1 : 1 for 1 h, and a second dilution of 1 part propylene oxide to 3 parts Spurr resin overnight, with continuous rotation. They were eventually embedded in 100% Spurr resin and left to harden at 60°C for two days. Material was sectioned on an LKB ultratome III using glass knives. Sections were cut at 90 nm thickness, mounted on grids, and stained with uranyl acetate and lead citrate. Sections were examined using a TEOL TEM 100 CX II electron microscope.

For SEM examination, specimens were fixed using the same method as for TEM, and subsequently dehydrated in an acetone series of 30%, 50%, 70%, 90%, and 3× 100%, for 25 min per change. Dehydrated specimens were critically point dried using CO_2 in an Emscope CPD 750, and mounted on specimen stubs using double sided adhesive tape. They were then sputter coated with gold in a Polaron E 5100 sputter coater and subsequently examined in a Jeol 25S scanning electron microscope using 12.5 KV accelerating voltage.

Results

Ultrastructure of body tegument

The tegument of *T. imbutiforme* possessed an outer syncytial layer connected to a subjacent layer of subtegumentary cells via cytoplasmic connectives. The apical syncytial layer of the body tegument was typically spinous, and possessed characteristic inclusions of a digenean tegument including mitochondria and secretory bodies (figs 1 and 11, m and sb). Also visible in some sections were small round or oval vacuoles which appeared 'empty' (fig. 17, v).

Undulating apical and basal plasma membranes defined the limits of the outer syncytial layer. The overall thickness of the layer ranged between 0.8 μm and 1.8 μm, with an overall mean thickness of 1.3 μm. The apical plasma membrane was generally smooth over the spinous regions, although where spines became less frequent or absent the surface was often pimpled (fig. 15, p).

A basal lamina was intimately associated with the basal plasma membrane, and in spinous regions ranged between 35 nm and 56 nm in thickness (mean = 44.8 nm) (see fig. 4, bl and bm). These two structures were occasionally observed contacting spine bases. At such junctions, the basal lamina and the basal membrane formed dense extensions, which invaginated inwards to reach the spine base (fig. 4, dbi). Below the basal lamina was an interstitial layer (fig. 1, inl) consisting of loosely arranged fibres separating the outer syncytial layer from underlying muscle layers.

The most prominent feature of the outer syncytium was an array of large serrated spines which characteristically overlapped creating an appearance in transverse sections similar to that of 'fish scales' (figs 2 and 3). A three dimensional representation of an individual spine was reconstructed from several longitudinal sections taken at variable angles (fig. 5). The spines appeared to possess a triangular base pointed towards the basal plasma membrane which it contacted at places where invaginations of the basal membrane and basal lamina occurred. As the triangular base expands upwards towards the surface, it reaches its widest point level with the external surface, and emerges as a flattened serrated triangular plate, orientated obliquely, and pointing backwards. The external part of the spine is closely invested by apical plasma membrane. The teeth of the protruding portion were united at their bases, but separated at the tips. Transverse sections through the spine close to the base created a triangular profile with an undulating external face, similar to a 'corrugated roof' (figs 2 and 3). Sections cut further along the protruding plate, gave rise to a biconvex profile with corrugations on both sides, due to the emerging form of the spine teeth (fig. 5). The teeth gradually separate, their number remaining constant in consecutive sections, but eventually decline as the teeth taper off near the tips. Two types of spines were identified according to the number of teeth arising from the base.

The first type possessed an average of 20 to 21 teeth, with a mean plate width of 2.3 μm (range 2.1–2.7 μm). The second spine type possessed less teeth, usually 8–9, with a plate width between 0.9 μm and 1.1 μm (mean width 1.0 μm) (for examples see fig. 2).

Examination of figs 2 and 3, both of which represent sections cutting the tegument in a plane approximately perpendicular to the apical surface, shows that they

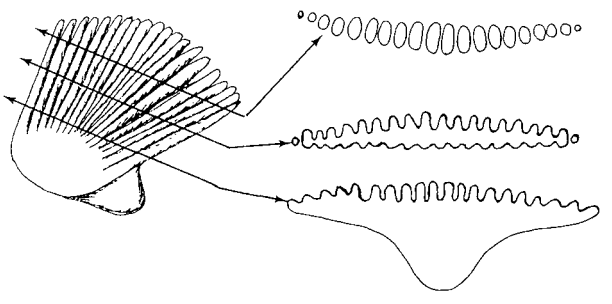
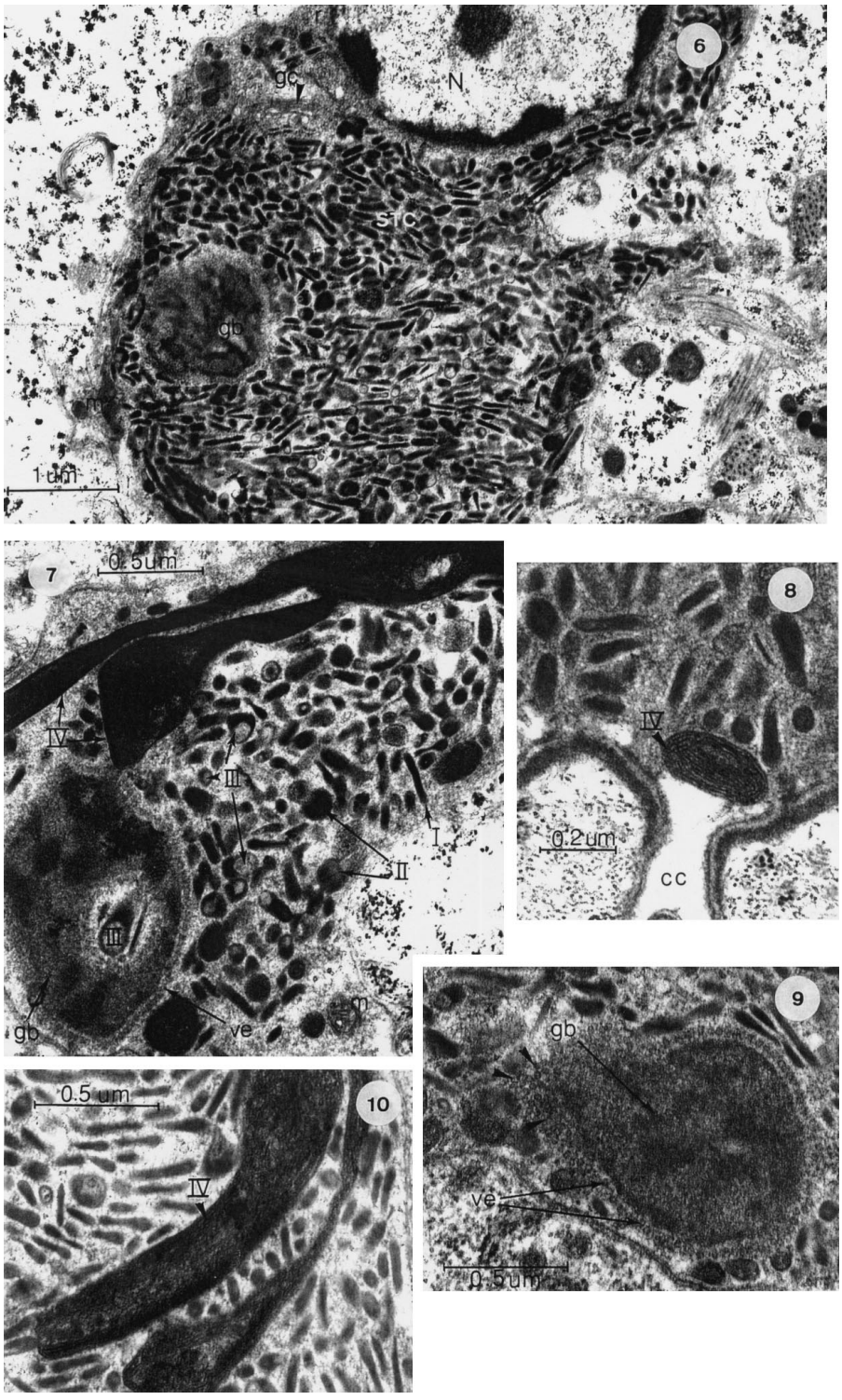


Fig. 5. Three dimensional image of the presumed shape of the body spines of *Timoniella imbutiforme*.

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press



Figs 6–10. Transmission electron micrographs of the metacercaria of *Timoniella imbutiforme*. 6. Subtegumentary cell containing abundant secretory bodies. 7. Part of a subtegumentary cell containing all four types of secretory bodies. 8. The junction between the cytoplasmic connective of the subtegumentary cell and the outer tegumental syncytium. 9. Detailed ultrastructure of the giant bodies showing the small vesicle envelope surrounding the dense core. Notice the concentration of vesicles in one area (arrow heads). 10. Details of the matrix structure of a Type IV secretory body. Giant body (gb), Golgi complex (gc), nucleus (N), subtegumentary cell (STC), Type I secretory body (I), Type II secretory bodies (II), Type III secretory bodies (III), Type IV secretory bodies (IV), vesicular envelope (ve).

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press

reveal different numbers of spine profile layers (five in fig. 2 and three in fig. 3). It is clear that such differences in layer number will depend on spine lengths, lateral separation of spines and the precise orientation of sectioning.

Both types of spines had similar internal crystalline organization composed of arrays of parallel and alternating light and dark bands of similar thickness and occurring at approximately 7 nm periodicity. The larger spines possessed arrangements of longitudinal and transverse periodicity (fig. 4). Distinctive thicker dark bands of greater electron density also occurred, but were distinguishable only at the apex of longitudinally sectioned spines (fig. 4). It was therefore not possible to determine which type of spine they belonged to. These bands occurred at a much wider periodicity (22 nm) to the less dense bands, and detailed examination of fig. 4 suggests that every fourth narrow dark band is enlarged to form a periodically thicker band at 22 nm intervals.

Numerous mitochondria were found throughout the basal and middle regions of the apical syncytium. Most were round or oval (fig. 1, m), although some elongate forms were occasionally encountered (fig. 17). All had visible cristae.

In common with other digenean teguments, the outer syncytial layer of *T. imbutiforme* also contained numerous membrane-bound secretory bodies. These were found scattered between the mitochondria, and were mainly located in the central and basal regions of the tegumental syncytium (figs 1 and 4). The two commonest types were an electron dense elongate or rod shaped form (Type I) (length $0.41 \pm 0.09\,\mu$m, width $0.04 \pm 0.003\,\mu$m SD), and a round form (Type II) of similar, or slightly less electron density (diameter $0.22 \pm 0.03\,\mu$m SD), with Type I being commoner than Type II. In most of the sections examined, the long axes of many of the secretory bodies were not orientated in any particular direction, except for Type I, which were often inclined at right angles to the apical surface (fig. 1). Also occurring in the syncytium were less prevalent Type III and IV secretory bodies.

Type III, a vacuolated secretory body, was mainly ovoid with an electron dense matrix similar to that of Type I and Type II (fig. 1). It was differentiated, however, by the presence of a centrally or peripherally situated electron lucent vacuole (fig. 7, III). Such bodies had a mean length of $0.19\,\mu$m (range $0.16–0.24\,\mu$m), and a mean width of $0.11\,\mu$m (range $0.08–0.12\,\mu$m). Some of the more elongate of this type of vesicle, however, measured between $0.28\,\mu$m and $0.32\,\mu$m long and $0.06\,\mu$m to $0.08\,\mu$m wide.

Type IV secretory bodies had a matrix of similar appearance to the internal structure of the multilaminate bodies of *Schistosoma* species (see Hockley, 1973). The limiting membrane enclosed a structure made up of dark and light bands tightly wrapped into a spiral or whirl of concentric rings (fig. 8, IV). This type of secretory body was much larger, and more electron dense, than the remaining types appearing round or oval in the outer tegumental syncytium (length $0.3 \pm 0.09\,\mu$m SD, width $0.2 \pm 0.03\,\mu$m SD), but larger and more elongate in the subtegumentary cells (figs 7 and 10, IV). The elongate profiles ranged between $1.1\,\mu$m and $3.0\,\mu$m long, possibly longer as it exceeded the micrograph margin, and $0.1\,\mu$m $0.5\,\mu$m wide (fig. 7). A central, slightly less dense core, was a feature of many of the transversely sectioned vesicles (figs 7 and 10).

The subtegumentary cells of *T. imbutiforme* contained all the characteristic cytoplasmic inclusions present in the outer tegumental cytoplasm, with the exception of the spines. Also present were nuclei, Golgi complexes (gc, fig. 6), abundant free ribosomes, and mitochondria (fig. 6, m). No RER, or smooth endoplasmic reticulum were detected in any of the examined micrographs.

The mitochondria of the subtegumentary cells resembled those found in the outer tegumental cytoplasm with respect to shape, size, and structure. They occupied a peripheral position in synthetically active cells (figs 6 and 7).

A characteristic and unusual inclusion in the cytoplasm of the subtegumentary cells was a large, round, or oval mass containing electron dense material with a patchy matrix. These have been termed here giant bodies (see figs 6, 7 and 9, gb). The core is surrounded by a distinct layer of vesicles (ve, fig. 9) which became clumped in a group at one end of the body (fig. 9 arrow heads). The vesicles had a mean diameter of 30 nm (fig. 9). Most subtegumentary cell profiles contained at least one such giant body, which had a mean diameter of $1.1\,\mu$m. Giant bodies were located at a distance from the nucleus, but close to the periphery of the cells, and always within the area occupied by the secretory bodies. In one of these giant bodies, there was a discernible electron lucent core containing a Type III secretory body and surrounded by another ring of vesicles (fig. 7).

Structures associated with the tegument

Musculature

The somatic muscles of *T. imbutiforme* located between the outer syncytium and the subjacent subtegumentary cells were non-striated and organized into three distinct layers. These consisted of a thin outer circular layer (mean thickness $0.2\,\mu$m) just below the interstitial layer, a median moderately thick longitudinal layer ($0.3\,\mu$m), and an even thicker inner circular muscle layer ($0.5\,\mu$m) (fig. 1). The outer circular muscle bundles were more densely packed (about 3–4 per μm) than those in the inner circular layer (with a density of 1 per μm) (fig. 1). Mitochondria were absent from the contractile areas of the myoblasts, but were conspicuous in the small areas of surrounding sarcoplasm due to their large size and electron dense matrix (fig. 1). Also present in the sarcoplasm were numerous clumps of electron dense glycogen particles. No sarcoplasmic reticulum (SR) was observed and the nucleated regions of the myoblasts were not found in close proximity to the fibrillar portions.

Nerves and sensory endings

A possible peripheral nerve bundle was encountered enmeshed by myocytes of the inner circular muscle layer. The bundle consisted of a group of 12 non-myelinated axons, containing electron lucent vesicles. These were concentrated mainly in axons associated with the neuromuscular junctions (fig. 1). The junctions were characterized by having dense material deposited only

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press

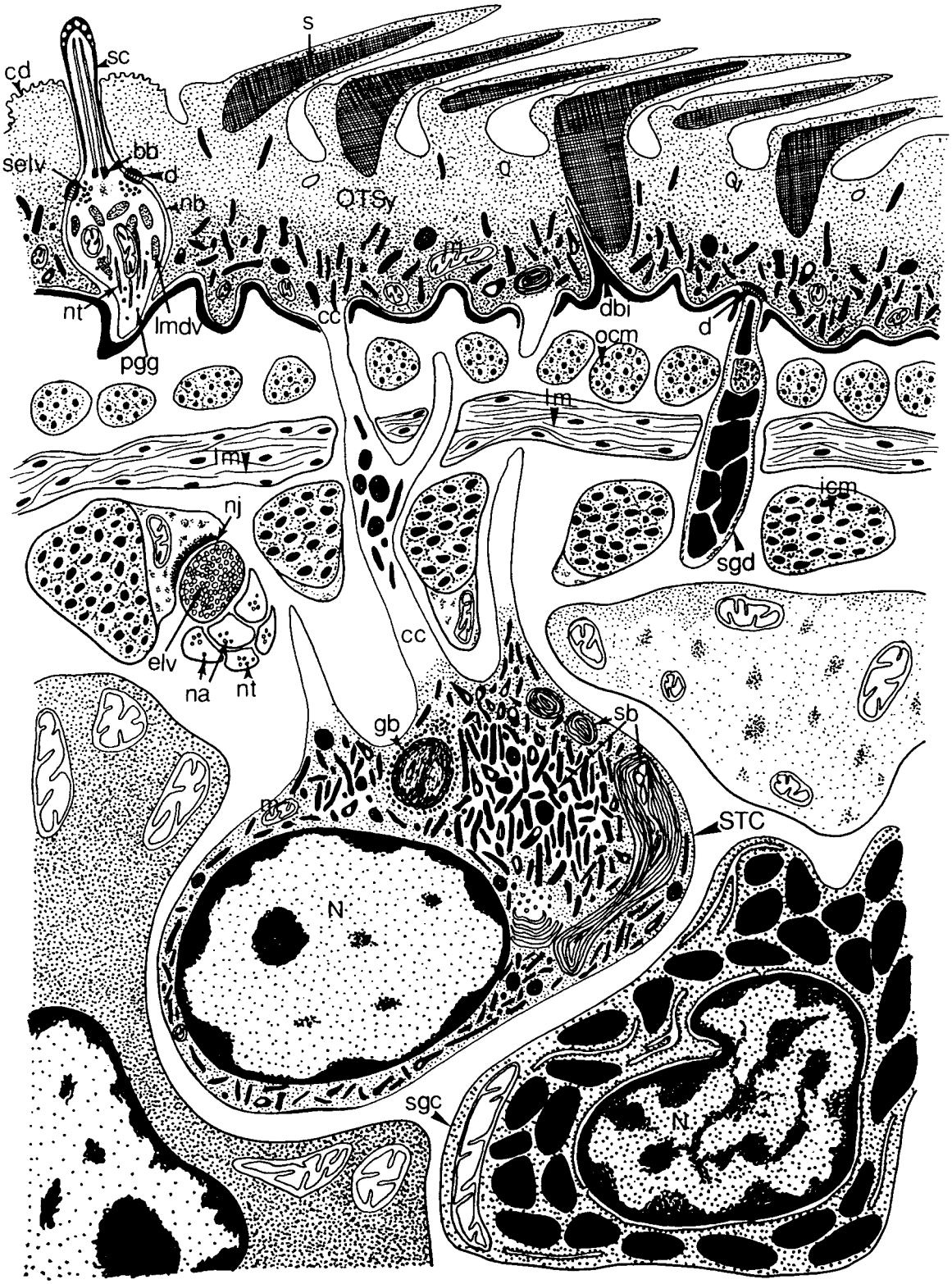
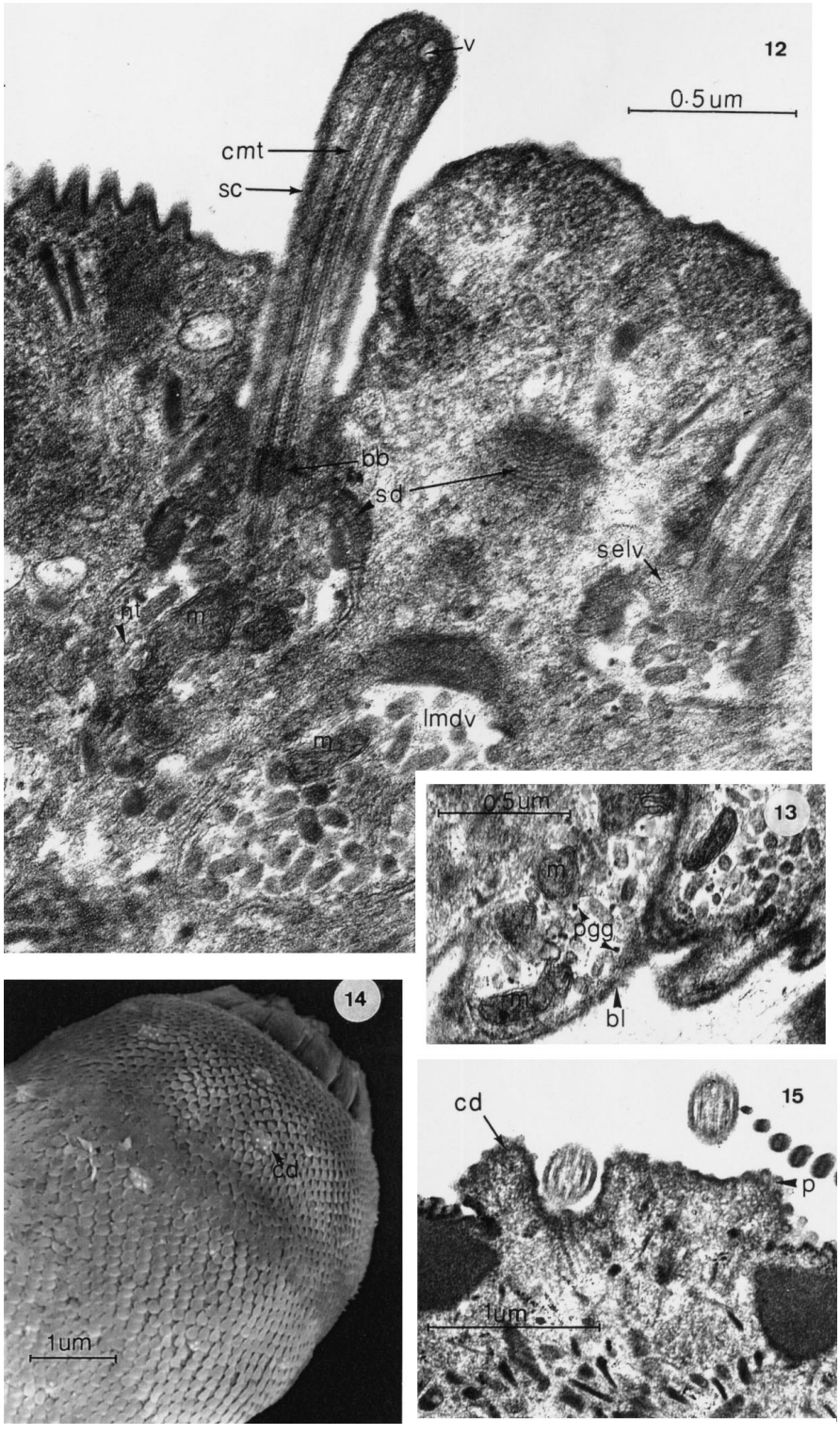


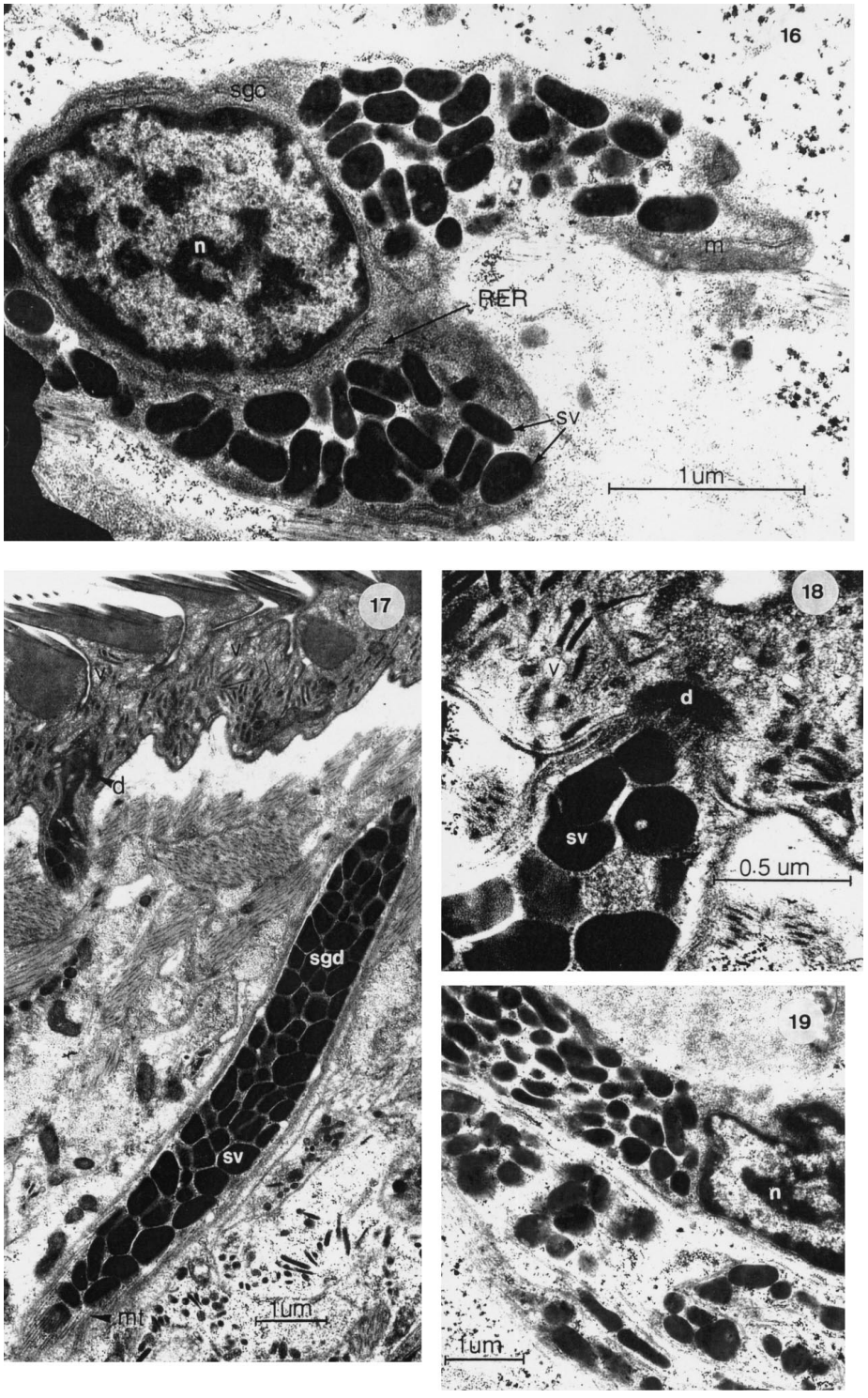
Fig. 11. Diagrammatical representation of the metacercarial tegument of *Timoniella imbutiforme*. Basal body (bb), ciliated domes (cd), cytoplasmic connectives of subtegumentary cells (cc), desmosome (d), dense basal invaginations (dbi), electron lucent vesicles (elv), giant bodies (gb), inner circular muscle layer (icm), longitudinal muscle layer (lm), large moderately electron dense vesicles (lmdv), mitochondria (m), nucleus (N), neural axon (na), neural bulb (nb), neuromuscular junction (nj), neurotubules (nt), outer circular muscle layer (ocm), outer tegumental syncytium (OTSy), presumed glycogen granules (pgg), spines (s), secretory bodies (sb), sensory cilium (sc), small electron lucent vesicles (selv), secretory gland cell duct (sgd), secretory gland cells (sgc), subtegumentary cells (STC), vacuoles (v).

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press



Figs 12–15. Transmission and scanning electron micrographs of the metacercaria of *Timoniella imbutiforme*. 12. TEM of the sensory endings in the body tegument. 13. TEM of the basal portion of the sensory ending process showing it distorting, but not penetrating the basal lamina. 14. SEM of the ciliated domes on the body. 15. TEM of the ciliated domes to show pimpled apical surface. Basal body (bb), basal lamina (bl), ciliated dome (cd), cilium microtubules (cmt), large moderately dense vesicles (lmdv), mitochondria (m), neurotubules (nt), pimples (p), presumed glycogen granules (pgg), sensory cilium (sc), septate desmosome (sd), small electron lucent vesicles (selv), cilium vacuoles (v).

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press



Figs 16–19. Transmission electron micrographs of the metacercaria of *Timoniella imbutiforme*. 16. Secretory gland cell. 17. Secretory gland cell duct. 18. Junction between a secretory gland cell duct and the outer syncytium demarcated by a desmosome. 19. Numerous adjacent gland cells. With an irregular nuclear profile in one of the cells. Desmosome (d), mitochondria (m), microtubules (mt), nucleus (n), rough endoplasmic reticulum (RER), secretory gland cell duct (sgd), secretory gland cell (sgc), secretory vesicles (sv).

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press

at the postsynaptic membranes in the non-fibrillar areas of the myocytes.

Scanning electron micrograph images revealed that uniciliate nerve endings occurred in groups in aspinous pimpled domes distributed over the body of *T. imbutiforme* (fig. 14, cd). These groups consisted of two to five uniciliate nerve endings in TEM sections (fig. 12), but as many as eight in SEM images (fig. 14). The former showed that each nerve ending consisted of a neural bulb embedded within the outer tegumental syncytium, and possessing a single cilium of approximately 1.3 μm length from the originating basal body. In cross-section the cilium possessed a '9 + 2' microtubule configuration, and in longitudinal section showed at its tip, distal to the terminus of the axoneme, several round vacuoles (fig. 12, v). The neural bulb was attached to the tegument by a circumferential septate desmosome just below the level of the basal body (fig. 12, sd). Each neural bulb profile contained one or two mitochondria (fig. 12, m), although more were observed in the neural processes (fig. 13, m). Apart from the mitochondria, three other types of inclusions were also present in the neural bulbs and processes, the most prominent of which were large discoid-shaped vesicles of moderate electron density (lmdv) (mean maximum diameter 0.12 μm, range 0.10–0.15 μm) (fig. 12). Also present were a few small, round, electron dense granules (pgg) (mean diameter 23.7 nm, range 10–43 nm) (see Hockley, 1973) (fig. 13), and clusters of small round, electron lucent vesicles (selv) (mean diameter 8.5 nm) occurring near the base of the cilium, between the basal bodies and the desmosomes (fig. 12). Neurotubules (nt) were also detected at the base of the neural bulb (fig. 12, nt).

Neural processes, seen to be continuous with the neural bulbs, were not observed passing through the basal membrane and lamina, although the distortions created by them in the two layers were encountered (fig. 13).

Secretory gland cells

Although secretory gland cells were mainly a feature of the parenchyma of *T. imbutiforme*, their ducts were connected to the outer tegumental syncytium. Such cell ducts were frequently observed passing through the body wall musculature towards the outer syncytium (fig. 17, sgd) and, on a few occasions, were actually found attached to the basal membrane by electron dense septate desmosomes (figs 17 and 18, d). The ducts were characterized by microtubules lining the periphery, and by the presence of large electron dense secretory vesicles, compressed into angular shapes (fig. 17, sv).

The secretory gland cells were readily recognizable by their contents of similar sized secretory vesicles to those found in the ducts, although appearing less compressed (mean maximum diameter 0.6 μm, range 0.5–0.8 μm, mean minimum diameter 0.3 μm, range 0.2–0.4 μm), and by their large and often folded nuclei (fig. 19).

Discussion

The results of the present ultrastructural examination of the tegument of the metacercaria of *Timoniella imbutiforme* (Molin, 1859), suggest that the overall structure, if not the detail, of the tegument corresponds to descriptions provided for other late juvenile digenean stages (see Hockley, 1973; Rees, 1974; Fujino *et al*., 1979; Apinhasmit *et al*., 1994).

The most striking feature of the body tegument of the metacercaria of *T. imbutiforme* was its characteristic spine arrangement and individual spine shape which allowed for an overlapping configuration. A sound structural attachment of the spines appears to be provided by the basal lamina. The basal lamina of *T. imbutiforme* was particularly thick in the areas associated with the spinous body tegument. Species which possess no spines generally have a thin basal lamina (Kuntz *et al*., 1982). The association of the interstitial layer with the basal lamina could also implicate it in the supportive function. The distance, however, between the lamina and the muscles, and the sparse nature of the connecting desmosomes, suggests an absence of a functional relationship between the muscles and the body spines, unlike that described for the circumoral spines of *T. imbutiforme* (McDowall & James, 1988).

In addition to the spines, four types of secretory bodies were identified in the body tegument of *T. imbutiforme*, the membranous vesicles, Type IV, perhaps being the most intriguing of the four. Multilaminate, or membranous vesicles have only been observed in *Schistosoma* species, where they have been studied extensively (Hockley, 1973: Wilson & Barnes, 1974), and in the redial tegument of *Paramphistomum epiclitum* (Dunn *et al*., 1992). However, the multilaminate bodies of *P. epiclitum* do not resemble those of *Schistosoma* in appearance, although Dunn *et al.* (1992) speculated on a possible similarity in function. The superficial resemblance of the membranous secretory bodies of *T. imbutiforme* to the multilaminate vesicles of *Schistosoma* might suggest a functional similarity. If the vesicles did indeed contain stored membrane, the functional significance may be comparable with that of the vesicles of *Schistosoma*, i.e. the membrane is inserted into the apical surface expanding its surface area, and simultaneously delivering a glycocalyx to protect against host immune attacks (Aikawa & Atkinson, 1990). *Schistosoma* species, however, occupy sites in the body (the lumina of blood vessels), where they would be in constant risk from host-mounted immune attacks, unlike *T. imbutiforme* adults which are inhabitants of the lumen of the rectum of the bass, *Dicentrarchus labrax* (Brooks, 1980).

The synthesis of the tegument's secretory bodies is the primary function of the subtegumentary cells of digeneans, which were found subjacent to the muscle layers of the body wall (Smyth & Halton, 1983). Apart from the main constituents of these cells, characteristic inclusions, the giant bodies, consistently appeared in the subtegumentary cells of *T. imbutiforme*. The form of the giant bodies, with an outer vesiculated envelope, and an inner dense core did not resemble any previous documented accounts. Køie (1971) reported finding bodies of a similar size composed of concentrically arranged material, and surrounded by rod bodies, and she believed them to represent waste products. Wilson & Barnes (1974) similarly observed residual bodies in the tegument and subtegumentary cells of *Schistosoma*, and accordingly

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press

suggested limited lysosomal activity in the system. Autophagosomes were observed by Mattison *et al.* (1994) in the subtegumentary cells of paramphistome juveniles, in which sequestered rough endoplasmic reticulum, polyribosomes, and sometimes secretory bodies were found. Despite this varied range of descriptions for other subtegumentary inclusions, none were structurally analogous to the giant bodies. Their appearance initially suggested that they might be an unconventional form of Golgi complex. The presence, however, of 'conventional' Golgi complexes within the subtegumentary cells makes this interpretation unlikely. It is more conceivable that these bodies represent a type of condensing vacuole (see Wilson & Barnes, 1974), and the surrounding vesicles represent small transporting vesicles from the Golgi complexes. Perhaps the vesicles are being imported into the centre of the giant body to produce a structure of proteinacious origin.

All of the four types of secretory bodies found in the distal cytoplasm are also seen in the subtegumentary cells and may be presumed to be transported up cytoplasmic connectives in this form from the cells to the distal cytoplasm. The only major distal cytoplasmic constituent not seen in the subtegumentary cells is spine material. Perhaps the giant bodies represent condensations of synthesized spine protein formed prior to their transport to the distal layer.

Acknowledgements

We would like to thank Mr Alan Maple for his technical help during the preparation of electron microscopical samples, Mr John Pacey and Ms Jane Storey for their invaluable assistance in the production of micrographs, Mr Reg Reed for assistance with fish sampling and the staff at Lots Road Power Station for making the fish sampling possible.

References

Aikawa, M. & Atkinson, C.T. (1990) Immunoelectron microscopy of parasites. *Advances in Parasitology* **29**, 151–214.

Apinhasmit, W., Sobhon, P., Saitongdee, P., Menayotin, S. & Upatham, E.S. (1994) *Opisthorchis viverrini*: Ultrastructure of the tegument of the first-week juveniles and adult flukes. *International Journal for Parasitology* **24**, 613–621.

Bennett, C.E. & Threadgold, L.T. (1973) Electron microscope studies of *Fasciola hepatica*. XIII. Fine structure of newly excysted juvenile. *Experimental Parasitology* **34**, 85–99.

Brooks, D.R. (1980). Revision of the Acanthostominae Poche, 1926 (Digenea: Cryptogonimidae). *Zoological Journal of the Linnean Society* **70**, 313–382.

Burton, P.R. (1966) The ultrastructure of the integument of the frog bladder fluke *Gorgoderina* sp. *Journal of Parasitology* **52**, 926–934.

Dunn, T.S., Dang, P.H., Mattison, G., Hanna, R.E.B. & Nizami, W.A. (1992) Ultrastructural observations on the redial tegument of *Paramphistomum epiclitum* from the planorbid snail, *Indoplanorbis exustus*. *Journal of Helminthology* **66**, 167–176.

El-Darsh, H. & Whitfield, P.J. (1999) Digenean metacercariae (*Timoniella* spp., *Labratrema minimus* and *Cryptocotyle concava*) from the flounder, *Platichthys flesus* L., in the tidal Thames. *Journal of Helminthology* **73**, 103–113.

Erasmus, D.A. (1967) The host-parasite interface of *Cyathocotyle bushiensis* Khan, 1962 (Trematoda: Strigeoidea) II. Electron microscope studies of the tegument. *Journal of Parasitology* **53**, 703–714.

Fujino, T., Ishii, Y. & Choi, D.W. (1979) Surface ultrastructure of the tegument of *Clonorchis sinensis* newly excysted juveniles and adults. *Journal of Parasitology* **65**, 579–590.

Hockley, D.J. (1973) Ultrastructure of the tegument of *Schistosoma*. *Advances in Parasitology* **11**, 233–297.

Køie, M. (1971) On the histochemistry and ultrastructure of the tegument and associated structures of the cercariae of *Zoogonoides viviparus* in the intermediate host. *Ophelia* **9**, 165–206.

Køie, M. (1973) The host-parasite interface and associated structures of the cercariae and adult *Neophasis lageniformis* (Lebour, 1910). *Ophelia* **12**, 205–219.

Kuntz, S.M., Font, W. & Wittrock, D.D. (1982) Ultrastructure of the tegument of *Amblosoma suwaense* metacercariae. *Proceedings of the Helminthological Society of Washington* **49**, 148–151.

LeFlore, W.B. & Bass, H.S. (1983) Observations on the morphology and hydrolytic enzyme histochemistry of excysted metacercariae of *Himasthla rhigedana* (Trematoda: Echinostomatidae). *International Journal for Parasitology* **13**, 179–183.

Mattison, R.G., Hanna, R.E.B. & Nizami, W.A. (1994) Ultrastructure and histochemistry of the tegument of juvenile paramphistomes during migration in Indian ruminants. *Journal of Helminthology* **68**, 211–221.

McDowall, A.A. & James, B.L. (1988) The functional morphology of the circumoral spines of *Timoniella imbutiforme* (Molin, 1859) Brooks, 1980 (Digenea: Acanthostomidae). *International Journal for Parasitology* **18**, 523–530.

Rees, G. (1974) The ultrastructure of the body wall and associated structures of the cercariae of *Cryptocotyle lingua* (Creplin) (Digenea: Heterophyidae) from *Littorina littorea* (L.). *Zeitschrift für Parasitenkunde* **44**, 239–265.

Robinson, R.D. & Halton, D.W. (1983) Functional morphology of the tegument of *Corrigia vitta* (Trematoda: Dicrocoeliidae). *Zeitschrift für Parasitenkunde* **69**, 319–333.

Smyth, J.D. & Halton, D.W. (1983) *The physiology of trematodes*. Cambridge, Cambridge University Press.

Threadgold, L.T. (1963a) The tegument and associated structures of *Fasciola hepatica*. *Quarterly Journal of Microscopical Science* **104**, 505–512.

Threadgold, L.T. (1963b) The ultrastructure of the 'cuticle' of *Fasciola hepatica*. *Experimental Cell Research* **30**, 238–242.

Threadgold, L.T. (1968) The tegument and associated structures of *Haplometra cylindracea*. *Parasitology* **58**, 1–7.

Wilson, R.A. & Barnes, P.E. (1974) The tegument of *Schistosoma mansoni*: observations on the formation, structure and composition of cytoplasmic inclusions in relation to tegument function. *Parasitology* **68**, 239–258.

(Accepted 12 May 1999)
© CAB *International*, 2000

https://doi.org/10.1017/S0022149X00700083 Published online by Cambridge University Press