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Ultrastructure of the tegument of *Saccocoelioides godoyi*

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Abstract

The tegument of adult *Saccocoelioides godoyi* Kohn & Froes, 1986 (Digenea: Haploporidae), specimens of which were collected from the intestine of the freshwater fish, *Leporinus friderici* (Bloch, 1794) (Anostomidae) from the reservoir of Itaipu Hydroelectric Power Station, Parana State, Brazil, was studied by transmission electron microscopy. The tegument comprises an external anucleate layer, covered by a surface plasma membrane and associated glycocalyx. The surface layer is bound by the basal plasma membrane and contains spines, two types of inclusion bodies and mitochondria. Tegumental cell bodies are located beneath the surface musculature and contain a single nucleus, cytoplasm with rough endoplasmic reticulum, mitochondria, ribosomes, and inclusion bodies similar to those found in the external layer. Cytoplasmic strands connect the cell bodies to the external surface layer, suggesting that the inclusion bodies are produced in these cells and pass up into the syncytium, as is known for other digeneans from experimental evidence.

Introduction

The tegument of digeneans plays a vital role in the survival of the parasites, performing important functions including absorption of nutrients and secretion or discharge of waste products (Lumsden, 1975). The ultrastructure of the digenean tegument has been reviewed by Lumsden (1975), Halton (1982) and Threadgold (1984). However, it appears that the tegument of intestinal digeneans, especially those in fish, has received little attention. Recently, the tegument of *Prosorhynchoides arcuatus*, (family Bucephalidae), a parasite of marine fish, was studied by scanning (Cohen *et al.*, 1995) and transmission electron microscopy (Cohen *et al.*, 1996).

The aim of the present study was to examine the ultrastructure of the tegument of adult worms of *Saccocoelioides godoyi*, a digenean parasite occurring in the intestine of the freshwater fish *Leporinus friderici*.

Material and methods

Fish samples from the reservoir of Itaipu Hydroelectric Power Station, locality of Guaira, Parana state, Brazil were collected with nets and kept alive until examination. Thereafter, specimens of *Saccocoelioides godoyi* were recovered from the intestine of *Leporinus friderici* (Bloch, 1794) (Anostomidae), fixed in 2.5% phosphate-buffered glutaraldehyde and post fixed for 1 h in 1% osmium tetroxide in the same buffer. After dehydration through a graded ethanol series, the material was embedded in Epon (Luft, 1961). Ultrathin sections were cut using a Reichert Ultracut E-microtome, constrasted with uranyl acetate and lead citrate (Reynolds, 1963) and observed under a Zeiss EM 900 electron microscope.

Results

The tegument of *Saccocoeliodes godoyi* is a syncytium, comprising an outer anucleated layer, connected at intervals by cytoplasmic strands to insunken nucleated areas, the tegumental cell bodies (figs 1, 2). The outer layer of the tegument bears irregular folds forming projections, and is covered by a surface plasma membrane and associated glycocalyx. Internally, the tegument is bound by the basal plasma membrane, below which resides the basal lamina. Pointed spines are present

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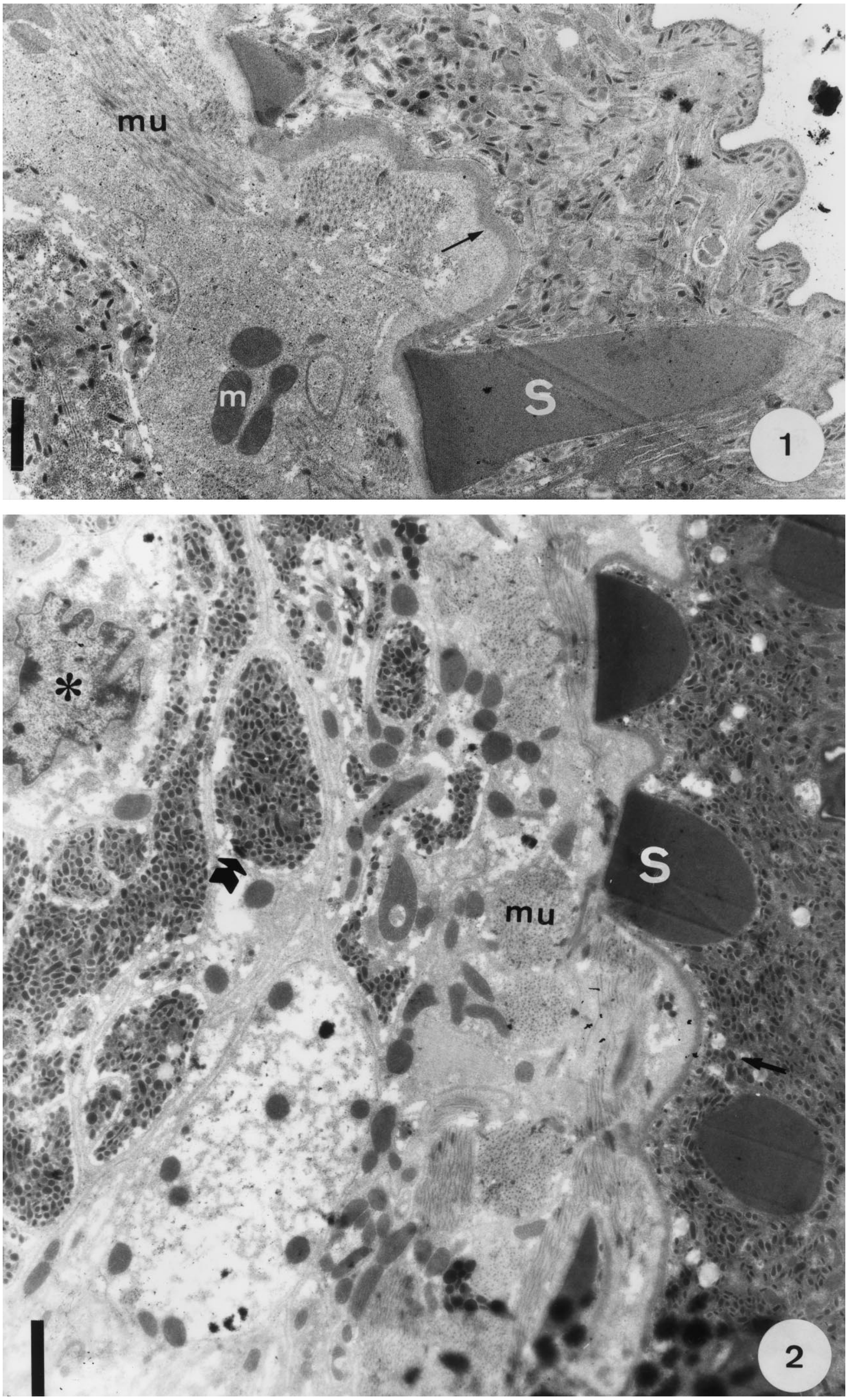

Figs 1–2. Transmission electron micrographs of the tegument of *Saccocoelioides godoyi*. 1. Transverse section of body tegument showing the external syncytial layer with pointed spine (S), bounded by the basal plasma membrane and, beneath it, the muscular layer (mu) and mitochondria (m). Scale bar = 2.2 μm. 2. General view of the tegument, to show external syncytial layer containing spines (S), muscular layer (mu), tegumental cells with nucleus (asterisk) and cytoplasm with inclusions (arrows). Scale bar = 3.4 μm.

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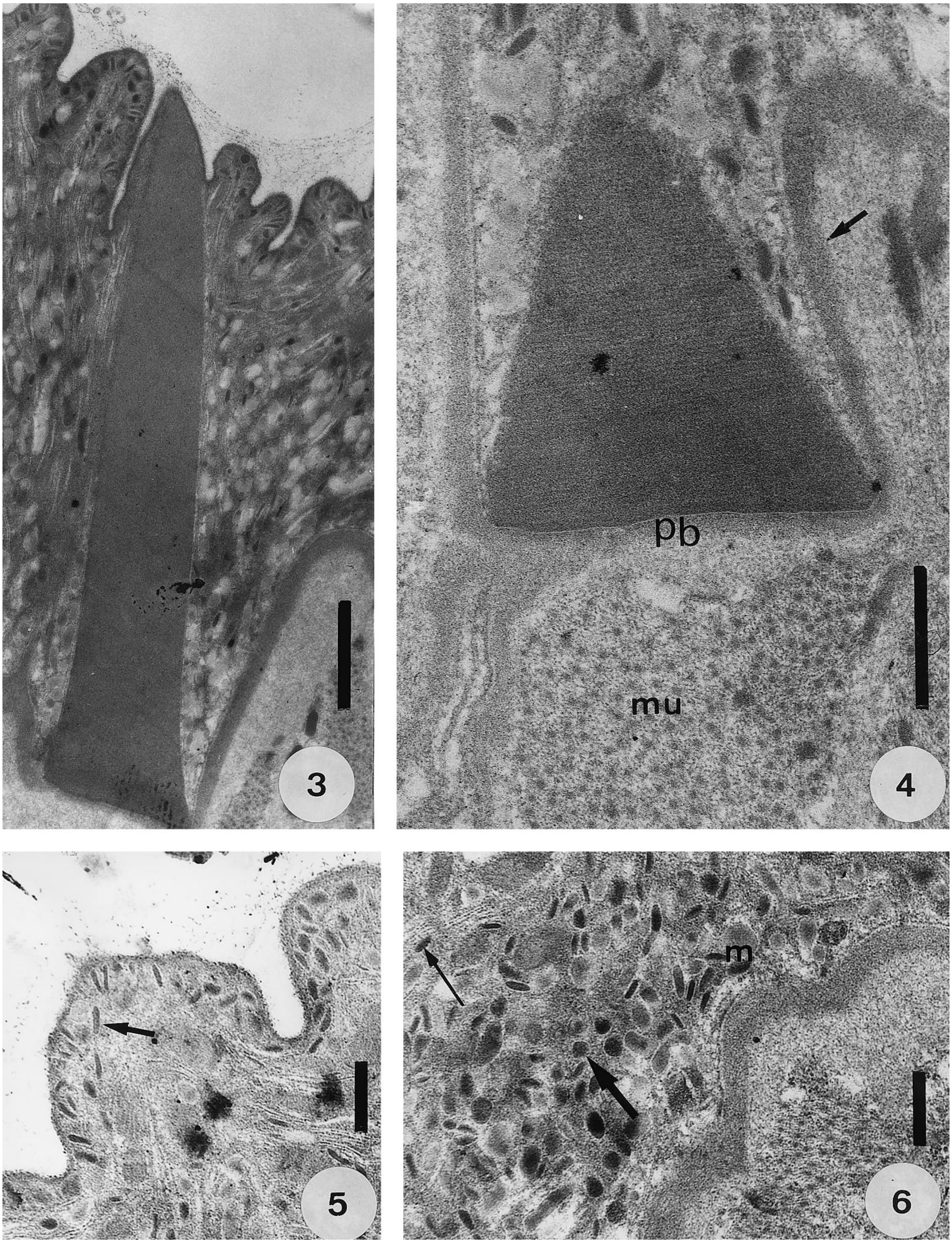

Figs 3–6. Transmission electron micrographs of the tegument of *Saccocoelioides godoyi*. 3. Pointed spine between the apical and basal plasma membranes. Scale bar = 2.7 μm. 4. Tegumental spine, showing the crystalline structure and basal plate (pb). Note the basal lamina (arrow) and circular muscular fibres (mu). Scale bar = 1.2 μm. 5. Apical region of the tegument showing rod shaped bodies perpendicular to the apical plasma membrane (arrow). Scale bar = 1.1 μm. 6. Basal region of the external syncytial layer near basal plasma membrane/lamina basal complex, showing the electron-dense bodies (large arrow), rod shaped bodies (narrow arrow) and mitochondria (m). Scale bar = 1.1 μm.

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inside the syncytium, between the surface and basal plasma membranes (fig. 3) The spines are elongate, with a crystalline appearance with dense, horizontally orientated lines. The area of the basal plasma membrane where the spines rest appears more electron-dense and represents the basal plate (fig. 4).

The ground substance of the surface layer is composed of granular material of moderate electron-density and presents two types of inclusion bodies: the first type is round to elongated, membrane-bound and electron-dense, refered to as small electron-dense bodies (seb), found irregularly dispersed throughout the syncytial layer. The second type are electron-dense, rod-shaped bodies which, in the vicinity of the surface plasma membrane, are orientated perpendicular to the surface. Mitochondria were also scattered in the external layer (fig. 5). The basal plasma membrane rests on a fibrous lamina of uniform thickness, that is folded, and follows the underlying musculature. A layer of fibrous interstitial material was observed between the basal lamina and the musculature (fig. 6). The surface layer presents no evidence of nuclei, Golgi apparatus, or endoplasmic reticulum. The subsurface musculature consists of alternating layers of circular and longitudinal fibres and associated mitochondria which are very large, dense, elongate and contain few cristae (fig. 7). The tegumental cell bodies, located beneath the muscle layer, are irregular in shape. The cytoplasmic strands connect the tegumental cells with the surface layer (fig. 8). The single nucleus of each of the tegumental cell bodies is irregularly-shaped with masses of chromatin distributed throughout, containing a central and ovoid nucleolus (fig. 9). The cytoplasm is filled with granular material, containing inclusion bodies that correspond to those found in the external layer (fig. 9); there is a well-developed rough endoplasmic reticulum, with dense, round to elongate mitochondria randomly distributed, and unattached ribosomes in small clusters, which fill the space between the organelles and inclusions (fig. 10).

Discussion

The ultrastructural organization of the tegument of *Saccocoelioides godoyi* resembles that described for other Digenea, where there is an external anucleate layer with spines and inclusion bodies, connected to the tegumental cell bodies by cytoplasmic processes. The folded apical surface provides an increase in the surface area of the tegument, presumably serving to enhance the transport of metabolites from the host's intestine and exchanges between the parasite and the host. The ultrastructure and arrangement of the tegument is consistent with an absorptive function.

The surface glycocalyx is believed to be involved in several roles, including absorption, transport and protection against mechanical, enzymatic and immunological activities of the host (Lumsden, 1975). Evidence that certain secretory vesicles produced in the tegumental cells may contribute to the glycocalyx in *Megalodiscus temperatus* has been presented by Bogitsh (1968) and Shannon & Bogitsh (1971).

The crystalline structure of the spines is characteristic of the majority of species of Digenea already studied (Zdarska *et al.*, 1990, Seo *et al.*, 1995, Cohen *et al.*, 1996, Ferrer *et al.*, 1996). These spines are believed to help the worms to attach to the host tissue (Senft *et al.*, 1961; Lumsden, 1975). According to Sharma *et al.* (1996), the crystalline nature of the spines and their presence in the external surface layer suggest they originate from the tegumental matrix. Abbas & Cain (1987) and Stitt *et al.* (1992) found filaments of actin in the tegumental spines of *Schistosoma mansoni* and *Fasciola hepatica*, respectively. These filaments were also present in the subtegumental musculature, confirming the hypothesis that the spines are motile structures.

The presence of mitochondria in the surface layer indicates the occurrence of metabolic exchanges that require energy. As in *Prosorhynchoides arcuatus*, studied by Cohen *et al.* (1996), the mitochondria found in the external layer are smaller than those associated with the musculature. The absence of mitochondria in the tegument of some digeneans, including *Orthocoelium scoliocoelium*, *Paramphistomum cervi* (studied by Sharma & Hanna, 1988) and *Gastrodiscoides hominis* (by Brennan *et al.*, 1991) indicate that the function of the tegument is related to chemical and mechanical protection of the worms, instead of an absorptive function. The fibrous interstitial material, lying beneath the basal lamina, can act as a mechanical integrator between it and the musculature, as suggested by Abbas & Cain (1987).

The inclusion bodies observed in the surface layer appear morphologically similar to those observed in other digeneans (Køie, 1971; Wilson & Barnes, 1974; Cohen *et al.*, 1996; Ferrer *et al.*, 1996; Sharma *et al.*, 1996). Several roles have been suggested for them, including the formation of the glycocalyx, maintenance of the surface plasma membrane or an involvement in immunoprotection (Lumsden, 1975; Hanna, 1980a,b). The characteristic arrangement of the rod-shaped bodies, perpendicular to the apical plasma membrane, indicates that they may contribute to the formation of this membrane.

The basal plasma membrane is invaginated, forming folds. These folds indicate a functional role in osmotic and ionic regulation, as suggested for *Fasciola hepatica* by Threadgold & Brennan (1978), besides the passage of material produced in the tegumental cells. The basal lamina follows the outline of muscle fibres and determines the organization and thickness of the surface layer.

Dislodgment of the parasite, due to contraction of the host body musculature during swimming, is probably prevented by the combined action of the tegumental spines and muscle fibres. The presence of numerous well-developed mitochondria in the musculature beneath the spines and the close association between muscular fibres and the base of the spines strengthen the suggestion that these structures are involved in an adhesive mechanism (Abdul-Salam & Sreelatha, 1992).

The tegumental cells present secretory organelles, such as endoplasmic reticulum and Golgi complex, indicating that they are secretory cells. The inclusion bodies are produced in the endoplasmic reticulum–Golgi complex of the tegumental cells and are exported to the surface layer (Cohen *et al.*, 1996).

It was observed that the tegument of *S. godoyi* presents only one type of tegumental cell that produces both of types of inclusion bodies. Nevertheless, there are

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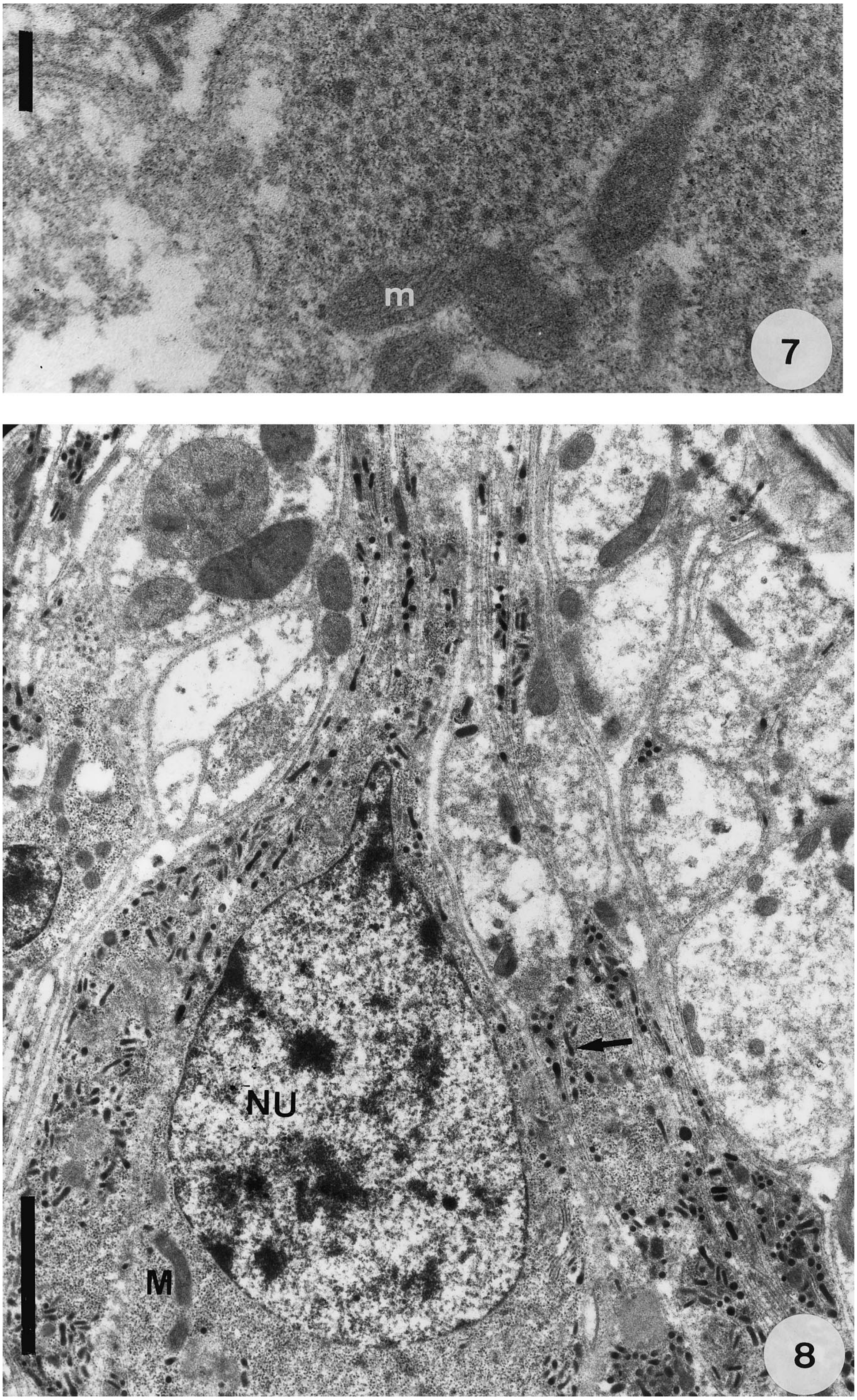

Figs 7–8. Transmission electron micrographs of the tegument of *Saccocoelioides godoyi*. 7. Transverse section of the muscular layer, containing large mitochondria (m) with few cristae. Scale bar = 0.6 μm. 8. Transverse section of a tegumental cell showing well-developed nucleus (NU) and defined regions of heterochromatin and cytoplasm with free ribosomes, inclusion bodies (arrow) and mitochondria (M). Scale bar = 4.6 μm.

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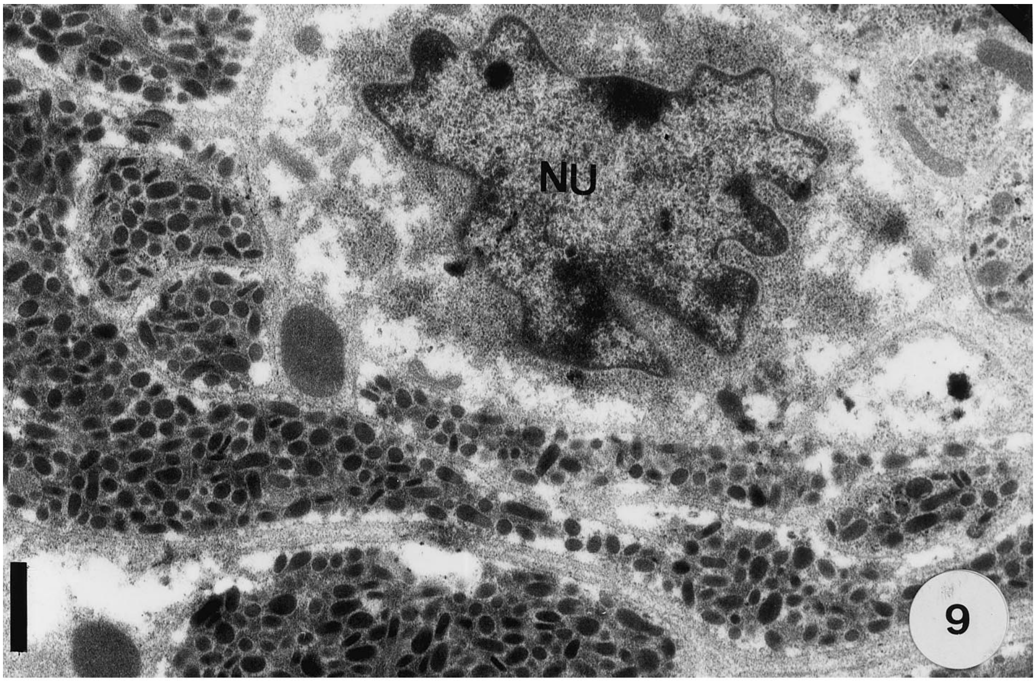

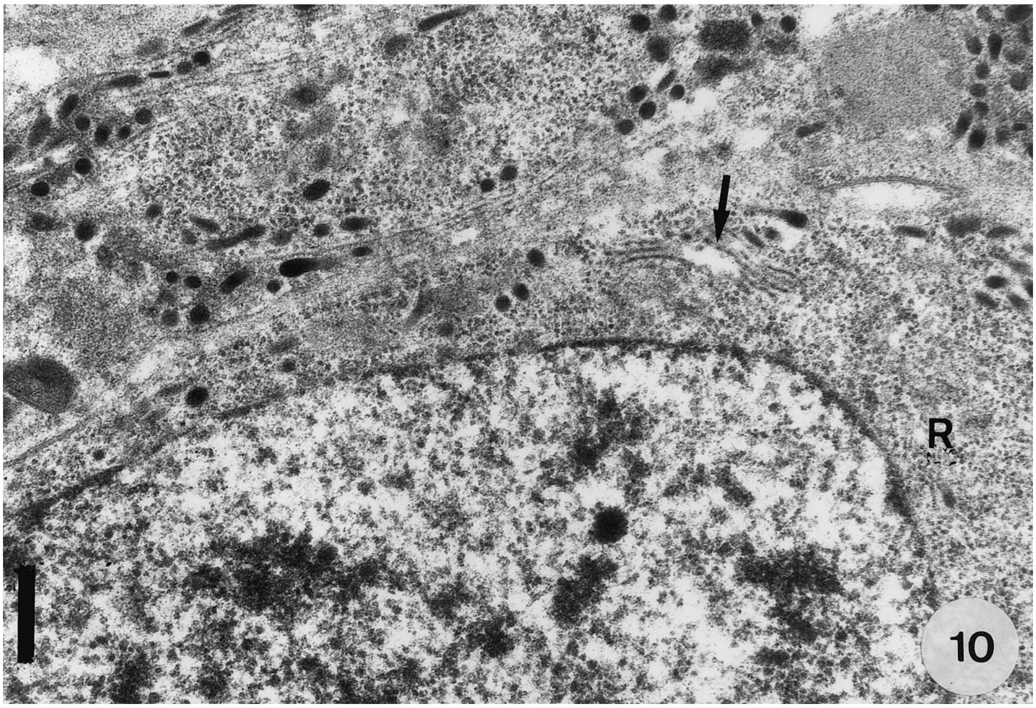

Figs 9–10. Transmission electron micrographs of the tegument of *Saccocoelioides godoyi*. 9. Tegumental cell with nucleus (NU) and cytoplasm filled with the inclusion bodies, similar to those found in the external syncytial layer. Scale bar = 1.7 μm. 10. Tegumental cell at higher magnification to show the nucleus, cytoplasm with Golgi complex/endoplasmatic reticulum (arrow), ribosomes (R) and inclusion bodies. Scale bar = 1.1 μm.

species of Digenea in different developmental stages that present two types of tegumental cells, each producing one of the inclusion bodies (Brennan *et al.*, 1991, Mattison *et al.*, 1994, Sharma *et al.*, 1996, Sobhon *et al.*, 1998)

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