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VERSITA

A transmission electron microscopical study of the tegument of *Maritrema feliui* (Digenea: Microphallidae)

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Abstract

The tegument of the microphallid digenean *Maritrema feliui,* examined by means of TEM, is described as a syncytial epithelium organised into two layers. The outer layer is an external anucleate, cytoplasmic region connected to a second region composed of nucleate perikarya (cytons) deeply embedded in the surrounding cortical parenchyma. The surface layer of the tegument is covered by a plasma membrane with many deep invaginations, which are apparently pinocytotic. This layer also bears numerous large, electron-dense spines of two types, which are intracellular and attached to the basal plasma membrane. Its cytoplasm is rich in free ribosomes, contains numerous mitochondria, disc-shaped granules frequently arranged in a rouleau, and several large, moderately electron-dense, membranous bodies. The subtegumentary perikarya and their nuclei, which are both flattened, are described in detail, as are their connections with the surface tegument. These perikarya appear to be the source of the disc-shaped granules and some of the other inclusions present in the surface layer. The main characteristics of the tegumental structure of *M. feliui* are commented upon in relation to the findings of previous publications and their suggested functions.

Keywords

Digenea, Microphallidae, *Maritrema feliui,* adult trematode, tegument, ultrastructure

Introduction

The functional ultrastructure and formation of the digenean tegument has long attracted the interest of parasitologists using electron microscopy for examining the surface layers of parasitic flatworms (for reviews, see: Threadgold 1963, 1984; Lee 1966; Świderski 1966; Hockley 1973; Lumsden 1975; McLaren 1980). The tegument of the sexual adult (marita) of digeneans, which represents the parasite's interface with its host, is of particular importance to the biology of these platyhelminths and their interrelationship with the host. This layer is involved not only in protection against the host's enzymes and immune system, but also in the absorption of nutritive substances and the processes of excretion and osmoregulation (Burton 1966; Lumsden 1975; Threadgold 1963, 1967, 1968, 1984; Halton 2004). A regional specialisation of the tegument occurs in most digeneans and is often correlated with a particular function or functions.

The great majority of the early ultrastructural studies on the digenean tegument were published on parasites of medical or veterinary importance, such as the blood flukes *Schistosoma* spp. (for a review, see: McLaren 1980) or the liver fluke *Fasciola hepatica* (for a review, see: Threadgold 1984). As shown by the numerous applied EM studies on the pharmacokinetics and therapeutic efficacy of different anthelmintic drugs against these blood and liver flukes (see: Mehlhorn *et al.* 1981; Moczoń and Świderski 1983, 1992; Shaw and Erasmus 1988), a rapid degeneration of the digenean tegument is generally accompanied not only by the ovicidal effects (a cessation of egg production) of the drug treatment but also by the death of the worms.

The aim of the present paper was to describe for the first time the ultrastructure of the tegument of a microphallid digenean, i.e. *Maritrema feliui* Gracenea, Montoliu et Deblock, 1993, a parasite of a wild host, the shrew *Crocidura russula* (Hermann) (Soricimorpha: Soricidae) in Spain.

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Materials and Methods

Live, sexually mature specimens of *Maritrema feliui* were collected from the intestine of the shrew *Crocidura russula* captured at La Ricarda Nature Reserve close to the estuary of the River Llobregat, Barcelona, Catalonia, Spain. The live worms were initially cleaned in a 0.9% NaCl solution. They were fixed in cold (4°C) 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4 for a minimum of 2 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.4, postfixed in cold (4°C) 1% osmium tetroxide with 0.9% potassium ferricyanide [$K_3Fe(CN)_6$] in the same buffer for 1 h, rinsed in milliQ water, dehydrated in an ethanol series and propylene oxide, and finally embedded in Spurr's resin. Ultrathin sections, about 60–70 nm thick, were obtained using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate. These were then examined using a JEOL 1010 TEM operated at an accelerating voltage of 80 kV.

Results

The thin, syncytial tegument of *Maritrema feliui* is of the usual neodermatan type, where junctions between cells are broken down and a single continuous cytoplasm surrounds the entire body. Furthermore, the tegumental nuclei are not located in the surface syncytium but within subtegumentary perikarya (cytons) situated in the cortical parenchyma beneath the superficial muscle layers of the body (Fig. 1). The tegumental surface is much infolded, with numerous surface pits and spines (Figs 2–5). It is covered by a plasma membrane with many deep invaginations, which appear to be pinocytotic. No evident glycocalyx layer was observed in this species.

Two types of spines are deeply embedded in the tegument and differ in the ultrastructure of their extremities which project through the tegument surface (Figs 1, 2, 5); these are referred to as type-1 and type-2 spines. In type-1 spines the exposed tip is sharp and conical, whereas in type-2 spines the tip is comb-shaped with 3–5 visible teeth, depending on the level of the section. The numerous spines pass through the lower part of the distal cytoplasm (Fig. 4A, B) and their enlarged base (Figs 2–5), which exhibits an increase in electron-density in its matrix, forms a basal plate, ~30 nm in thickness, that is attached to the basal plasma membrane (Figs 2–4).

The surface tegumental cytoplasm (Figs 2–5) consists of an electron-dense granular matrix with three main types of inclusion: small mitochondria; biconcave, disc-like bodies; and large, irregular, moderately electron-dense bodies. The

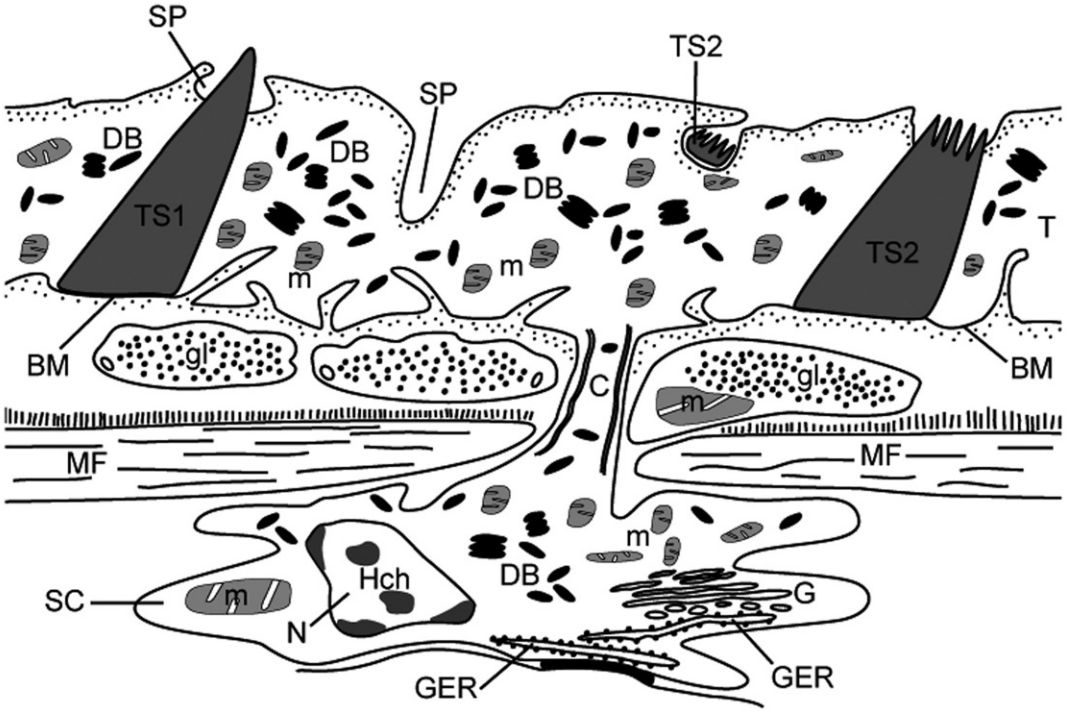

Fig. 1. Diagram illustrating the connection (C) of a subtegumentary perikaryon (cyton) (SC) to the tegument (T). **Abbreviations to all figures: BM** – basal membrane; **C** – connection between the syncytial cytoplasm of the tegument and a subtegumentary perikaryon; **DB** – dense bodies; **G** – Golgi complex; **GER** – granular endoplasmic reticulum; **gl** – glycogen; **Hch** – heterochromatin islands; **L** – lipid droplets; **LB** – large bodies; **m** – mitochondria; **MF** – muscle fibres; **N** – nucleus; **P** – parenchyma; **SC** – subtegumentary perikaryon; **SP** – surface pits; **T** – tegument; **TS1** – tegumental spine type-1; **TS2** – tegumental spine type-2

biconcave, disc-like bodies vary in diameter and electron-density (Fig. 3 and Inset to Fig. 3), as they are filled with a granular, more or less electron lucent material, and are frequently arranged in the form of a rouleau (Fig. 3 and Inset to Fig. 3).

The subtegumentary perikarya (Figs 1 and 5) are irregular in shape, much flattened and sometimes multinucleate. Their large, flattened nuclei contain numerous heterochromatin islands in a moderately electron-dense karyoplasm. The perikaryon cytoplasm contains Golgi complexes, a well-developed granular endoplasmic reticulum (GER), free ribosomes and a few mitochondria. In addition to these organelles, it also contains two characteristic types of tegumental inclusion, i.e. the same (disc-like and irregular) bodies as occur in the external cytoplasm of the tegument, which are apparently secreted by these cells. The subtegumentary perikarya are linked to the outer tegument by one or more long, tortuous cytoplasmic connections (bridges) (Figs 1 and 5), which are reinforced by a single ring of numerous longitudinally arranged microtubules. Secretory vesicles migrating from the perikarya to the distal cytoplasm pass through these

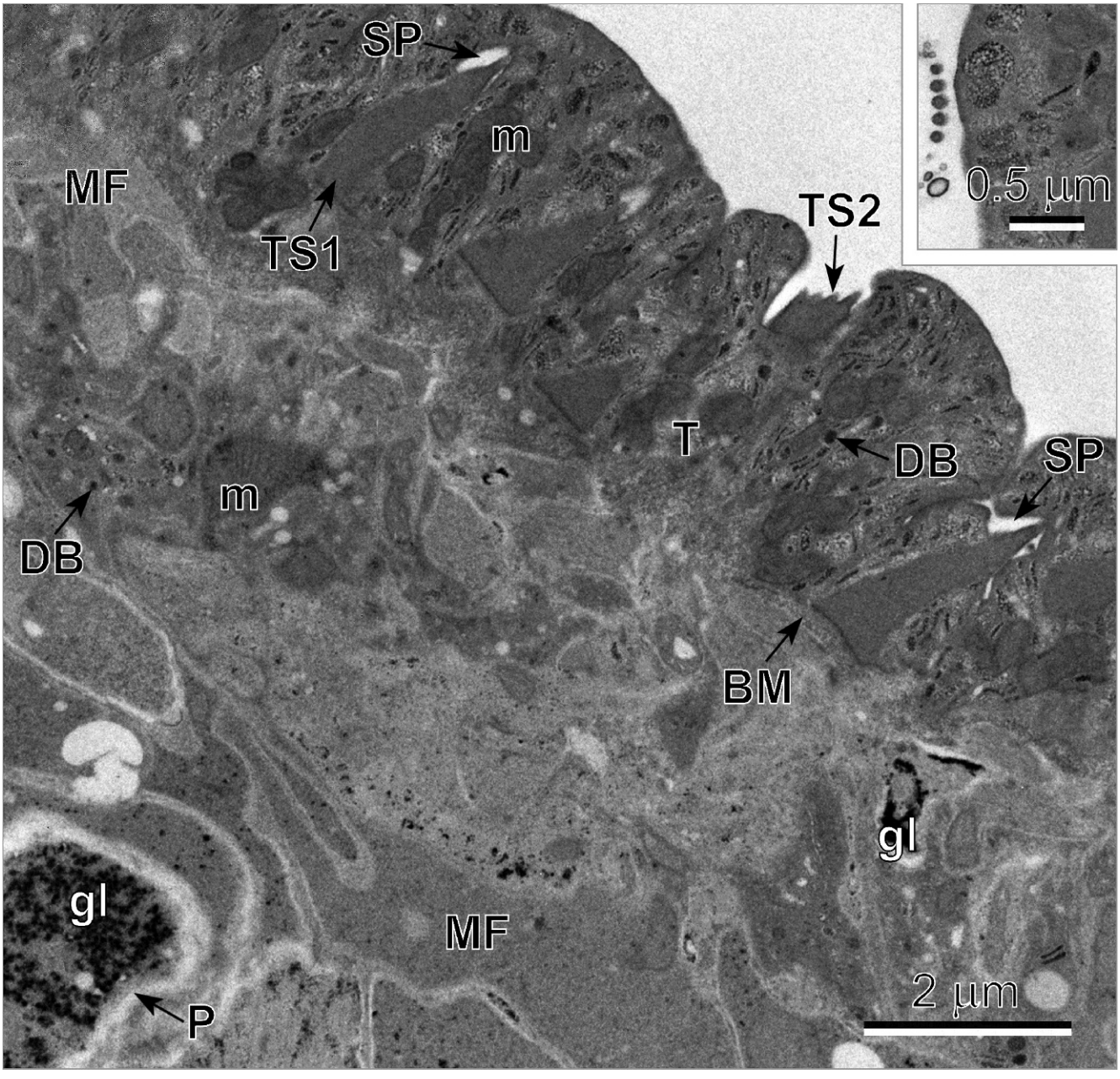

Fig. 2. Low power TEM micrograph showing the general topography of the tegument, basal membrane, several layers of muscle fibres and parenchyma

cytoplasmic connections (Fig. 1). A direct continuity between these perikarya and the outer, cytoplasmic layer of the tegument is seen only rarely in sectioned material, due to the tortuous course of these connections. Both the parenchyma surrounding the perikarya and the cortical musculature contain a high concentration of densely stained glycogen particles and rosettes (Figs 2–5). An extracellular matrix, composed of a basal matrix and an interstitial matrix, occurs between the perikarya and the distal cytoplasm. The interstitial matrix connects the basal matrix to the circular muscle layer beneath, resulting in a slight invagination of the basal matrix (Figs 2 and 5). Mitochondria and a high accumulation of glycogen particles are present in the muscle layers (Fig. 2).

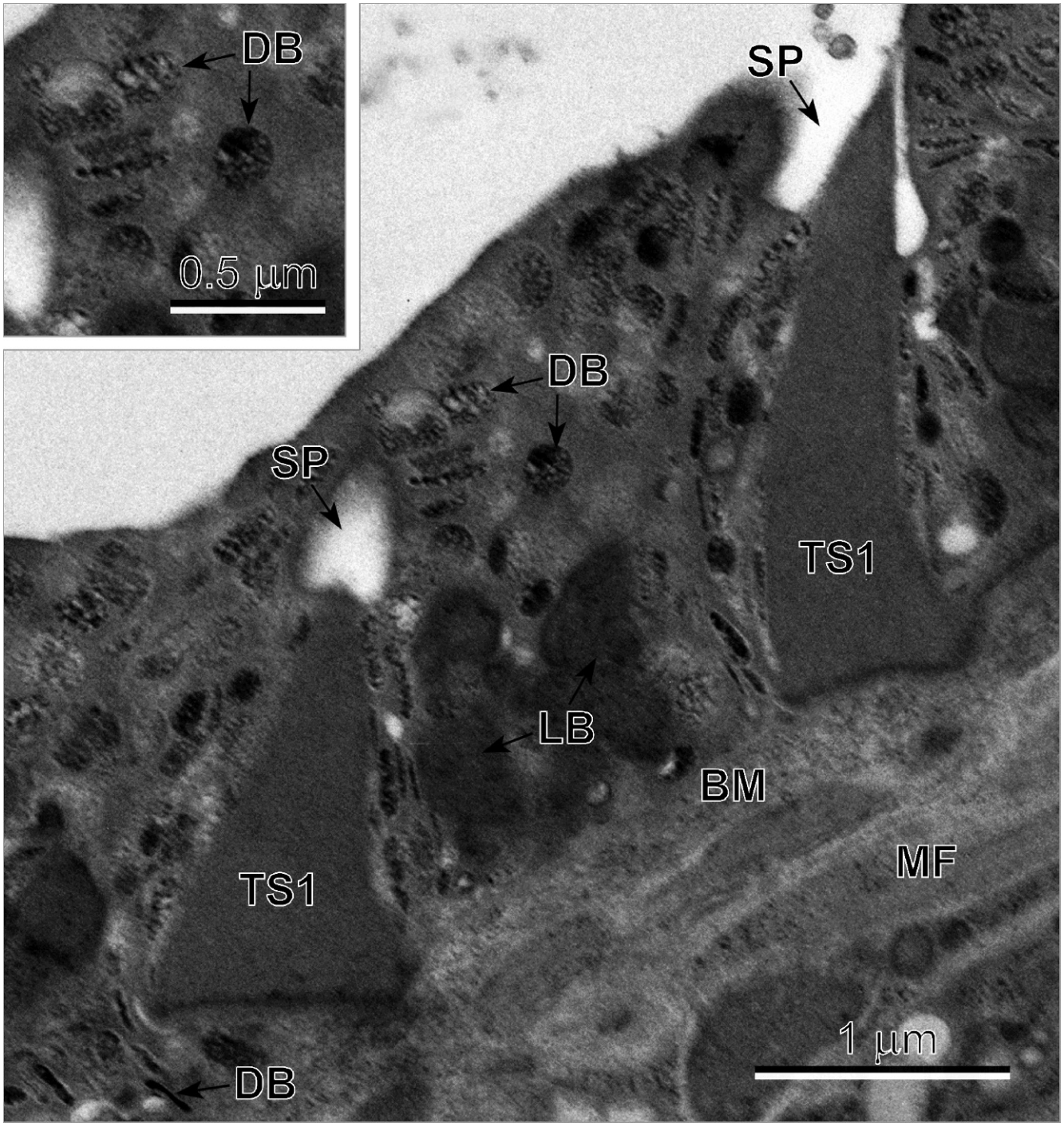

Fig. 3. Higher power TEM micrograph showing details of tegumental spines of type-1 and the outer syncytial cytoplasm with numerous dense granules and several moderately electron-dense membraneous bodies. Inset: Detail of dense granules arranged in a rouleau formation

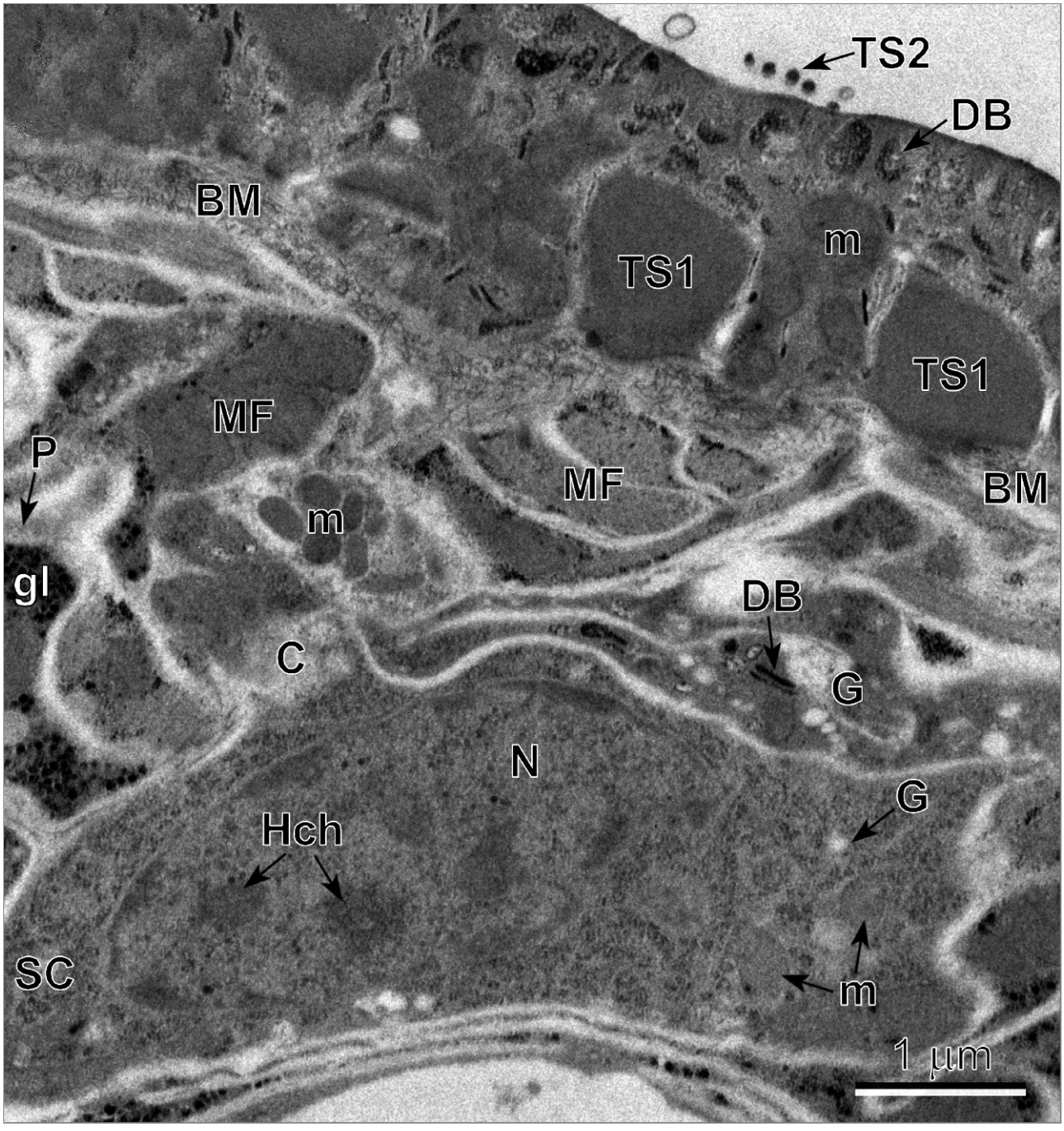

Fig. 4. TEM micrograph illustrating both parts of the tegumentary epithelium: (a) the outer syncytial cytoplasm of the tegument in the upper region of the micrograph; and (b) a subtegumentary perikaryon situated in the lower part of the micrograph and several obliquely sectioned bridges linking it to the syncytial tegument in the middle region. Note a section of the apical part of a type-2 spine, showing its comb-shaped structure with five teeth

Discussion

The general topography and ultrastructural organisation of the tegument in *Maritrema feliui* is essentially similar to that previously described for other digeneans by, *inter alia*, Threadgold (1963, 1968, 1984), Lee (1966), Burton (1966), Świderski (1966), Bogitsh (1968), Hockley (1973), Lumsden (1975), McLaren (1980), Choi and Yoo (1985), Brennan *et al.* (1991), Cohen *et al.* (1996), Ferrer *et al.* (1996) and, more recently, by Filippi *et al.* (2010, 2012, 2013). The nature of the tegumental surface of *M. feliui*, with infolding, pits and spines, inevitably increases the total surface area, which is probably

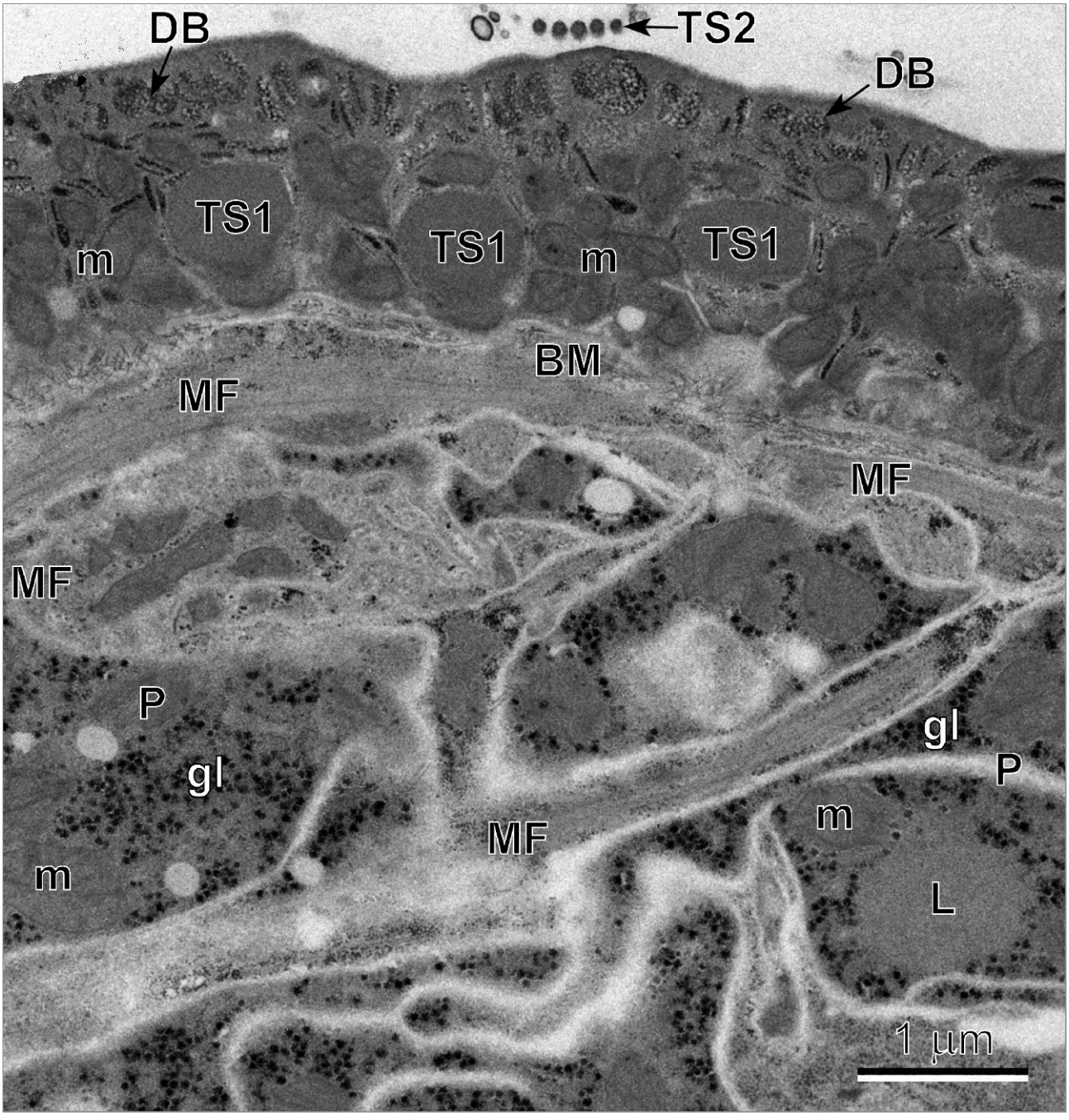

Fig. 5. Oblique section through both the tegument and the outer region of the body wall. Note: (a) the different orientations of the muscle fibres; (b) a heavy accumulation of glycogen in the parenchyma; and (c) a section of the comb-shaped extremity of a type-2 tegumental spine with five teeth at its apex (upper part of micrograph)

important for the inward and outward transmission of nutrients and waste products, respectively. Consequently, with regard to functional ultrastructure, our results appear to support previous hypotheses on the three the most important functions of the tegument, i.e. (1) the protection of the parasite; (2) the absorption and transport of nutrients from the environment; and (3) the evacuation of metabolic waste products. The main functions of the anucleate surface layer of the tegument are: (1) the transmission of nutritive and other resources via an increased surface area resulting from the numerous infoldings of the surface membrane (Lee 1966; Świderski 1966; McLaren 1980; Threadgold 1984); (2) the supplementary role of the two types of tegumental spines in the maintenance of attachment and the locomotion of the parasite in relation to the gut-wall or other substrate (Threadgold 1963, Lee 1966, Lumsden 1975); and (3) a protective role in preventing damage to the

worm by the host's digestive enzymes (McLaren 1980; Smyth and Halton 1983). The characteristic disc-like, granular bodies in the external cytoplasm of the *M. feliui* tegument resemble the 'secretory vesicles' described in *Fasciola hepatica* and other digeneans. These structures are thought to be involved in the renewal of the surface membrane, which represents a protective layer (for a review, see: McLaren 1980; Threadgold 1984).

Two types of tegumental spines were observed in the tegument of *M. feliui.* Differences in spine structure and their distribution may be related to differences in the host-parasite relationship (Filippi *et al.* 2010, 2012, 2013). For example, Køie (1977) has suggested that large, pointed spines may be involved in feeding, with these spines being used for abrading host tissues, although Lumsden (1975) was of the opinion that the main function of spines is to assure and help maintain the parasite's attachment to the host.

In *M. feliui*, the subtegumentary perikarya, as in all studied digeneans (for reviews see: McLaren 1980; Threadgold, 1984), are connected to the distal cytoplasm via cytoplasmic bridges and lie beneath the superficial layers of circular and longitudinal muscles. An extracellular matrix, composed of a basal matrix and an interstitial matrix, as seen in other digenean species (Filippi *et al.* 2010, 2012, 2013) and in other parasitic platyhelminths (Conn 1993), occurs between the perikayra and the distal cytoplasm.

Despite the fact that *M. feliui* is distantly related to the vast majority of digeneans whose fine structure has been examined, the tegument of this species is fundamentally similar to that of the others. Nevertheless, some characteristic differences were observed. These include: (1) the tegumental layer of *M. feliui* forms a relatively thin layer in comparison with that reported for the majority of digeneans (Lumsden 1975; Smyth and Halton 1983; Threadgold 1984); (2) the subtegumental perikarya always have a much flattened shape and contain large flattened nuclei with several heterochromatin islands of moderate electron density; (3) the granular cytoplasm of the subtegumental perikarya is relatively thin, rich in free ribosomes and contains a few Golgi complexes, short profiles of GER, several mitochondria and dense bodies; and (4) the latter dense bodies occur much more frequently in the long, tortuous cytoplasmic bridges between the perikarya and the syncytial surface layer, where they accumulate in a much greater concentration.

Although the tegument of different adult trematode species has long remained a topic of interest for numerous SEM and TEM studies and this layer is fundamentally similar in the taxa studied, our results show that some differences, even at the interspecific level, are likely to occur. A good example of this is the shape of the body spines, which are too small to be seen in detail with the light microscope. Thus ultrastructural characteristic of the tegument may provide taxonomically informative criteria that are otherwise not available.

In view of the fact that the digenean tegument represents the parasite's main interface with its host, it will likely prove worthwhile for this feature to be studied in as wide a variety of taxa as possible. Indeed, as indicated by Mehlhorn *et al.* (1981), Moczoń and Świderski (1983, 1992) and Shaw and Erasmus (1988), a better knowledge of the functional biology of the body surface of the digeneans is likely to have applied importance, especially for species of medical and veterinary significance. In fact, the tegument of digeneans, such as schistosomes, due to its very high metabolic activity, represents a very sensitive target for examining the effects of anthelmintic drugs upon the parasite. It is also worth noting that, according to the recent studies on the concept of vaccination, the most efficacious schistosome vaccines are directed against tegumental structures (Schulte *et al.* 2013), which opens up a new way of looking at the tegument.

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