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FISH PARASITOLOGY - ORIGINAL PAPER

# Unique ultrastructural characteristics of the tegument of the digenean blood fluke *Aporocotyle simplex* Odhner, 1900 (Digenea: Aporocotylidae), a parasite of flatfishes

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**Abstract**

This paper includes the first transmission electron microscopical (TEM) study of the tegument of a member of the basal digenean family Aporocotylidae. Scanning electron microscopical investigations of the fish blood fluke *Aporocotyle simplex* show that each boss on the lateral body surface bears 12–15 simple, uniform spines which extend from 0.5–2.7 μm above the surface of the boss. TEM observations revealed that these spines reach deep beneath the distal cytoplasm of the tegument for much of their length (9–12 μm) and are surrounded by a complex of diagonal muscles in each boss. This is the first record of any digenean with so-called 'sunken' spines. The results suggest that aporocotylid spines arise from within the sarcoplasm of the boss diagonal muscles. The sunken cell bodies (perikarya) of the tegument are connected to the distal cytoplasm via ducts (specialised processes lined by microtubules); this in contrast to other digeneans studied, where they are connected via non-specialised cytoplasmic processes. Within the distal cytoplasm, the tegumental ducts of *A. simplex* are surrounded by invaginations of the basal membrane and release their cytoplasmic inclusions into the distal cytoplasm. These apparently unique morphological features of the tegument, especially the deep origin of the spines, may represent useful characteristics for understanding aporocotylid relationships, especially in view of the known variation in the spine patterns of aporocotylids.

**Keywords** Basal group · Ultrastructure · *Aporocotyle* · Sunken spines · Tegument

## Introduction

The superfamily Schistosomatoidea Stiles & Hassall, 1898 is regarded as one of the most basal digenean groups (Olson et al. 2003; Cribb et al. 2017). It includes three families, one of which is a group of fish blood flukes, the Aporocotylidae Odhner, 1912 (syn. Sanguinicolidae von Graff, 1907), containing 38 accepted genera (WoRMS 2019). Aporocotylids comprise a collection of somewhat bizarre digeneans that usually lack functional attachment organs (suckers) and infect the heart, branchial vessels, mesenteric vessels, and other parts of the vascular system of both marine and freshwater fishes (Smith 2002; Bullard and Overstreet 2008; Orélis-Ribeiro et al. 2014). They represent an ancient and taxonomically diverse group and one of the few containing members that have adults, eggs, and miracidia all capable of harming the definitive fish host (Bullard and Overstreet 2008; Orélis-Ribeiro et al. 2014). Some marine aporocotylids, including *Aporocotyle simplex* Odhner, 1900, a parasite of the heart and branchial vessels of flatfishes in northern North Atlantic waters (Thulin 1980a; Gibson 1996), are extremely unusual for digeneans in that their asexual generations develop in a polychaete rather than a mollusc (Køie 1982; Cribb et al. 2017).

Only three transmission electron microscopical (TEM) studies have previously been published on the fine morphology of aporocotylid species. The ultrastructure of the female reproductive ducts was studied in *Aporocotyle simplex* by Thulin (1982), the eggs of the freshwater species *Sanguinicola inermis* Plehn, 1905 by McMichael-Phillips

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et al. (1992), and the digestive system in the cercaria of the same species by McMichael-Phillips et al. (1994). Scanning electron microscopical (SEM) studies have also been published on the surface topography of rediae and cercariae of *A. simplex* by Køie (1982) and its adult by Thulin (1980b). On the basis of light microscopical (LM) taxonomic studies on different aporocotylid species, it has been possible to evaluate the significance of diversity in the morphology, nature, and location of the tegumental spines found in this group (Bullard and Overstreet 2008; Bullard et al. 2008; Orélis-Ribeiro et al. 2013; Ruiz et al. 2013). Hence, in some taxonomic papers on aporocotylids, only a limited number of authors have included SEM views of spines and other surface features (e.g. Nolan and Cribb 2004, 2006; Bullard et al. 2008, 2012; Orélis-Ribeiro et al. 2013). This is unfortunate because these characteristics may be useful for an understanding of phylogenetic interrelationships, both within the family Aporocotylidae and between it and other digenean groups (Orélis-Ribeiro et al. 2013; Cribb et al. 2017).

The main topographical SEM characteristics of adult *A. simplex* have already been reported by Thulin (1980b). In the present study, we draw attention to additional, hitherto undescribed, characteristics of the spines which may be unique to aporocotylid blood flukes. This study represents the first TEM observations on the ultrastructure of the tegumental spines and cytoarchitecture of an aporocotylid, with the aim of adding diagnostic characteristics for the members of the genus *Aporocotyle* Odhner, 1900 and searching for useful phylogenetic traits among the three basally distinct lineages (Cribb et al. 2017) of the Aporocotylidae. It also forms part of a series of publications on the ultrastructure of basal neodermatans, representing a search for phylogenetically useful characteristics which may contribute to a better understanding of their phylogenetic position and origin (e.g. Poddubnaya et al. 2015, 2017; Justine and Poddubnaya 2018).

## Materials and methods

Twenty-three specimens of *Aporocotyle simplex* were obtained from the branchial arteries of the long rough dab (American plaice) *Hippoglossoides platessoides* (Fabricius, 1780) (Pleuronectidae) trawled from the Norwegian Sea off Tromsø, Norway, during June 2017. For electron microscopy, live specimens of *A. simplex* were fixed using 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 10 days at 5 °C, rinsed 4 times for 20 min periods in the same buffer and postfixed in 1% osmium tetroxide for 1 h. For SEM observations, 12 fixed specimens were dehydrated in a graded ethanol series, with a final change to absolute acetone and then critical-point-dried. Later, the specimens were mounted on stubs, sputter-coated with gold-palladium, and examined using a JEOL-JSM-6510LV microscope operating at 30 kV. For TEM observations, after fixation and dehydration as above, 11 specimens were embedded in a mixture of Araldite and Epon. Ultrathin sections were then stained with uranyl acetate and lead citrate, and examined using a JEM 1011 microscope operating at 80 kV.

## Results

### SEM observations of the body surface

The body of *Aporocotyle simplex* is dorso-ventrally flattened and fusiform in shape (Fig. 1a). Around the ventrally subterminal mouth, there is a collection of small, irregularly localized spines (Fig. 1c). Commencing a short distance (~ 90 μm) from the apical extremity, two fields of small bosses are also present laterally and sublaterally throughout the length of the body (Fig. 1a). These bosses are shallow, irregular, closely packed (~ 14 μm apart) and bear 12–25 spines in each boss (Fig. 1e, g). The bosses are ~ 7.0 × 12 μm in size and distributed irregularly in uniformly spaced rows (Fig. 1a, e); their number decreases gradually from the anterior to the posterior end of the body. The distance between the spines on each boss is 1.2–2.0 μm (Fig. 1b–g). The exposed region of the spines is simple and can reach up to 0.7 μm above the surface of the bosses (Fig. 1b, g); moreover, the tips of bifurcate spines (see below) can be observed on the boss (Fig. 1b). Uniciliate sensory endings are also visible on each boss (Fig. 1g). In the lateral-most regions close to the posterior extremity of the body, there can be seen bosses containing large spines reaching up to 2.7 μm above the surface (Fig. 1f, h). The surface layer of each spine appears to be a continuation of the whole body surface of the worms, which is covered with shallow, knob-like outgrowths similar to those observed on the surface of the bosses (Fig.1b, h). The greatest density of knob-like surface outgrowths is found in the anterior region of the worm (Fig. 1b, c). Medial regions of the ventral and dorsal body surfaces of the body are aspinous but bear scattered, uniciliate sensory endings with short (~ 1.3 μm) or long (~ 2.7 μm) cilia (Fig. 1a, d).

### TEM morphology of the tegumental spines

The tegumental spines are uniform in shape, simple, straight, and only the very tip protrudes through the tegument in these observed specimens (Figs. 2a–h and 4). They have a lanceolate shape, with the distal extremity tapering to a sharp point (Fig. 2b–h). Internally, they are electron-dense and exhibit the typical linear patterned crystalline structure of all digenean spines (Fig. 2e). Very infrequently, spines possessing a bifurcate distal extremity are visible (Fig. 2i). Much of the length of each spine occurs very deep beneath the level of the distal cytoplasm (Figs. 2c–h and 4). In sections, the visible length

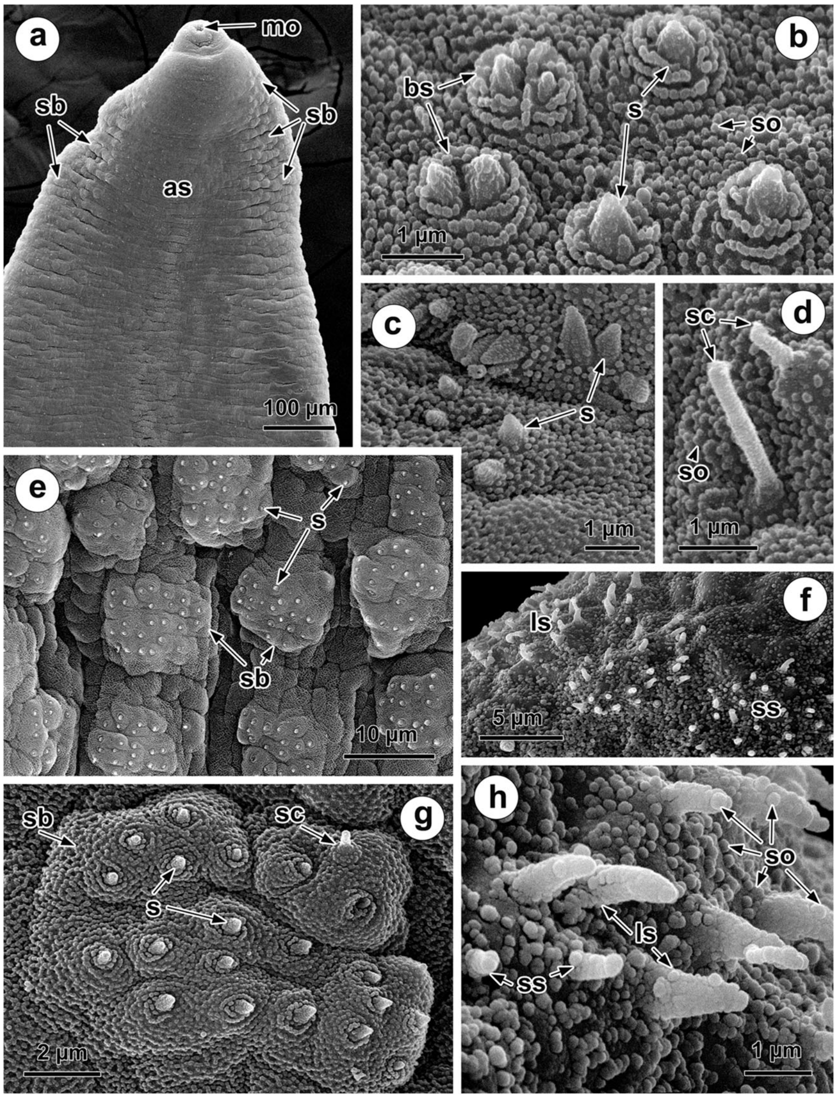

**Fig. 1** SEM observations of the body surface. (a) Ventral view of the anterior body surface, note the subterminal mouth and ventro-lateral spine bosses on both sides of the body. (b) Close-up of the spines visible arising from bosses on the anterior body, note both simple and bifurcate spines. (c) Irregularly localized spines around the mouth. (d) Uniciliate sensory endings on the ventro-medial body surface. (e) Ventro-lateral bosses bearing spines. (f) Bosses on the lateral region of the body close to the posterior extremity, note the large spines. (g) Single boss showing both spines and a uniciliate sensory ending. (h) Close-up showing both large and small spines on a boss near the posterior extremity of the body. Abbreviations: *as* region of body surface without bosses or spines, *bs* bifurcate spine, *ls* large spine, *mo* mouth, *s* simple spine, *sb* spine boss, *sc* sensory cilium, *so* knob-like surface outgrowths, *ss* small spine

of the body spines ranges from 9 to 12 μm, with a diameter of 0.7–1.3 μm (Fig. 2c, h), and the spine tips protruding from the body are ~ 0.5–0.7 μm in length (Fig. 2b, g). The distal region of the spine projecting above the tegument is covered by the surface plasma membrane of the syncytial tegumental cytoplasm (Figs. 2b, g and 4). At the point where the spine passes through the lower region of the distal cytoplasm, it is surrounded by invaginations of the basal membrane and underlying extracellular basal matrix (Figs. 2b, g and 4).

In each tegumental spine boss, a well-developed network of diagonal muscle bundles is associated with each spine (Figs. 2c–h and 4). There is at least one muscle cell forming the muscle complex of each boss (Figs. 2d and 4). The spines are enveloped by muscle bundles, and the base of the spines merges with the prolongations of the dense filamentous material present within the sarcoplasm (Fig. 2k). The hemidesmosomes are located at the tapering ends of the muscle fibres and spine bases (Fig. 2j, k).

## TEM features of the tegument

The body surface is bounded by the tegumental syncytial layer of anuclear cytoplasm (also referred to as the distal cytoplasm) limited by surface and basal membranes (Figs. 3a–g and 4). Numerous invaginations of the basal membrane extend into the basal regions of this cytoplasm in the form of thin folds (Figs. 3f and 4). A layer of extracellular basal matrix separates the tegument from the underlying muscle layers (both circular and longitudinal) and sunken tegumental perikarya (Figs. 3a–g and 4). The distal cytoplasm of the anterior, middle, and posterior regions of the body is ~ 1.2–2.0 μm in thickness (Fig. 3a–g). Its surface bears regular protuberances, referred to here as 'knob-like outgrowths' on the basis of SEM observation (Figs. 1b–h, 3b, f, and 4). These protuberances are ~ 0.1–0.3 μm in length and ~ 0.1 μm in diameter at their base (Fig. 3b, f). A moderately dense fibrous zone (~ 0.2–

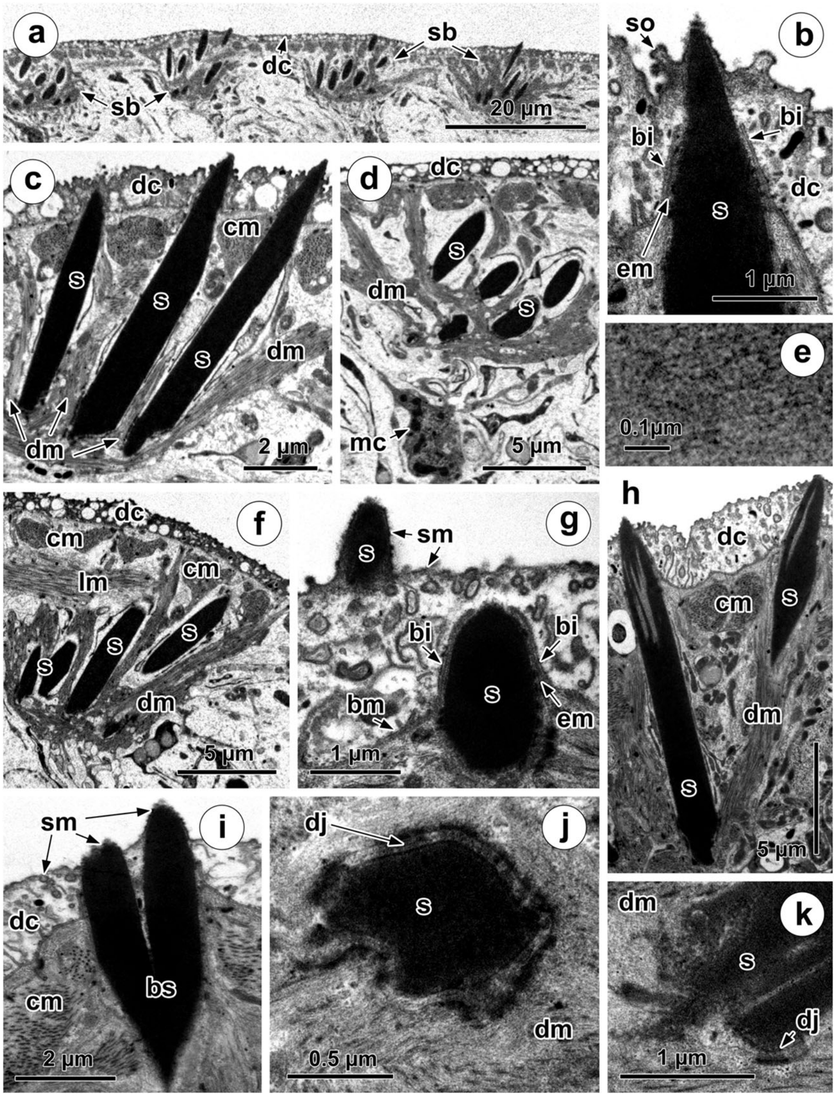

**Fig. 2** TEM observations of the tegumental spines in the bosses. (a) Section through four spine bosses. (b) Distal region of a spine within the distal tegumental cytoplasm, note the protrusion of a spine tip covered with surface plasma membrane and the presence of invaginations of the basal membrane on either side. (c), (f), (h) Longitudinal sections of the spines within a boss, note the network of diagonal muscles. (d) Muscle cell associated with the musculature of the boss. (e) Details of the spine body contents; (g) Distal regions of two spines, note one spine surrounded by invaginations of the basal membrane of the distal cytoplasm and the protrusion of another spine above the surface tegument. (i) Bifurcate spine. (j), (k) Spine base within the sarcoplasm of the diagonal muscles, note the desmosomal junctions. Abbreviations: *bi* basal invagination, *bm* basal membrane, *bs* bifurcate spine, *cm* circular muscles, *dc* distal tegumental cytoplasm, *dj* desmosomal junction between sarcoplasmic membrane and spine surface, *dm* diagonal muscles, *em* extracellular basal matrix, *lm* longitudinal muscles, *mc* muscle cell, *s* simple spine, *sb* spine boss, *sm* surface membrane, *so* knob-like surface outgrowths

0.3 µm in thickness) is localized immediately beneath the surface plasma membrane (Figs. 3b, d, and 4). The remaining distal cytoplasmic matrix is electron-lucent in appearance (Fig. 3b, d). Three types of inclusions are recognizable within the distal cytoplasm throughout the whole length of the body; these are electron-dense bodies and two kinds of vesicular inclusions (Figs. 3b–g and 4). Vesicles of the first type (v1) are rounded (~ 0.1 µm in diameter) or oval (~ 0.1–0.3 µm in their longest axis) with a slightly fibrous content and are limited by an electron-dense wall (Figs. 3b–f and 4). Some sections clearly indicate that adjacent vesicles connect to form channels (Figs. 3d, f and 4). Such channels, and separate vesicles, occur in close proximity to the surface membrane with which they unite, resulting in the release of their contents onto the outer surface of the worm (Figs. 3b, d, f and 4). Vesicular inclusions of the second type (v2) are electron-lucent, larger (0.4–0.9 µm in diameter) and rounded in shape (Figs. 3a, g and 4). The electron-dense bodies are rod-shaped or oval, homogeneous, and range in size from 0.05–0.1 µm in diameter and to 0.2–0.5 µm in their longest axis (Figs. 3a–g and 4).

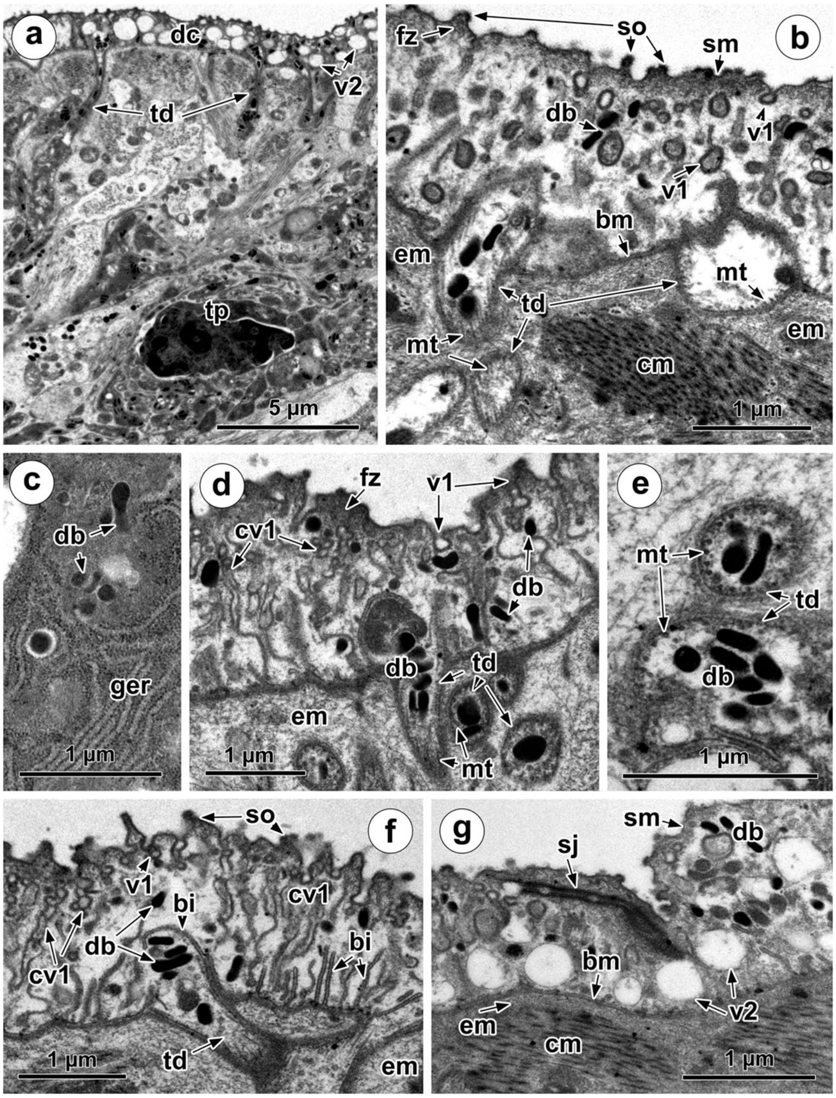

**Fig. 3** TEM observations of the tegument. (a) View of the surface layer of the body, note the sunken perikaryon. (b) Section through the distal cytoplasm, note two microtubule-lined ducts in the basal region and two types of inclusions (dense bodies and v1 vesicles). (c) Perinuclear cytoplasm of a tegumental perikaryon. (d), (f) Distal cytoplasm in the middle of the body showing vesicles (v1) connected to form channels. (e) Cross-section of the tegumental ducts, note their peripheral microtubules. (g) Section through the distal cytoplasm in the anterior region of the body, note the large electron-lucent vesicles (v2) and septate junction. Abbreviations: *bi* basal invagination of basal membrane, *bm* basal membrane, *cm* circular muscles, cv1 connecting v1 vesicles, *db* dense bodies, *dc* distal cytoplasm, *em* extracellular basal matrix, *fz* fibrous zone, *ger* granular endoplasmic reticulum, *mt* peripheral microtubules, *sj* septate junction, *sm* surface membrane, *so* knob-like surface outgrowths, *td* tegumental duct, *tp* tegumental perikaryon, *v1* first type of vesicle, *v2* second type of vesicle

Tegumental perikarya are located deep beneath the subtegumentary muscle layers (Figs. 3a and 4). Each perikaryon is irregular in shape with an irregularly shaped nucleus (Fig. 3a). Their perinuclear cytoplasm is filled with granular endoplasmic reticulum and both dense bodies and vesicles (Figs. 3a, c and 4). These perikarya give rise to cytoplasmic processes; the distal portion of which form specialised ducts through which the cytoplasmic inclusions are conveyed to the distal cytoplasm (Figs. 3a–f and 4). The membranous walls of each duct are lined by a ring of peripheral microtubules (Figs. 3b–e and 4). Close to the distal cytoplasm, the tegumental ducts are surrounded by prolongations of the basal membrane with a dense, fibrous content (Figs. 3b–f and 4). These ducts perforate the distal cytoplasm of the tegument, where they are surrounded by invaginations of the basal membrane and release their cytoplasmic inclusions into the distal cytoplasm (Figs. 2 and 4b–f). No special junctions were observed between the distal cytoplasmic layer and the ducts (Figs. 3b and 4). Occasionally, in the anterior region of the body, septate junctions can be seen to penetrate the full width of the distal cytoplasm (Fig. 3g).

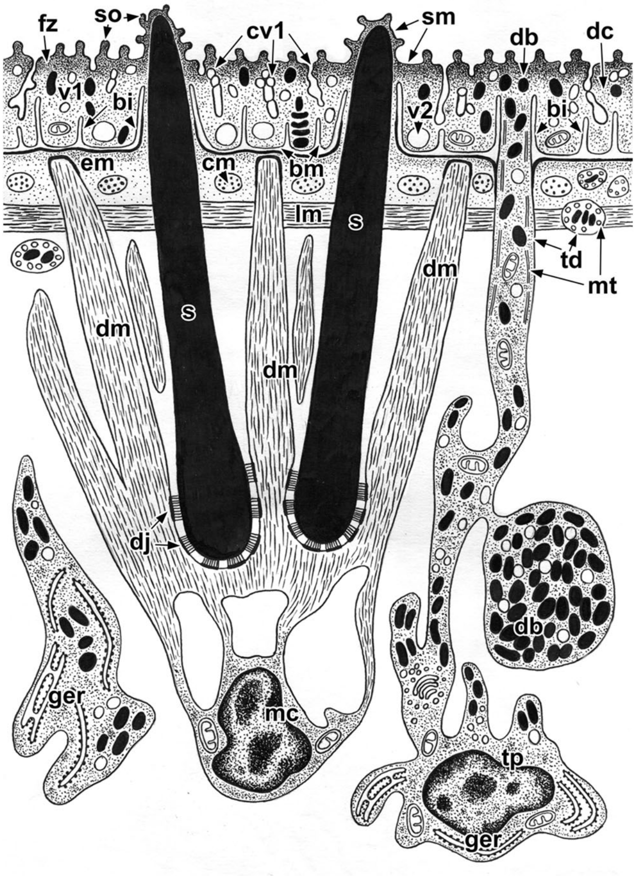

**Fig. 4** Diagrammatic illustration of the tegumental structure and spines of a boss. Abbreviations: *bi* basal invagination, *bm* basal membrane, *cm* circular muscles, cv1 connecting v1 vesicles, *db* dense bodies, *dc* distal tegumental cytoplasm, *dj* desmosomal junction between sarcoplasmic membrane and spine surface, *dm* diagonal muscles, *em* extracellular basal matrix, *fz* fibrous zone, *ger* granular endoplasmic reticulum, *lm* longitudinal muscles, *mc* muscle cell, *mt* peripheral microtubules, *s* simple spine, *sm* surface membrane, *so* knob-like surface outgrowths, td tegumental duct, tp tegumental perikaryon, *v1* first type of vesicle, *v2* second type of vesicle

## Discussion

### Body spines

A characteristic topographical feature of the body surface of *Aporocotyle simplex* is the presence of tegumental spines which are not distributed in equally-spaced, ventro-lateral, transverse rows but occur within spine bosses present on the ventro-lateral surface of the body (Thulin 1980b; Bullard and Overstreet 2008; Present study). In this study, the ultrastructure of the tegument of an adult aporocotylid is described for the first time using transmission electron microsopy (TEM). Our investigation revealed two distinctive and unique ultrastructural features. Firstly, in contrast to other digeneans studied to date, spines of *A. simplex* have a characteristic position deep beneath the distal cytoplasm of the tegument for much of their length and are surrounded by diagonal muscles of the boss within which they occur. In most TEM observations of digenean spines, spines occur within the distal syncytial layer of the tegument, are anchored to the basal plasma membrane and underlying extracellular basal matrix, and are covered apically by the surface plasma membrane (Clegg 1972; Erasmus 1967, 1970; Hockley 1973; Fujino et al. 1979; Køie 1982; Fukuda et al. 1987; Žd'arská et al. 1990; Sharma et al. 1996; Podvyaznaya 1999; Scholz et al. 1991; Cohen et al. 2001; Jamjoon and Shalaby 2006; Conn et al. 2008; Poddubnaya et al. 2010; Filippi et al. 2013; Świderski et al. 2013; Antonelli et al. 2014). The abovementioned publications show that the shape, size, and distribution of the spines over the worm surface varies considerably, and their pattern is often used as a taxonomic feature of the different digenean groups. No similar subtegumentary spine position, which occurs in *A. simplex*, appears to have been reported for any other digenean which has been studied.

Aporocotylids are said to have a variety of tegumental spines (Bullard and Overstreet 2008); however, the ultrastructural features of these spines have remained undocumented. The vast majority of ultrastructural studies on blood flukes have been on members of the family Schistosomatidae, especially the species of *Schistosoma* Weinland, 1858. In specimens of these taxa, the body of the spines has been described as enclosed within the distal cytoplasm and arising from the basal membrane, with only their pointed tip projecting beyond the tegument; but, as in *A. simplex*, there are invaginations of the basal membrane around the lower half of the spine (Hockley 1973; Sobhon et al. 1984; Abbas and Cain 1987). However, in a more recent paper by Gobert et al. (2003) on the ultrastructural architecture of adult *Schistosoma japonicum* Katsurada, 1904, there is an indication that the spines of female worms originate below the first (circular) tegumental muscle layer, unlike those of the gynaecophoric canal of the male; the spines of which are located above the basal lamina. Unfortunately, Gobert et al. (2003) did not discuss the different genesis of the spines in female *S. japonicum*. Based on the structural characteristics observed, Hockley (1973) assumed that *Schistosoma* spines are movable within the tegument, rather than flexible, and that the worm is capable of changing its position within the blood vessel of its host without causing extensive damage to the endothelial cells. In view of the complex musculature surrounding the spines of *A. simplex*, this assumption also seems likely for aporocotylid spines; i.e. the well-developed network of diagonal muscle bundles surrounding these spines is likely correlated with the protrusion and retraction of the spines, not only within each boss but also in different bosses, as the associated musculature in each boss is independent. Our SEM observation revealed variation in the degree of the spine protrusion within each boss and a different level of protrusion by spines of different bosses.

The crystalline structure of the spines is a characteristic of those in the majority of species of the Digenea (see Cohen et al. 2001). According to Fukuda et al. (1987) and Sharma et al. (1996), the crystalline nature of spines and their presence in the distal cytoplasm suggest an origin in the tegumental matrix. Nevertheless, with the aid of the direct fluorescence of FITC-phalloidin (fluorescein isothiocyanate) and indirect immunofluorescence using monoclonal anti-actin antibody (MAA) staining, hexagonally packed actin filaments have been shown to occur in the spines of both schistosomatid and fasciolid digeneans (Cohen et al. 1982; Abbas and Cain 1987; Stitt et al. 1992). It is worth noting that actin in a filamentous form is associated with the actin filaments of flatworm smooth muscles (Reger 1976; Stitt et al. 1992; Sulbarán et al. 2015; Grano-Maldonado et al. 2018). Our ultrastructural investigation of the spines of *A. simplex* is a morphological confirmation of studies on the immunocytochemistry and immunofluorescence of digenean spines, suggesting that aporocotylid spines may be derivatives of the filamentous, actin-like material in the sarcoplasm of the diagonal muscles of the bosses.

Our SEM observations revealed that around the mouth of adult *A. simplex*, there is a collection of small, irregularly arranged spines; further posteriorly, surface bosses bearing spines are distributed along the lateral and sublateral surface of the body. In an SEM study of the cercaria of *A. simplex* by Køie (1982), she showed that the anterior extremity of the worm (which she termed the 'cephalic organ') is surrounded by spines (termed 'cephalic spines') arranged in 6–9 rings. Identical spines are scattered on the anterior half of the cercarial body dorsally but almost to the posterior end of the body ventrally. She noted the presence of unchanged cephalic spines in immature specimens of *A. simplex* from the gill arteries of the flatfish host and the appearance of small bosses on the lateral margins of the body. In contrast to our SEM results, prior to Køie's (1982) study, Thulin (1980b) indicated the presence of six rings of small perforations surrounding the anterior extremity in small, possibly immature, specimens of *A. simplex* obtained from flatfish gill arteries; this suggests the recent loss of cercarial cephalic spines. According to Conn et al. (2008), tegumental structures of larval trematodes play a role in migration and site selection, and subsequently these structures regress following the location of the worm within a new site.

## Characteristics of the tegumental structure

The entire body surface of *A. simplex* is covered with regularly distributed, uniform, knob-like outgrowths (SEM) or surface protuberances (TEM), including the surface of both the bosses and the spine extremities. Furthermore, in *A. simplex*, a dense fibrous layer (termed the terminal web) occurs beneath the surface membrane of the distal cytoplasm. Another feature worthy of comment is the presence of rare septate junctions, which we observed in the distal cytoplasm of the anterior body region of *A. simplex*. According to Rieger et al. (1991), the existence of such a fibrous layer and the presence of epithelial intercellular junctions are characteristics of the basic organization of platyhelminths.

The TEM investigation of the tegument revealed a very unusual ultrastructural trait in *A. simplex*, which differs from the previously reported organization of the tegument in digeneans and, indeed, all parasitic flatworms. In *A. simplex*, the sunken cell bodies (perikarya) are connected to the distal cytoplasm via ducts (lined by microtubules), in contrast to other parasitic flatworms which have been studied, where these cell bodies are connected to the distal cytoplasm via cytoplasmic processes. As a rule in parasitic flatworms, ducts are the specialised processes of unicellular secretory glands, which are strengthened by longitudinal microtubules, pass through the distal cytoplasm, and discharge their granules via small pores (in an eccrine-like manner), each duct being attached to the distal cytoplasm by a ring-like septate junction (McCullogh and Fairweather 1989). However, in *A. simplex*, the tegumental cell ducts within the distal cytoplasm are surrounded by invaginations of the basal membrane, release their cytoplasmic inclusions into the distal cytoplasm, and do not penetrate the surface membrane to open to the exterior of the worm. There appear to be no previous descriptions of parasitic platyhelminths using specialised ducts as a connection between the sunken tegumental cell bodies and the distal cytoplasm. Taking into account the fact that the present investigation is the first TEM description of the tegumental structure of an aporocotylid, it is important to clarify whether this feature is a characteristic of the tegumental cytoarchitecture of all aporocotylids or of species of *Aporocotyle* only?

The distal cytoplasm of the tegument of adult *A. simplex* contains three distinct types of inclusions, i.e. membrane-bound electron-dense bodies, electron-lucent vesicles limited by a dense wall, and large, electron-lucent vesicles. However, the most remarkable tegumental feature is the fact that the vesicles with a dense wall become connected to each other to form channels; these, in turn, fuse with the surface membrane of the tegument. It is worth noting that the same three types of inclusions and the connecting channels have also been observed in the distal cytoplasm of the tegument of another blood parasite, *Schistosoma mekongi* Voge, Bruckner & Bruce, 1978, in a detailed ultrastructural work by Sobhon et al. (1984). To our knowledge, such channels have not been described in other digeneans. According to Pappas and Read (1975), the transport of cytoplasmic bodies within the tegument to the surface plasma membrane has been reported for many platyhelminths, and their production is an active process induced by specific physiological and biochemical triggers in different regions of the body. In some digeneans, there are two types of the tegumental inclusions, both produced by the same cells (Morris and Threadgold 1968; Cohen et al. 2001; Świderski et al. 2013; Filippi et al. 2013; Antonelli et al. 2014) or two types of inclusions produced in different cells (Threadgold 1967; Sharma et al. 1996). However, as in the present case, in some digeneans, three types of tegumental inclusions have been demonstrated (Sobhon et al. 1984; Abdul-Salam and Sreelatha 1992; Present study).

## Phylogenetic remarks

A recent phylogenetic analysis of the 28S rDNA region by Cribb et al. (2017) has provided evidence for the recognition of three basally distinct lineages of aporocotylids: (1) chondrichthyan-infecting species with bivalves as intermediate hosts, (2) freshwater teleost-infecting species with gastropods as intermediate hosts, and (3) marine teleost-infecting species with polychaetes as intermediate hosts. The known life-history data for *A. simplex* (see Køie 1982) clearly show that it fits into the third lineage. The first TEM results on the tegumental structure of an aporocotylid presented here show the presence of unique morphological characters, such as the position and origin of the tegumental spines. Considering the facts that neodermal sclerites are known to constitute useful characteristics for phylogenetic analyses and that aporocotylids are known to have huge variations in spine patterns, ultrastructural investigations in species of all three distinct lineages of aporocotylids may serve as a morphological basis for an understanding of their phylogeny and relationships. However, little in the way of analysis can currently be carried out, since of three families of schistosomatoids, only the schistostomatids, one aporocotylid (Present study), and no spirochiids have been studied.

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**Compliance with ethical standards** This study was conducted in compliance with all institutional, national, and international guidelines on the care and use of animals.

**Conflict of interest** The authors declare that they have no conflict of interest.

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