Parasitology International 59 (2010) 539–548

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Functional ultrastructure of the hexacanth larvae in the bothriocephalidean cestode *Eubothrium salvelini* (Schrank, 1790) and its phylogenetic implications

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ARTICLE INFO

Article history:
Received 22 April 2010
Received in revised form 6 July 2010
Accepted 7 July 2010
Available online 14 July 2010

Keywords:
Eubothrium salvelini
Bothriocephalidea
Cestoda
TEM
Functional ultrastructure
Cell
Oncosphere

ABSTRACT

Functional ultrastructure and its phylogenic implications in the bothriocephalid cestode *Eubothrium salvelini* (Schrank, 1790) are described and discussed. The infective hexacanth shows bilateral symmetry in cellular organization. The mature hexacanth is armed with three pairs of oncospheral hooks of a heterogeneous electron density. It is covered by a thin layer of the oncospheral tegument, possessing characteristic bubble-like processes at the surface. Within the infective hexacanth larva five cell types were distinguished: (1) a binucleated subtegumental cell; (2) the U-shaped, tetranucleated penetration gland; (3) two nerve cells; (4) three types of somatic cells represented by: i) myocytons of both somatic and hook musculature, ii) numerous degenerating micromeres with pycnotic nuclei and iii) a new oncospheral cell type, the interstitial cell, that has never been observed in any other hexacanth; and (5) large germinative cells with characteristic prominent nucleoli in their large spherical nuclei. Functions of all the cell types are described on the basis of the obtained ultrastructural characteristics and previously published reports. The mode of the penetration gland secretion is classified as apocrine. Flame cells have never been observed within the hexacanth of *E. salvelini*. The results of the present study, comparing the functional aspects of the ultrastructure of the hexacanths of *E. salvelini* with literature data on the oncospheres of other bothriocephallideans and diphyllobothriideans, suggest potential phylogenetic and evolutionary criteria for determining relationships among these groups of tapeworms.

1. Introduction

The cestode *Eubothrium salvelini* (Schrank, 1790) (Bothriocephalidea: Triaenophoridae) is a widely distributed parasite of salmonid fishes [1–3]. Adult *E. salvelini* inhabits the intestine and pyloric caeca of the definitive host [4]. Infection by this parasite may have negative effects on the growth and fitness of the fish host [5,6]. Moreover, heavy infestations of *E. salvelini* cause reduced growth in trout and salmon, but rarely death.

Although numerous studies have been published on the oncospheres in various cyclophyllideans, very little information is available on cellular organization and ultrastructure of infective oncospheres of the diphyllobothriideans and bothriocephalideans [7]. Our results on egg envelopes of *E. salvelini* [8] and literature data [7,9–13] seem to indicate that the oncosphere of this species differs ultrastructurally from those in other members of the Diphyllobothriidea and Bothriocephalidea. New ultrastructural data on tapeworm oncospheres have the potential to be useful criteria in phylogenetic analysis of "lower cestodes". As shown in the oncospheres of other species [14–16] the ontogenetic characters may indicate phylogenetic relations. In addition, ultrastructural analyses of cestode larvae are essential to our understanding of cestode biology, parasite and host interactions and finally prevention as was demonstrated most recently by Jabbar et al. [17,18].

The purpose of the present study is to describe the ultrastructural aspects of cellular organization of the hexacanth of *E. salvelini* (Schrank, 1790) and compare them with literature data on the hexacanths of other lower cestodes to suggest potential phylogenic implications.

2. Materials and methods

Adult *E. salvelini* (Schrank, 1790) were collected from the intestine of *Oncorhynchus mykiss* (Pisces: Salmonidae) from Loch Awe (Scotland, UK). Specimens were placed in cold fresh water to stimulate release of eggs from gravid proglottids. Free eggs, sedimented at the bottom of a cultivation vessel, were collected with a pipette and transferred into the fixative.

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1383-5769/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2010.07.001

For transmission electron microscopy (TEM), the small pieces of gravid proglottids containing intrauterine eggs, and the isolated eggs were both fixed in ice-cold 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 1 month and then postfixed in 1% OsO_4 for 2 h. The material was dehydrated in a graded acetone series and embedded in Spurr's epoxy resin. For general topography of eggs the semithin sections were stained with 1% toluidine blue in borax solution. The ultrathin sections were cut using a Leica Ultracut UCT ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate. The grids were examined in a JEOL 1010 TEM operated at 80 kV.

3. Results

3.1. General topography and cellular organization of the infective hexacanth

The schematic diagram (Fig. 1) shows our interpretation of the general topography and bilateral symmetry in cellular organization of the infective larvae. As in our previous papers [19,20] we follow the oncospheral terminology proposed by Ogren [21]. Such terms as "anterior pole" and "posterior pole" of the oncosphere are used in this paper with respect to hexacanth's invasive activity. It uses its hooks, generally in conjunction with penetration gland secretion, to penetrate through host tissue with the hooks oriented in the direction of movement. Therefore, the hook region, directed forward during movement, is considered as the anterior part of the larva and functionally as the "somatophore" (Fig. 2). The opposite part, containing germinative cells, is considered as posterior and functionally as "germatophore" (Fig. 2). The mature hexacanth is armed with three pairs of hooks (Fig. 1): one pair of median hooks and two pairs of lateral hooks interconnected by a complex hook muscle system, responsible for coordination of their synchronic movements. The fully formed hooks, when observed at cross sections, show a heterogeneous structure and are composed of three layers of different electron densities (see Inset to Fig. 2). In *E. salvelini* the infective oncosphere consists of very numerous cells which are arranged symmetrically (Figs. 1, 2). They include also two multinucleated structures: the tetranucleated penetration gland and the binucleated perikaryon of the tegument (Fig. 1). Within the infective hexacanth larva the following cell types were distinguished: (1) binucleated subtegumental cell; (2) U-shaped, tetranucleated penetration gland; (3) two nerve cells; (4) three types of somatic cells, including i) myocytons of both somatic and hook musculature, ii) numerous degenerating micromeres with pycnotic nuclei and iii) a new cell type, which we call the interstitial cell that has never been observed in any other hexacanth; and (5) germinative cells with characteristic prominent nucleoli in their large spherical nuclei, surrounded by a thin layer of granular cytoplasm.

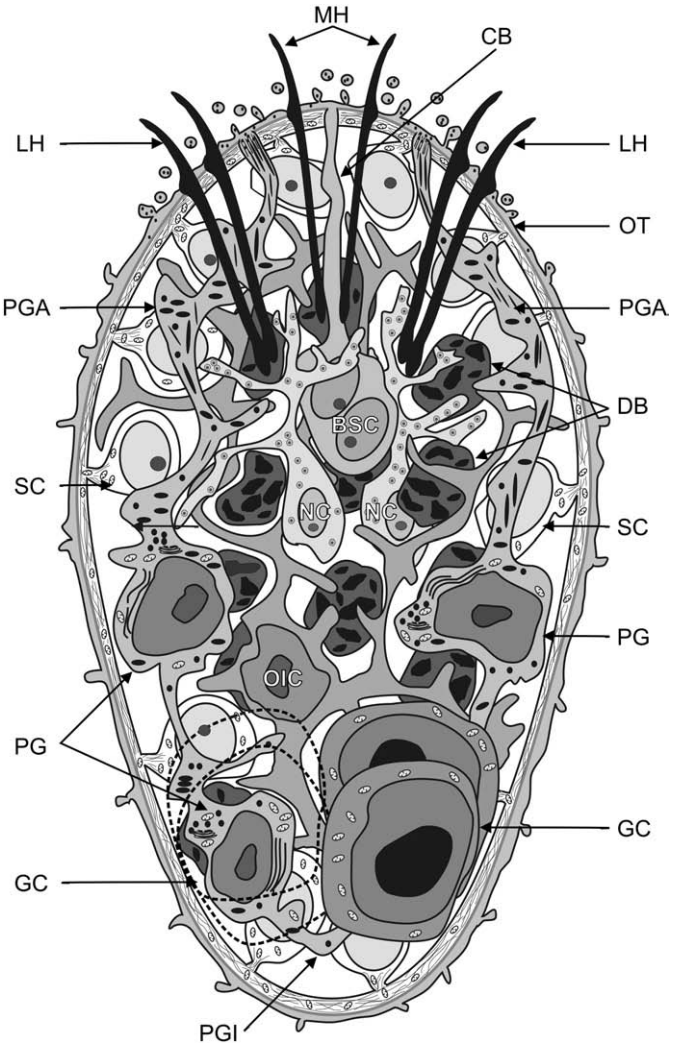

Fig. 1. Schematic diagram illustrating cellular composition and bilateral symmetry in the internal organization of the hexacanth of *E. salvelini*. BSC, binucleated subtegumental cell; CB, cytoplasmic bridge; DB, degenerating blastomeres; GC, germinative cells; LH, lateral hook pair; MH, median hook pair; NC, nerve cells; OIC, oncospheral interstitial cell; OT, oncospheral tegument; PG, penetration gland; PGA, arm of the penetration gland; PGI, isthmus of the penetration gland; and SC, somatic cells.

3.2. Oncospheral tegument

A thin anucleated cytoplasmic layer of oncospheral tegument covers the surface of the infective hexacanth of *E. salvelini* (Figs. 1–3). It is underlain by the moderately electron-dense basal lamina (Fig. 3a). At the tegumental surface numerous characteristic bubble-like processes are present (Fig. 3a,c). Some of them are in the process of detaching from the surface of the tegument and forming large vesicles with granular content (Fig. 3a,c). However, some of them may represent villi sectioned at different angles. This outer anucleated layer of the tegument is connected directly with its binucleated perikaryon situated deeper in the oncosphere body by a narrow cytoplasmic process (Figs. 1, 3b). This syncytial cell shows two closely adjacent nuclei, both containing electron-dense nucleoli (Fig. 3b). All peripheral, somatic musculature, composed of differently oriented muscle bundles and responsible for oncospheral body movements, is situated below the basal lamina (Fig. 3a,c). Muscles are attached to the basal lamina in highly electron-dense areas of muscle attachment zones (Fig. 3a). Numerous mitochondria and β-glycogen-like particles were observed in nearby cytoplasm of the myocyton projections connecting myofibers to their cell bodies or perikarya (Figs. 3c, 6b).

3.3. Penetration gland

The oncospheral penetration gland forms a U-shaped, tetranucleated syncytium with numerous cytoplasmic processes or ramifications (Fig. 1) containing secretory granules of different shapes and sizes (Fig. 4). Gland exits open into the cytoplasmic layer of the tegument in the hook region of the oncosphere (Figs. 1, 4a and inset). The oblique and longitudinal sections of gland exits and arms usually show a single row of microtubules, reinforcing their plasma membranes (inset to Fig. 4a). The cytoplasm of gland arms and exits contains elongated secretory granules that become fragmented into much smaller vesicles at the point of connection of exits to the tegumental cytoplasm (Fig. 4a and inset). The irregularly shaped nuclei of the penetration gland contain numerous large heterochromatin islands and prominent nucleoli (Fig. 4b,c). They are surrounded by a granular syncytial cytoplasm, rich in free ribosomes, Golgi