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Ultrastructure of oncospheral hook formation in the cestode *Mosgovoyia ctenoides* (Railliet, 1890) Beveridge, 1978 (Cyclophyllidea, Anoplocephalidae)

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Abstract

The ultrastructure of oncospheral hook formation in the anoplocephalid cestode *Mosgovoyia ctenoides* (Railliet, 1890) Beveridge, 1978, is described. The hook morphogenesis takes place inside the six symmetrically arranged hook-forming cells, the oncoblasts. They show characteristic large nuclei of semilunar shape, localized at one pole of the embryo. At the beginning of the hook formation, the "hook-forming centre" appears in the cytoplasmic part of each oncoblast. It consists of numerous free ribosomes and polyribosomes surrounded by several mitochondria and Golgi complexes. The hook-forming centre is involved in synthesis of an electron-dense, undifferentiated hook primordium, which undergoes progressive differentiation and elongation into the fully developed hook. A fully formed oncospheral hook consists of the three parts: blade, shank, and base. Each hook, at the site of its protrusion from the oncosphere, is surrounded by two electron-dense rings interconnected by a circular septate junction. The hook material consists of two or three layers that differ in electron density: (1) a moderately electron-dense core, (2) a middle layer of low electron density, and (3) a highly osmiophilic cortex. Wide bands of hook muscles are attached to the basal and collar parts of the hook. The hook blades project outside of the oncospheral body into a large cavity delimited by the hook region membrane attached at this pole directly to the oncospheral surface. In the fully developed oncosphere of *M. ctenoides*, the three pairs of oncospheral hooks and their muscles form a complex "hook muscle system", responsible for coordinated hook action. The differentiation and ultrastructure of oncospheral hooks in the oncospheres of *M. ctenoides* are compared to those described in other cestode species.

Key words

Mosgovoyia ctenoides, Cestoda, Cyclophyllidea, Anoplocephalidae, ultrastructure, oncospheral hook, oncoblast

Introduction

Embryonic development and cellular differentiation in *Mosgovoyia ctenoides* leads to the formation of the infective oncosphere (hexacanth larva). Each fully formed oncosphere is protected by envelopes and passes out of the host into the environment. Before further development into the cysticercoid (juvenile) form, the hexacanth larva must be eaten by an oribatid mite (Acarina) as the intermediate host specific for anoplocephalids. In the mite it must first pierce the mite's gut barrier. For this purpose, oncospheres of *M. ctenoides* are armed with three pairs of oncospheral hooks and a penetration gland, which play an important and active role during penetration through the intestinal tissues of the mite.

Despite several studies on different cestode species (for references see Ogren 1961; Rybicka 1966; Świderski 1973, 1983; Ubelaker 1983; Świderski and Tkach 1997a, b; Świderski *et al*. 2000a, b, 2004) still little is known about the structure and morphogenesis of hooks among cestodes belonging to the family Anoplocephalidae (Świderski 1976, Świderski *et al*. 2001, Świderski and Tkach 2002). Only one species, *Inermicapsifer madagascariensis*, has been considered in this respect (Świderski 1976). Nothing is known of the differentiation of oncospheral hooks in *M. ctenoides*, a para-

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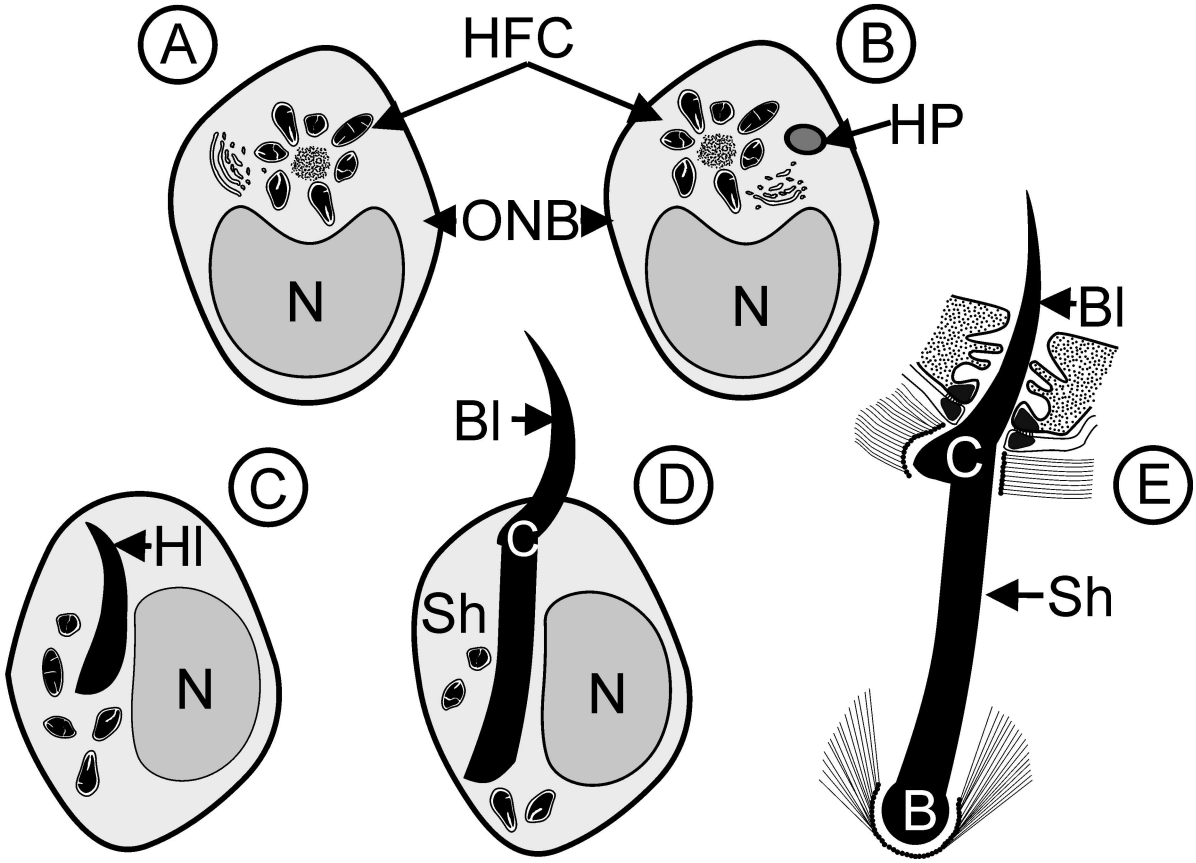


Fig. 1. Schematic diagram of consecutive stages of hook formation in *Mosgovoyia ctenoides*: **A** – early oncoblast with peripherally situated kidney-shaped nucleus and hook-forming centre in the invagination of the nucleus; **B** – early oncoblast with hook primordium within the hook-forming centre; **C** – early oncoblast with intracellular hooklet outline; **D** – late oncoblast with hook blade protruding outside and early shank myofibrils attached to the collar and basal part; **E** – fully developed oncospheral hook. **Abbreviations to all figures:** B – hook base, Bl – hook blade, C – hook collar, CHP – central hook pair, D – septate junction, G – Golgi complex, H – hooks, HFC – hook-forming centre, Hl – hooklet, HM – hook muscles, HMA – hook muscle attachment zones, HP – hook primordium, HRM – hook region membrane, LHP – lateral hook pair, m – mitochondria, N – nucleus, nsg – neurosecretory-like granules, OM – oncospheral membrane, ONB – oncoblast, ONC – oncosphere, OT – oncospheral tegument, PG – penetration gland, SC – somatic cells, Sh – hook shank, 1–3 – consecutive layers of hook material showing different electron density

site of wild and domestic rabbits (*Oryctolagus cuniculus*) and sometimes hares (*Lepus* sp.).

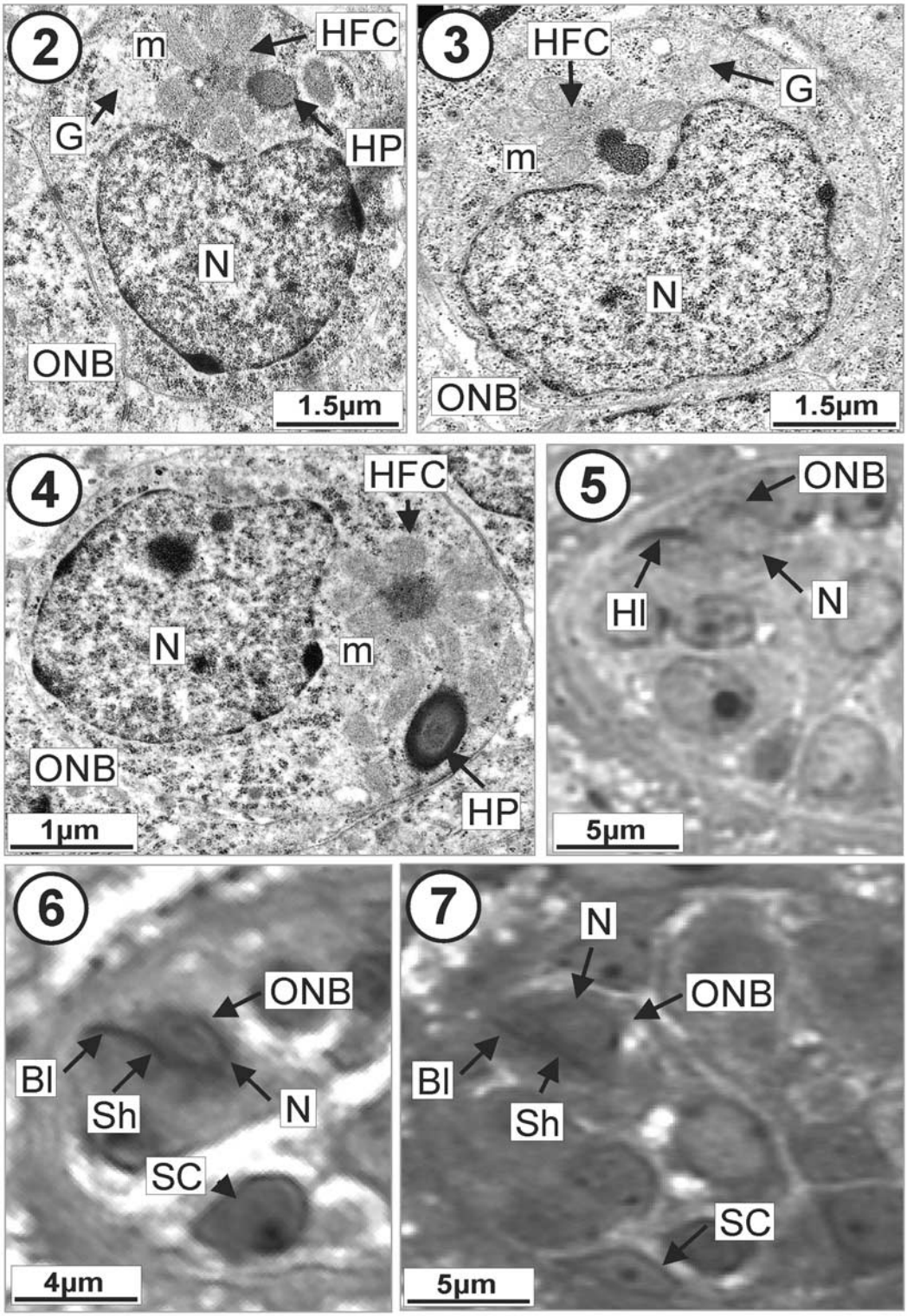
The purpose of the present paper is to describe various aspects of oncospheral hook morphogenesis in the anoplocephalid cestode *M. ctenoides*, and thus to extend our understanding of this important morphogenetic process.

Materials and methods

Adult specimens of *Mosgovoyia ctenoides* (Railliet, 1890) Beveridge, 1978 were obtained from the small intestine of naturally infected wild rabbits (*Oryctolagus cuniculus*) collected in Quiaios, Portugal. The cestodes were isolated from the intestine, washed in NaCl solution and cut into small pieces. Tissue samples of mature and gravid proglottids were fixed for 2 hrs in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), washed in the same buffer and postfixed in 1% OsO_4 for 1 hr. Material was dehydrated in a graded ethanol series and propylene oxide and embedded in Spurr's epoxy resin. Semithin sections, stained with 1% methylene blue in borax solution, were used for light microscope study of the consecutive stages of oncospheral hook development. Ultrathin sections, double stained with uranyl acetate and lead citrate, were examined under a JEM 100 B transmission electron microscope operated at accelerating voltage of 80 kV.

Results

Oncospheral hooks in *M. ctenoides* are formed during the early preoncospheral phase. They appear in six symmetrically arranged hook-forming cells, localized at one pole of the



Figs 2–7. Transmission electron microscopy (TEM) and light microscopy (LM) sections illustrating the consecutive stages of hook formation. **Figs 2** and **3.** TEM of early oncoblast with undifferentiated hook primordium near the hook-forming centre. Note high accumulation of free ribosomes and mitochondria. **Fig. 4.** TEM of early oncoblast with cross-section through the hook primordium. Note: (1) two zones of different electron density within hook material; (2) concentration of numerous free ribosomes and polyribosomes surrounded by several mitochondria. **Fig. 5.** LM of early oncoblast with the intracellular, comma-shaped outline of the hooklet. **Fig. 6.** LM of oncoblast with hook blade protruding outside the hook-forming cell. Note the first stages of shank formation. **Fig. 7.** LM of late oncoblast with a long intracellularly situated shank

embryo. The consecutive stages of oncospheral hook morphogenesis are illustrated in Figure 1. The oncoblasts are characterized by displacement of their large nuclei to one pole of the cell and simultaneous changes in their shape from oval or spherical into kidney-shaped (Figs 1A, B and 2–4). At the beginning, a high accumulation of free ribosomes and polyribosomes appears in the cytoplasmic pole of the oncoblast near the invaginated part of the nucleus (Fig. 1A). This aggregation of ribosomes becomes surrounded by several mitochondria and Golgi complexes; this region is the hook-forming centre (Figs 1A, B and 2–4). A small, dense hook primordium appears first inside this hook-forming centre (Figs 1B, 2 and 4).

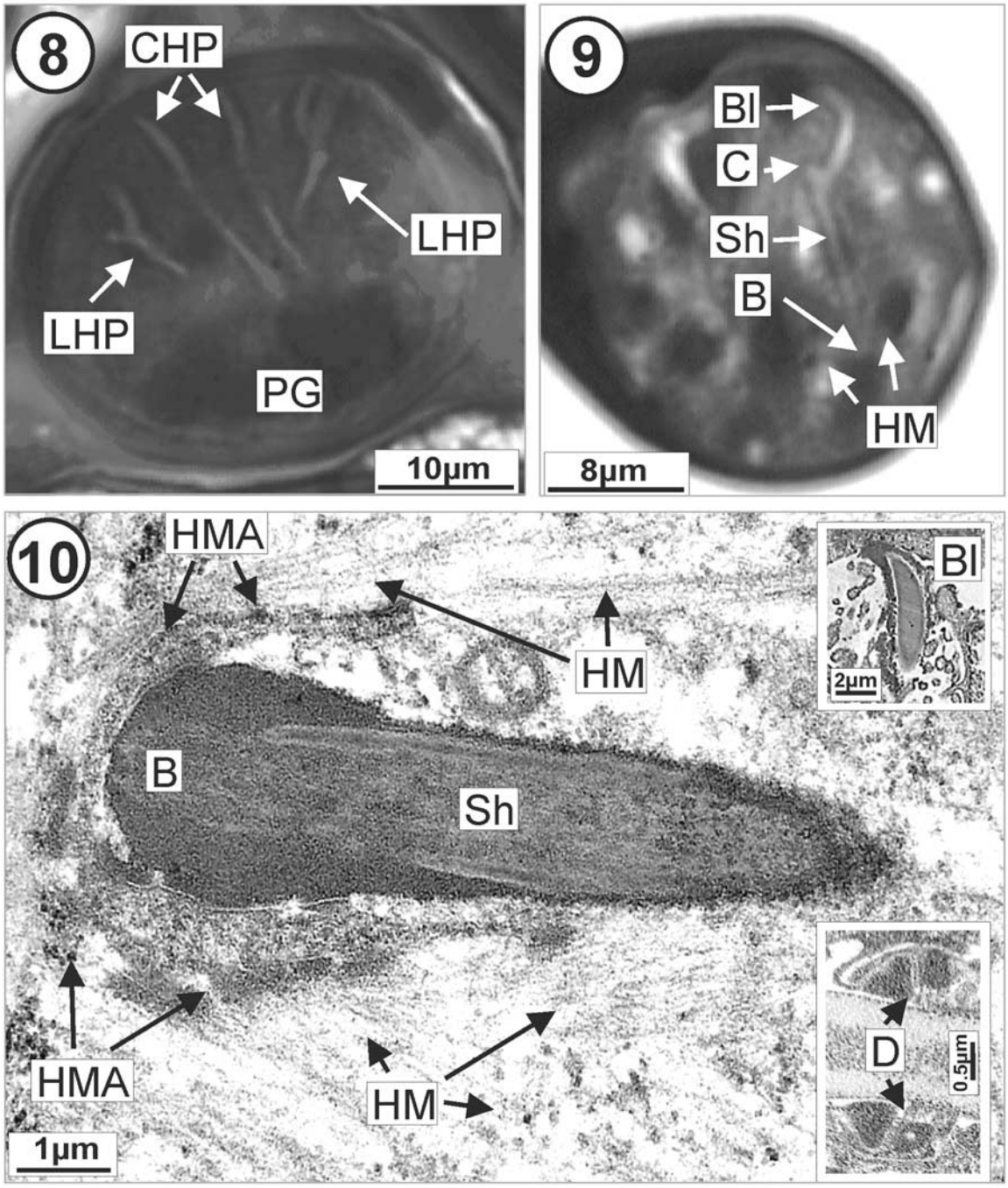
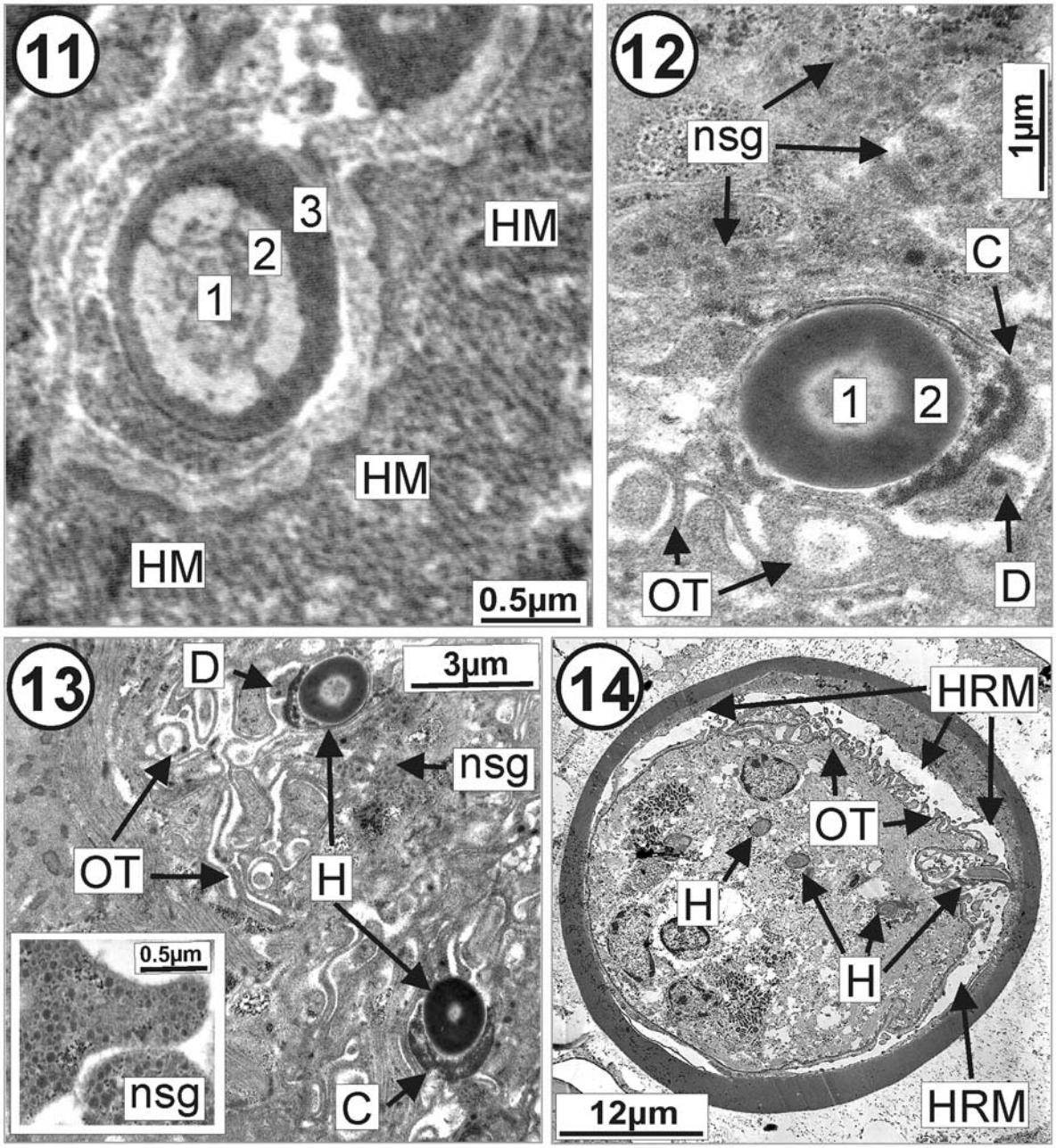


Fig. 8. LM of semithin section showing three pairs of oncospheral hooks in the fully developed, infective oncospheres of *M. ctenoides*. **Fig. 9.** LM of fully developed oncospheral hook composed of base, shank, collar and blade. **Fig. 10.** TEM of oblique section through the hook base. Note the fibrous layer of hook muscle attachment zone. Inset in right upper corner: detail of blade exit covered by a cup of electron-dense material. Inset in right lower corner: details of two electron-dense rings connected by circular septate junctions surrounding blade exit

Initially, the hook primordium consists of undifferentiated electron-dense material (Fig. 2). It grows rapidly in diameter and becomes differentiated into two layers clearly visible in cross- and oblique sections (Fig. 4). The outer layer is an electron-dense cortex, which covers a core of lower electron density. The primordia undergo further elongation and differentiation (Figs 1C, D and 5–7). At the beginning, each unit undergoes elongation into a comma-shaped hooklet, which is the first step of blade formation (Figs 1C and 5). In more advanced stages, the hook shank is progressively formed (Figs



Figs 11 and **12.** TEM of cross-sections through fully developed oncospheral hooks showing evident differences in the number of concentric heterogeneous layers (2 or 3) at different cross-section levels. **Fig. 13.** TEM of peripheral part of the oncosphere showing long projections of the oncospheral tegument near the sites of the hook blade exit from the oncosphere. Note: (1) a septate junction ("desmosome") and hook collar adjacent to hook blade; (2) neurosecretory-like granules in the cytoplasmic projections near the hook. Inset: high power magnification of cell processes containing numerous neurosecretory-like granules. **Fig. 14.** TEM of the general topography of the infective oncosphere showing hook region membrane on the anterior pole of the hexacanth covering the protruding hook blades. Note the long tegumental processes below the hook region membrane

1D and 6–7). The elongating blade leads to its protrusion through the oncoblast cell membrane and thus protrusion outside the hook-forming cell occurs (Figs 1D, 5–7). The part between the blade and shank increases in thickness, forming a well-developed guard or collar clearly visible in the fully developed hook (Figs 1D, E, 9 and 12–13). Subsequently, after the shank length increases, an enlarged knob-like base of the hook forms at its end (Figs 1E, 9 and 10).

The fully developed oncospheres are armed with three pairs of oncospheral hooks: one medial pair and two pairs of lateral hooks, interconnected by a complex hook muscle system (Fig. 8), responsible for coordination of the synchronized hook movements. Each fully developed oncospheral hook consists of three parts: the blade, the collar, and a long shank or handle, which terminates in knob-like base (Fig. 9). Each hook, at the site of its exit from the oncosphere, is always surrounded by two electron-dense rings interconnected by circular septate junction (Figs 1E, 12 and 13).

At the ultrastructural level, the fully developed oncospheral hooks of *M. ctenoides* show a heterogeneous nature of the hook-forming material, well visible in cross- and oblique sections. Two or three layers of hook material can be distinguished in the section (Figs 11 and 12): (1) a moderately electron-dense core, (2) a middle layer of low electron density, and (3) a highly osmiophilic cortex.

Following full hook development, wide bands of differentiating hook muscles become attached directly to the base and collar parts of each hook (Figs 1E and 9–11). These hook muscle attachment (HMA) zones are formed as thick structures consisting of a fibrous substance (Fig. 10). Moreover, numerous intracellular granules with an ultrastructure consistent with that of neurosecretory vesicles were observed close to oncospheral hooks and their musculature (Figs 12, 13 and inset).

The hook region membrane (HRM) is present at one pole of the oncosphere, above the hook blades (Fig. 14). The lower cytoplasmic layer of this cavity represents the newly formed oncospheral tegument (Fig. 14), with clearly visible long tegumental processes (Figs 13 and 14).

Discussion

In *M. ctenoides*, as in other tapeworms, the oncospheral hooks and penetration gland cooperate to play a main role during the invasion of the intermediate host. The viewpoint that they are very important structures in the infection mechanism has been published in numerous papers (for review see Rybicka 1966; Świderski 1973, 1976; Ubelaker 1983; Świderski *et al*. 2000a, b).

The first studies based on light microscope observations (Ogren 1957, 1958, 1961; Moczoń 1971), indicated that hook morphogenesis takes place inside specialized cells, the oncoblasts, during the preoncospheral phase of embryogenesis. In all cestodes studied to date, the oncoblasts are symmetrically arranged near the anterior pole of the preoncosphere and occur in three pairs, one medial and two lateral. The course of hook formation inside the oncoblasts was first described by Ogren (1961) in his work on *Hymenolepis diminuta* and confirmed by more recent TEM studies (Świderski 1973, 1976; Świderski and Tkach 1997a; Świderski *et al*. 2000a, b). Ogren (1961) distinguished five stages of hook development: (1) early oncoblast, initiating hook synthesis, (2) early oncoblast, with blade outline completed, (3) late oncoblast during shank synthesis, (4) late oncoblast with shank completed, and (5) oncoblast degeneration when the fully developed hook is completely formed. In *M. ctenoides* similar stages were observed, but the formation of the hook base and the process of hook muscle attachment were not examined in detail. Nevertheless, some details described in the early light microscopical studies (Ogren 1957, 1958, 1961; Moczoń 1971) appear, incorrect due to the inherently low resolution, and are not supported by the more recent TEM studies (Świderski 1973, 1976; Świderski and Tkach 1997a; Świderski *et al*. 2000a, b). Ultrastructural aspects of hook morphogenesis were previously described in other tapeworms by Świderski (1973, 1976), Świderski and Tkach (1997a), Kornakova (1999), Świderski *et al*. (2000a).

The first electron microscopical and histochemical analysis of hook morphogenesis were in *Catenotaenia pusilla* and added some new information including determining shapes of oncospheral hooks and chemical aspects of hook formation (Świderski 1973). These results suggested that the shapes of oncospheral hooks are determined genetically and not by the profile of the oncoblast plasma membrane as suggested by Ogren (1961). In *M. ctenoides*, as in other species studied to date, the hook blade touches the cell membrane in only one point and never follows its shape. Histochemical analysis of *M. ctenoides* hook formation has not yet been performed, and should be a subject for further studies. The present study on *M. ctenoides* indicates that the hook musculature is directly attached only to the hook collar and base, not to the surface of the oncoblast. The direct attachment of hook muscle to the collar end base was also observed in *I. madagascariensis* by Świderski (1976), *Pseudhymenolepis redonica* by Tkach and Świderski (1997), *Nematotaenia dispar* by Świderski and Tkach (1997a), *Hepatocestus hepaticus* by Świderski *et al*. (2000b), *Dilepis undula* by Świderski *et al*. (2000a), *Anoplocephaloides dentata* by Świderski *et al*. (2001), and *Joyeuxiella echinorhyncoides* by Świderski *et al*. (2004).

Kornakova (1999) described unusual features in *Paserilepis crenata*. According to her interpretation, the fully developed oncospheral hooks never protrude through the oncoblast plasma membrane and the entire hook-forming cell always remains intact. She argued that the penetration of the hook blade through the cell plasma membrane of the oncoblast would destroy the plasma membrane potential and ionic balance of the cell, resulting in its immediate death. Kornakova's (1999) arguments, however, are not supported by our data. It is difficult to explain how the hook muscle system would function if the entire hook remained embedded in the oncoblast, including both its hook muscle attachment

points at the hook collar and base. Thus, the hook must emerge from its formative cell at some time. The exact mechanism for this process has not been demonstrated conclusively, but may involve a specialized form of exocytosis that would result in externalization of the hook while leaving the oncoblast intact. In *M. ctenoides*, as in other species examined to date (Collin 1968; Świderski 1973, 1976; Chew 1983; Świderski and Tkach 1997a; Tkach and Świderski 1997; Świderski *et al*. 2000a, b), the hook muscle system forms a very complex pattern of muscle arrangements and attachment zones. In all species examined the muscles are attached only to the guards or collars and bases of hooks.

Another of Kornakova's (1999) viewpoints that is not supported by our study is her opinion on the role of mitochondrial accumulation in the hook-forming cells. Our data demonstrate that the oncoblasts are characterized by high synthetic activity, based on the accumulation of free ribosomes, polyribosomes, Golgi complexes and mitochondria. Energy supplied by mitochondria is necessary for protein synthesis, which supplies construction materials for the process of hook formation (Nieland 1968; Świderski 1973, 1976). Thus, the viewpoint of Kornakova (1999) has overlooked the synthetic role of mitochondrial energy metabolism.

Despite some similarities in the general pattern of hook morphogenesis among cestodes, differences are evident among species. Despite the fact that the oncoblasts have never been observed around the entire fully developed hooks of *I. madagascariensis*, *Echinococcus granulosus*, *N. dispar*, *Hepatocestus hepaticus* and *M. ctenoides*, (Świderski 1976, 1983; Świderski and Tkach 1997a; Świderski *et al*. 2000b; and present study, respectively), their remnants are often still visible in the fully developed infective oncospheres of some species. For example, the nucleated oncoblasts surrounding the hooks in infective oncospheres were described in the dilepidid tapeworm *D. undula* and in the dipylidiid cestode, *J. echinorhyncoides* by Świderski *et al*. (2000a, 2004). Similar observations were reported previously by Collin (1968), Moczoń (1971), Furukawa *et al*. (1977), Chew (1983) and Tkach and Świderski (1997). In the fully developed oncospheres of *Staphylocystoides stefanskii*, only the thin layers of anucleated cytoplasm around the hook shank regions remain from the oncoblast, which seems to be a common feature for the mammalian hymenolepidids (Świderski and Tkach 1997b, 1999). However, in this kind of interspecific comparison, two criteria must be taken under consideration: (1) the degree of oncosphere and hook development, and (2) the presence of representative cross, oblique and longitudinal sections along the hook shank, blade and base. The cases of total degeneration of oncoblasts as observed in *C. pusilla*, showing only five nuclei in fully developed oncospheres (Świderski 1972, 1973) and in the present study on *M. ctenoides*, appear to be among the characteristic features associated with evolutionary trends in tapeworm larval simplification. Świderski (1983) has formulated the hypothesis that progressive reduction in the number of oncospheral cells is an adaptative feature in cestode evolution. Further details on the degeneration of nuclei in oncospheres have recently been presented by Świderski and Mackiewicz (2004).

The formation of oncospheral hooks in *M. ctenoides*, as in other cestode species examined so far (see Świderski *et al*. 2000a) evidently differs from that of the rostellar hooks (Mount 1970) which originate from a fusion of specialized tegumental microtriches and a progressive deposition of proteins on the differentiating rostellar hook surface. Rostellar hooks are not individually connected to myofibrils, and function in attachment to the host. Conversely, oncospheral hooks are directly attached to myofibrils and function in host invasion. Thus, although the hooks may have slight similarity at the gross level, they are neither analogous nor homologous.

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