Electron microscope study on oncospheral envelope morphogenesis in the dilepidid cestode, *Dilepis undula* (Schrank, 1788)

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Abstract

The origin, differentiation and ultrastructural characteristics of oncospheral envelopes surrounding invasive oncospheres of the dilepidid cestode *Dilepis undula* are described. In the early preoncospheral phase three primary embryonic envelopes are formed: (1) the capsule; (2) the outer envelope formed by two macromeres; and (3) the inner envelope originating from fusion of two or three mesomeres. Both the outer and inner envelopes of *D. undula* are therefore cellular in origin and syncytial in nature. Mature eggs of *D. undula* are slightly oval, measuring 40–50 × 56 µm in diameter. Within fully formed eggs, the mature, invasive oncospheres, 36–40 µm in diameter, are surrounded by five oncospheral or egg envelopes: (1) outer shell; (2) outer envelope; (3) inner envelope; (4) oncospheral membrane; and (5) hook region membrane covering only one pole of the hexacanth. The ultrastructural characteristics of *D. undula* oncospheral envelopes are discussed in comparison with those of previously examined dilepidids and other cyclophyllideans.

Key words

*Dilepis undula*, Dilepididae, Cestoda, oncospheral envelopes, morphogenesis, cestode eggs, ultrastructure

Introduction

Little information is available on the ultrastructural aspects of embryogenesis in dilepidid cestodes; only eight species have been examined in this respect (see Gabrion 1981a, b; Świderski et al. 2000, 2003, 2004; Świderski and Salamatin 2002). The family Dilepididae Railliet et Henry, 1909 represents one of the largest groups of cyclophyllidean cestodes and is characterized by a remarkable diversity in the morphology, development and life cycles represented (Bona 1994). Several attempts have been made to find suprageneric arrangements for this group (Spasskaya and Spasskiy 1977, 1978; Spasskiy 1994). However, this requires further study and hypotheses, taking into account the significance of new ultrastructural characters, better knowledge of traditional morphological characters, ranges of variation and trends in shape, anatomical homologies and revision of the generic allocation of many species. According to the recent morphological and molecular phylogenetic studies (Hoberg et al. 1997, 1999; Mariaux 1998) dilepidids are placed among the most derived cestode families. Regarding egg envelopes and their morphogenesis in Dilepididae, Gabrion (1981a, b) provided some data on the ultrastructure of oncospheral envelopes in two bird dilepidids, *Spiniglans constricta* (= *Anomotaenia constricta*) and *Paricterotaenia porosa*, as well as *Molluscotaenia crassiscolex*, a parasite of mammals. Świderski et al. (2000) presented data on the ultrastructure and origin of the oncospheral envelopes in *Hepatocestus hepatitis*, a parasite of mammals. A detailed description of the morphogenesis and ultrastructure of egg envelopes was recently presented by Świderski and Salamatin (2002) for *Spassytaenia platyrhyncha*, and briefly reported in an abstract by Świderski et al. (2003) for *Dichoanotaenia clavigera*, both parasites of aquatic birds. The aim of this paper is to describe the ultrastructural aspects of oncospheral envelope morphogenesis in the cestode *Dilepis undula*, a parasite of terrestrial birds and a type species of the genus *Dilepis*, which is, in turn, a type genus in the family Dilepididae. New data on this subject may present useful criteria for phy-
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logenetic analysis of this group, as the ontogenetic characters have been proposed as phylogenetic indicators in studies on cestode evolution.

**Materials and methods**

Adult specimens of *Dilepis undula* (Schrank, 1788), were removed from the intestine of the fieldfare, *Turdus pilaris* and the blackbird, *Turdus merula*, collected in Teremki, suburb of Kiev, Ukraine. Tissue samples from mature and gravid proglottids were rinsed quickly in saline, cut into small pieces and prefixed in 2.5% glutaraldehyde in sodium cacodylate buffer (pH 7.4). After overnight washing in the same buffer and post-fixation for 2 hrs in 1% OsO₄, they were dehydrated in an ethanol series, infiltrated with propylene oxide and embedded in Spurr’s resin. Semithin sections stained with 1% methylene blue in borax solution, were used to study general topography of eggs in the mature and gravid proglottids. Ultrathin sections, mounted on uncoated copper grids, double stained with lead citrate and uranyl acetate, were examined under a JEM-1200EX transmission electron microscope (TEM) operated at an accelerating voltage of 80 kV.

**Results**

**General topography of the eggs**

The eggs of *Dilepis undula* are usually slightly oval, 40–50 × 56 µm in diameter on the total preparations and semithin sections. The mature oncospheres measure about 36–40 µm. The eggs are tightly packed within the uterus, which is coarsely reticulate or labyrinthine.

Each egg consists of an oncosphere surrounded by an outer egg shell, the outer and inner envelopes, and the oncospheral membrane (Figs 3–6). In addition to these four envelopes entirely surrounding the hexacanths, the hook region membrane surrounds only one pole of the oncosphere and is attached directly to its surface. The embryophore is of the discontinuous type, formed within the cytoplasm of the inner envelope, and is composed of randomly dispersed moderately electron-dense structures, which are most probably arranged three-dimensionally into a loose network (Figs 5, 7–9, 12 and 14).

**Morphogenesis of embryonic envelopes**

In the early preoncospheral phase (Fig. 1A–C), three primary embryonic envelopes are formed: (1) the capsule originating from vitellocyte material; (2) the outer envelope formed by two macromeres; and (3) the inner envelope originating from a fusion of two or three mesomeres. Both the outer and inner envelopes of *D. undula* are therefore cellular in origin and syncytial in nature (Figs 2, 4 and 5). The delicate membranous capsule progressively decreases in volume (Fig. 1B and C), becomes impregnated by a moderately electron-dense materi-
Fig. 2. Part of the early preoncosphere composed of numerous differentiating blastomeres. Note the inner envelope containing numerous ribosomes and polyribosomes, few mitochondria and Golgi complexes in its granular cytoplasm as well as the elongated flattened nucleus of the mesomere with prominent nucleolus. Figs 3 and 4. Low power electron micrographs showing the secondary egg envelopes surrounding fully formed invasive oncospheres in mature eggs of *D. undula*. In Fig. 3, note: (1) large number of lipid droplets of different sizes and electron densities in the cytoplasm of the outer envelope; and (2) the hook region membrane separated by delamination from the oncospheral tegument, visible on one pole of the hexacanth (on the left side); the laguna or cavity between these two structures contains numerous fine lamellae. In Fig. 4, note: (1) large, irregularly shaped nucleus of macromere with prominent electron-dense nucleolus, surrounded by granular cytoplasm of the outer envelope, rich in GER, ribosomes and polyribosomes; and (2) the infoldings of the hook region membrane situated below the oncospheral membrane (in the lower right corner)
Oncospheral envelope morphogenesis in *Dilepis undula* and is reduced and transformed into the outer egg shell (Figs 5, 6 and 13). The outer envelope remains the thickest layer. The large volume of the cytoplasm of this envelope contains characteristic elongated dense aggregates, granular endoplasmic reticulum (GER) and ribosomes in the external granular sublayer (Fig. 4). In some sections (Figs 4 and 5) this envelope also shows the presence of large macromere nuclei, each with a prominent nucleolus. In addition, in the fully

Figs 5 and 6. TEM showing parts of the oncosphere surrounded by the envelopes. Note: (1) a very thin layer of moderately electron-dense outer egg shell; (2) nucleated (Fig. 5) and anucleated parts (Fig. 6) of the outer envelope composed of an external granular sublayer rich in ribosomes and elongated dense aggregates and an evidently less granular amorphous internal sublayer; (3) characteristic outer surface of the inner envelope covered by numerous pinocytotic-like vesicles or pits; (4) a discontinuous type of embryophore, within the cytoplasm of the inner envelope, composed of randomly dispersed moderately electron-dense structures, which apparently are arranged three-dimensionally into a loose network; and (5) the long cytoplasmic processes at the inner surface of inner envelope, interdigitating with similar cytoplasmic processes of the oncospherical tegument.
formed eggs this envelope contains a large number of lipid droplets of different sizes and electron densities (Fig. 3).

Ultrastructure of oncospheral envelopes

The capsule/outer egg shell: The delicate capsule in *D. undula* (Fig. 1A), formed at the beginning of embryonic development, decreases rapidly in volume (Fig. 1B) and is replaced by the egg shell observed in this species in the advanced stages of the preoncosphere in a form of moderately to highly electron-dense material encrusting the surface of mature eggs (Fig. 1B and C). Therefore, the outer egg shell in the mature eggs of *D. undula* (Fig. 1C) consists of an inner zone of electron-

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**Figs 7–9.** Ultrastructural details of the inner envelope. Note: (1) numerous pinocytotic-like vesicles or pits at the surface of the inner envelope; (2) the long cytoplasmic processes at the inner surface of inner envelope, interdigitating with similar cytoplasmic processes of the oncospheral tegument (Figs 7 and 9); and (3) a discontinuous type of embryophore composed of moderately electron-dense subunits.
Fig. 10. Ultrastructural details of the outer and inner envelopes. Note: (1) a smooth (agranular) endoplasmic reticulum in the cytoplasm of the outer envelope; and (2) several large mitochondria in the cytoplasm of the inner envelope containing moderately electron-dense subunits of a discontinuous embryophore (compare Figs 10, 11 and 12). Fig. 12. High power detail of the subunits of a discontinuous embryophore. Fig. 13. Details of the outer egg shell; note several large mitochondria in the outer and inner envelopes. Fig. 14. Details of the pits or pinocytotic-like vesicles and obliquely sectioned subunits of a discontinuous embryophore.
dense material with an irregular surface of bud-like projections.

The outer envelope of eggs consists of a layer of cytoplasm containing large, more or less flattened nuclei of macromeres with prominent electron-dense nucleoli (Figs 4 and 5), few mitochondria (Figs 4 and 13), GER (Fig. 4), Golgi complexes (Fig. 2), free ribosomes (Figs 4–6) and a few areas of focal cytoplasmic degradation (Fig. 3).

The inner envelope of *D. undula* is generally much thinner than the outer one and at the beginning of its cytodifferentiation (Fig. 2) contains flattened nuclei of mesomeres with predominant nucleoli, several mitochondria, GER, Golgi complexes, free ribosomes and polyribosomes. During egg maturation, the external surface membrane of the inner envelope forms numerous pinocytotic-like vesicles or pits (Figs 5–8 and 14), while its internal layer develops long cytoplasmic processes that establish an interdigitating pattern with similar cytoplasmic extensions of the oncospheral tegument (Figs 5–7 and 9). At the same time small moderately electron-dense subunits of discontinuous embryophore appear in its deeper layer. Such a discontinuous, unique type of embryophore of *D. undula* represents the most characteristic structure of the inner envelope of this species. It is composed of small dense subunits that show a great variety of shapes and sizes in cross (Figs 10–12) and oblique sections (Figs 5, 7, 8, 14). When examined under high power (Fig. 12), these embryophoral subunits have an average diameter of 0.07–0.1 µm, and resemble in most cases the "incomplete microtubules" or triangularly arranged large, dense fibres showing discontinuous arrangement. In the mature eggs of *D. undula*, numerous ribosomes of discontinuous embryophore and several large mitochondria were still observed in some parts of the cytoplasm of this layer (Figs 10 and 13).

The oncospheral membrane and hook region membrane: In the mature eggs of *D. undula*, both the oncospheral membrane (Figs 4, 10 and 11) and the hook region membrane (HRM) (Figs 3 and 4) are already well defined. As shown in Figure 3, the HRM and the oncospheral tegument originate from a delamination of an initially solid mass of peripheral cytoplasmic complex. The large cavity between the oncospheral tegument and HRM contains numerous fine lamellae (Fig. 3). The oncospheral membrane, formed by a delamination of the inner surface of the inner envelope (Fig. 1B), consists of two closely apposed plasma membranes. In the mature eggs, it is usually detached from the inner envelope (Figs 10 and 11), but in some regions can be closely adjacent to the oncospheral surface or to the inner plasma membrane of the inner envelope.

**Discussion**


The interrelationships between the ultrastructure of the oncospheral envelopes, cestode life cycles, and habitats of the intermediate hosts were illustrated and discussed by Chomicz and Świderski (2004) for twelve species of hymenolepidids with aquatic life cycles. It was concluded by the authors that the structural and chemical composition of infective egg envelopes is dependent upon the character of the habitats where final and intermediate hosts live and the presence of conditions that facilitate direct contact of eggs with the intermediate hosts. In hymenolepidids with terrestrial life cycles, e.g., *Hymenolepis diminuta*, *H. microstoma*, *H. citelli* or *H. nana*, egg envelopes are essentially similar (Pence 1970, Rybicka 1972, Świderski 1975, Conn 1993, Conn and Forman 1993); two layers that play an important role are the outer egg shell and the embryophore. The outer egg shell in *D. undula* eggs, as in the above mentioned hymenolepidids, consists of an inner zone of electron-dense material with an irregular surface of bud-like projections. As demonstrated by Pence (1970), in *H. diminuta* the main chemical component of the outer egg shell is apparently a neutral mucopolysaccharide or glycoprotein. Vogel and Berntzen (1961) and Lethbridge (1980) reported that the outer egg shell is refractory to host digestive enzymes and must be removed mechanically before hatching can proceed. The same probably is true for *D. undula* eggs, in which the outer shell is relatively thin and electron-dense.

As mentioned in the introduction, the dilepidids represent not only one of the largest groups of cyclophyllidean cestodes, but at the same time they are characterized by a remarkable diversity of their morphology, developmental biology and life cycles. The terrestrial life cycle of *D. undula* is well known (see Gulyaev 1997); it takes place in a relatively humid environment and the anrellids of the family Lumbricidae serve as intermediate hosts. This may explain why there is no risk of desiccation for the infective eggs and thus no need for excessive development of the oncospheral protective structures such as very thick and electron-dense outer egg shells and/or embryophores. This type of life cycle and habitat of the intermediate hosts of this tapeworm remain in direct correlation with the functional ultrastructure described above for the oncospheral envelopes in the mature infective eggs of *D. undula*.

The most characteristic feature of dilepidid eggs is the unique structure of the external surface membrane of the inner envelope forming numerous pits, pinocytotic-like vesicles or short bulbous projections that fit tightly into corresponding pits of the outer plasma membrane of the inner envelope and act as “anchoring” structures for both egg envelopes. Such structures are characteristic not only for *D. undula* eggs, as they have been described previously in the eggs of *Hepatocestus hepaticus* by Świderski et al. (2000), *S. platyrhyncha* by Świderski and Salamatin (2002) and in *D. clavigera* by Świderski et al. (2003). In these three species, as well as in...
three other dilepidid species, namely two bird dilepidids, Spini-
giglans constricta (= Anomotaenia constricta) and Paricter-
rotataenia porosa, and one species Molluscticaenia crassico-
lex, a parasite of mammals (examined previously by Gabron1
1981a and described in his unpublished PhD thesis), despite
the low power of the micrographs, one can observe the pres-
ence of pits on the border between the outer and inner enve-
lves, precisely on the external plasma membrane of the inner
envelope. A great number of such bulbous pits have also been
described by Śviderski et al. (2003) on the surface of two
hook-like polar processes of the inner envelope in Dichomo-
taenia clavigera eggs. The exact function of these structures
remains enigmatic and can only be elucidated by means of
experimental studies. They may be involved in transport of
substances by endo- and exocytosis and therefore participate
in supplying the developing embryos with nutritive or struc-
tural materials; alternatively, they may also participate in
the elimination of metabolic waste products. Based on present
data, it is our opinion that the most probable function of these
pits is the reinforcement of the oncospheral envelopes, as they
represent anchoring structures for outer and inner envelopes.
Until now, however, there is no clear and convincing evidence
for any of the above hypotheses, so the questions of origin,
nature and function of these pits remain open for further stud-
ies.

Another ultrastructural character, apparently specific for
D. undula eggs and never reported before in the eggs of other
cestode species, is the presence of very long cytoplasmic proc-
ces formed by the innermost sublayer of the inner enve-
lope, which establish an interdigitating pattern with similar
cytoplasmic extensions of the oncospheral tegument as shown
on Figures 5, 7 and 9. Such an interdigitating system of these
structures probably helps in maintaining a very close, intimate
contact between the hexacanth larvae and the oncospheral
envelopes surrounding them.

The evident differences in the fine structure of the onco-
spheral envelopes of dilepidid eggs are, however, well reflect-
ed by the thickness and electron density of their outer egg
shells and their embryophores, which show a great variety in
different species examined so far (Gabron 1981a; Śvi-
derski and Salamatin 2002; Śviderski et al. 2003, 2004). For
example, both the egg shells and the embryophores of S. pla-
tyrryncha eggs (Śviderski and Salamatin 2002) are very ro-
 bust, showing a great thickness and a very high electron den-
sity. In D. clavigera eggs, the outer shell does not exist, the
outer envelope is reduced to a rudimentary, very thin, infold-
ed membranous remnant structure, and a continuous, moder-
ately electron-dense and relatively thin embryophore is still
present. In D. undula eggs, as presented results show, the outer
egg shell consists of a thin inner zone of electron-dense mate-
rial with an irregular surface of bud-like projections, provid-
ing very limited protection. The discontinuous type of the
embryophore, which apparently forms a very loose and fine
embryophoral network surrounding the infective hexacanth
in this species, appears also to be a very delicate and fragile
structure. It may be concluded therefore that both of these
essentially protective components of egg envelopes of D. un-
dula remain very soft in the mature eggs in comparison with
a very thick and electron-dense structure of the outer egg shell
and robust, very compact and dense fibrillar embryophore
observed in S. platyrhyncha eggs (Śviderski and Salamatin
2002).

Our data presented here show that in D. undula eggs, both
the outer egg shell and the dispersed islands of the embry-
ophore material of discontinuous type are very delicate and thin
and show rather moderate electron density. From these obser-
vations on the reduced volume and density of all oncospheral
envelopes, it may be concluded that their protective func-
tion in this species is very limited, as has been described for
some species of other cestode families (Conn and Etges 1984,
Conn et al. 1984, Conn 1999).

It should be emphasized, however, that further detailed
investigations on egg envelope differentiation and ultrastruc-
ture in other representative species of Dilepididae with aquat-
ic and terrestrial life cycles will provide material for fruitful
comparison and analysis of pathways of adaptation of these
tapeworms to their diverse life cycles. Such data would be
very useful for phylogenetic and systematic studies of this
large family and Cyclophyllidea as a whole.

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