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Développement embryonnaire intra-utérin précoce du cestode bothriocéphale Clestobothrium crassiceps *(Rudolphi, 1819), parasite du téléostéen* Merluccius merluccius *(L., 1758) (Gadiformes : Merlucciidae)*

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ABSTRACT

The early intrauterine embryonic development of the bothriocephalidean cestode *Clestobothrium crassiceps* (Rudolphi, 1819), a parasite of the teleost *Merluccius merluccius* (L., 1758), was studied by means of light (LM) and transmission electron microscopy (TEM). Contrary to the generic diagnosis given in the CABI *Keys to the cestode parasites of vertebrates*, the eggs of *C. crassiceps*, the type of species of *Clestobothrium* Lühe, 1899, are operculate and embryonated. Our LM and TEM results provide direct evidence that an operculum is present and that the eggs exhibit various stages of intrauterine embryonic development, and in fact represent a good example of early ovoviviparity. The intrauterine eggs of this species are polylecithal and contain numerous vitellocytes, generally ~ 30, which are pushed to the periphery and remain close to the eggshell, whereas the dividing zygote and later the early embryo remain in the egg centre. During early intrauterine embryonic development, several cleavage divisions take place, which result in the formation of three types of blastomeres, i.e. macro-, meso- and micromeres. These can be readily differentiated at the TEM level, not only by their size, but also by the ultrastructural characteristics of their nuclei and cytoplasmic organelles. The total number of blastomeres in these early embryos, enclosed within the electron-dense eggshells, can be up to ~ 20 cells of various sizes and characteristics. Mitotic divisions of early blastomeres were frequently observed at both LM and TEM levels. Simultaneously with the mitotic cleavage divisions leading to blastomere multiplication and their rapid differentiation, there is also a deterioration of some blastomeres, mainly micromeres. A similar degeneration of vitellocytes begins even earlier. Both processes show a progressive degeneration of both

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vitellocytes and micromeres, and are good examples of apoptosis, a process that provides nutritive substances, including lipids, for the developing embryo.

Mots clés:
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Apoptose

R É S U M É

Le développement embryonnaire intra-utérin précoce du cestode bothriocéphale *Clestobothrium crassiceps* (Rudolphi, 1819), parasite du téléostéen *Merluccius merluccius* (L., 1758), a été étudié en microscopie photonique (MP) et microscopie électronique à transmission (MET). Au contraire de la diagnose générique donnée par CABI, les œufs de *C. crassiceps*, espèce-type de *Clestobothrium* Lühe, 1899, sont operculés et embryonnés. Nos résultats en MP et MET donnent des preuves directes de la présence d'un opercule et aussi du fait que les œufs exhibent des stades variés de développement embryonnaire intra-utérin, représentant un bon exemple d'ovoviviparité précoce. Les œufs intra-utérins de cette espèce sont polylécithes et contiennent de nombreux vitellocytes, au nombre de 30 environ, qui sont poussés vers la périphérie et restent proches de la coque de l'œuf, alors que le zygote en division et ensuite l'embryon jeune restent au centre de l'œuf. Pendant le développement intra-utérin précoce, plusieurs divisions de clivage interviennent et produisent trois types de blastomères, c'est-à-dire des macro-, méso- et micromères. Ceux-ci peuvent être aisément distingués par microscopie électronique à transmission (MET), non seulement par leur taille, mais aussi par les caractéristiques ultrastructurales de leurs noyaux et de leurs organites cytoplasmiques. Le nombre total de blastomères dans ces embryons jeunes, enveloppés dans les coques de l'œuf qui sont denses aux électrons, peut atteindre approximativement 20 cellules de tailles et de caractéristiques diverses. Les divisions mitotiques des jeunes blastomères ont été fréquemment observées en MP et MET. Simultanément aux divisions mitotiques de clivage qui amènent à la multiplication des blastomères et leur différenciation rapide, on observe aussi une détérioration de certains blastomères, surtout des micromères. Une dégénération similaire des vitellocytes commence même plus tôt. Les deux processus de dégénération des vitellocytes et des micromères sont de bons exemples d'apoptose, un processus qui procure des substances nutritives, dont des lipides, à l'embryon en développement.

1. Introduction

Clestobothrium Lühe, 1899 comprises five species, *C. crassiceps*, the types-species, *C. neglectum, C. gibsoni*, *C. splendidum* and *C. cristinae*. Formerly in the suppressed order "Pseudophyllidea", *Clestobothrium* was transferred to the newly erected order Bothriocephalidea [1,2]. Morphological, molecular and ecological data showed that the order "Pseudophyllidea" consisted of two unrelated clades [1,2]. The members of the Bothriocephalidea are parasites of teleost fishes and comprise 46 genera distributed into four families, the Bothriocephalidae, the Echinophallidae, the Philobythiidae, and the Triaenophoridae [2]. *Clestobothrium* is a member of the family Bothriocephalidae, which includes seven other genera.

Numerous transmission electron microscope (TEM) studies have been published on the ultrastructure of cestode embryonic development, the important role of vitellocytes and the nourishment of cestode embryos, as well as on the great diversity of mature tapeworm eggs [3–15]. As far as we are aware, there have been TEM studies of the embryonic development and eggs of only four bothriocephalidean species, i.e. *Bothriocephalus clavibothrium* [5,12], *B. gregarious* and *B. barbatus* [16], and *Eubothrium salvelini* [8,17].

The aims of the present study are to describe the functional ultrastructure of the eggs, the associated vitellogenesis and the early intrauterine embryonic development of the bothriocephalidean cestode *C. crassiceps* (Rudolphi, 1819), to compare the results with those of similar studies on other lower cestode taxa, and in particular bothriocephalideans, and to consider any possible phylogenetic implications.

2. Materials and methods

Live adult specimens of *C. crassiceps* were collected from the intestine of the hake *Merluccius merluccius* (L., 1758) (Gadiformes: Merlucciidae) caught off Roses, Girona, Spain.

2.1. High-pressure freezing

The live cestodes were examined under a stereomicroscope and pieces of uterus were excised into small Petri dishes in PBS with 20% BSA and transferred into the cavity of a 200-μm-deep flat specimen carrier. The specimen holder was then inserted into the rapid transfer system, and high pressure frozen using a Leica EM PACT and stored in liquid nitrogen.

2.2. Freeze substitution and infiltration with resin

For freeze substitution, sample holders were transferred into pre-cooled cryovials (−120 °C) and freeze substitution was performed in anhydrous acetone containing 2% of osmium tetroxide. Using a Leica EM AFS, the samples were maintained for 24 h at −90 °C. Hereafter, the temperature was raised at a rate of 2 °C/h to −60 °C and then to −30 °C. The samples were maintained at each level for 9 h