

## Ultrastructure of intrauterine embryonic and larval stages of *Ityogonimus lorum* (Digenea: Brachylaimidae) involving transitory development of ciliated miracidia

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This report presents the first detailed ultrastructural analysis of the consecutive stages of embryonic development and miracidial morphogenesis taking place in the intrauterine eggs of the brachylaimid digenean trematode, *Ityogonimus lorum*. Specimens for this study were collected from an Iberian mole, *Talpa occidentalis*, captured in Priesca (Asturias, Spain). Adult trematodes were prepared by standard methods for transmission electron microscopy (TEM).

The results provide ultrastructural evidence that the embryogenesis and larvigenesis of this species are generally similar to those of other digeneans in which miracidial morphogenesis is fully completed within the intrauterine eggs. In contrast to other digeneans, the development of mature larvae of *I. lorum* involves transitory development of cilia on the tegumental surface of the miracidium stage, with zones of cilia separated by zones of thin tegumental processes, but with subsequent autolytic degradation of the cilia to result in a non-ciliated final larval stage. The transitory ciliated miracidia occur in the most posterior uterine region, but the final non-ciliated larvae occur in a more anterior location in the uterine region closest to the uterine pore. In this terminal uterine region, all ciliary structures in the larva appear to have been eliminated by several lysosome-like structures, appearing as areas of focal degradation in the final stages of larval development, which are localized between the eggshell and the miracidial body surface.

The ultrastructural characteristics of various organelles and cell types in the fully formed ciliated miracidia and in subsequent un-ciliated larvae are very similar. Two types of miracidial glands were observed: a single apical gland and two, more elongated, lateral glands. Each has characteristic but different types of secretory granules. The anterior end of each miracidium consists of an apical papilla on which are situated the individual exits of these three larval glands. Germinative cells, grouped together in a sac-like germinative follicle, are situated in the medioposterior part of the larva, the germatophore. Large nuclei of germinative cells contain large spherical nucleoli and numerous electron-dense heterochromatin islands arranged in a network having a chain-like pattern, distributed mainly in the karyoplasm adjacent to the nuclear membrane. A thin layer of granular cytoplasm is rich in free ribosomes and contains a few small mitochondria. It can be concluded that the presence of these three structures in the egg of this brachylaimid provides convincing evidence for completion of the entire larval maturation

in the intrauterine eggs of *I. lorum*. Unlike the case of most digeneans, flame cells, neurons, and nerve centers are absent from all these developmental stages of *I. lorum*. Absence of these may be related to a fully terrestrial life cycle of *I. lorum*, in which a free-swimming miracidium is not required, as eggs containing infective larvae are ingested directly by terrestrial gastropod hosts..