The use of different microscopic techniques for the study of monogenean parasite *Eudiplozoon nipponicum*

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Eudiplozoon nipponicum (Goto, 1891) (Monogenea, Diplozoidae) is a parasite from the gills of carp (*Cyprinus carpio* L.). Members of the family Diplozoidae are unique among platyhelminths in so far as two adult worms are fused in permanent copula, each individual being unable to survive alone. The life cycle of *E. nipponicum* begins when the invasive larval stage – oncomiracidium – hatches from the egg in water. After attachement on the gill the oncomiracidium develops into the unpaired post-larval stage – diporpa. Two diporpae come into contact, pair and fuse. The typical shape of the two fused parasites is like the letter X. The study of these stages has been made with the use and comparison of different microscopic techniques.

Light microscopy (LM). The live specimens of *E. nipponicum* were studied and documented using the light microscope and stereomicroscope systems Olympus BX 51 and, SZH 10 equipped with differential contrast (DIC according to Nomarski) and digital image analysis system (analySIS auto 5.0).

Histology. The whole specimens were fixed in AFA, dehydrated and embedded in paraffin medium (Histoplast). Sections were stained with Haematoxylin and Eosin (H&E) or with Masson´s Trichrome to make visible collagen fibers in tissues. The study was focused on internal parasite body structures and their changes during the development.

Confocal laser scanning microscopy (CLSM). Using FITC and TRITC-conjugated phaloidin as a specific probe for F-actin, applied to whole-mount preparations of *E. nipponicum*, the organization of the major muscular structures was examined. The body wall musculature is well-developed and highly organized, with lattice-like outer circular, intermediate longitudinal and inner diagonal muscle fibres. The buccal suckers, glandulomuscular organs and the pharynx are dominant muscle structures of the parasite's forebody. The buccal suckers are primarily formed by dense radial muscle fibres. The muscles of the pair glandulo-muscular organs are arranged basket-like. In the hindbody, two haptors, each with four pairs of clamps which enable the worm to secure its attachment to fish gills. Every clamp has groups of the muscle bundles of muscles, which ensure a sufficient mobility of the clamp's skeletal jaws.

Scanning electron microscopy (SEM). The SEM study of the surface has been made to describe tegument and tegumentary structures of all parasite developmental stages. The fixation methods and preparation of specimens were optimised. The optimal fixation method was preservation in hot 4% formaldehyde and 3% glutaraldehyde that fixed the parasite body in a straight position and conserved surface structures with the exception of fine cilia. For conservation of ciliate structures the use of the same fixation but at room temperature is more suitable. After fixation, the samples were dehydrated, critical point dried, sputter-coated with gold and observed under VEGA SEM (Tescan). The egg with long filament is smooth and without superficial structures. The attachment apparatus of unpaired diporpa parasitic the gills of the host fish is not fully developed. Typical structures of this parasite stage are the

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dorsal papilla and ventral sucker which are responsible for the first contact between diporpae during their pairing. On the ventral side of the forebody of the adult specimen is situated the large mouth. The uniciliated structures that may be sensory are found arround the mouth. The tegument of the parasite´s forebody is highly folded forming transverse ridges. The dorsal and ventral surfaces of the forebody are covered with many ciliated structures. The uterus openings of both partners are situated on the ventral surface in the region of fusion. Transverse tegumentary ridges on the parasite hindbody are highly developed. Running longitudinally along each lateral side of the hindbody, there is a row of papillae-like structures.

Transmission electron microscopy (TEM). The parasites were fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide, dehydrated and embedded in eponaraldite resin. The ultrathin sections were examined in Morgagni TEM (FEI). A detailed TEM study was necessary to clarify the ultrastructure and function of the tegument and structures recognized by SEM.

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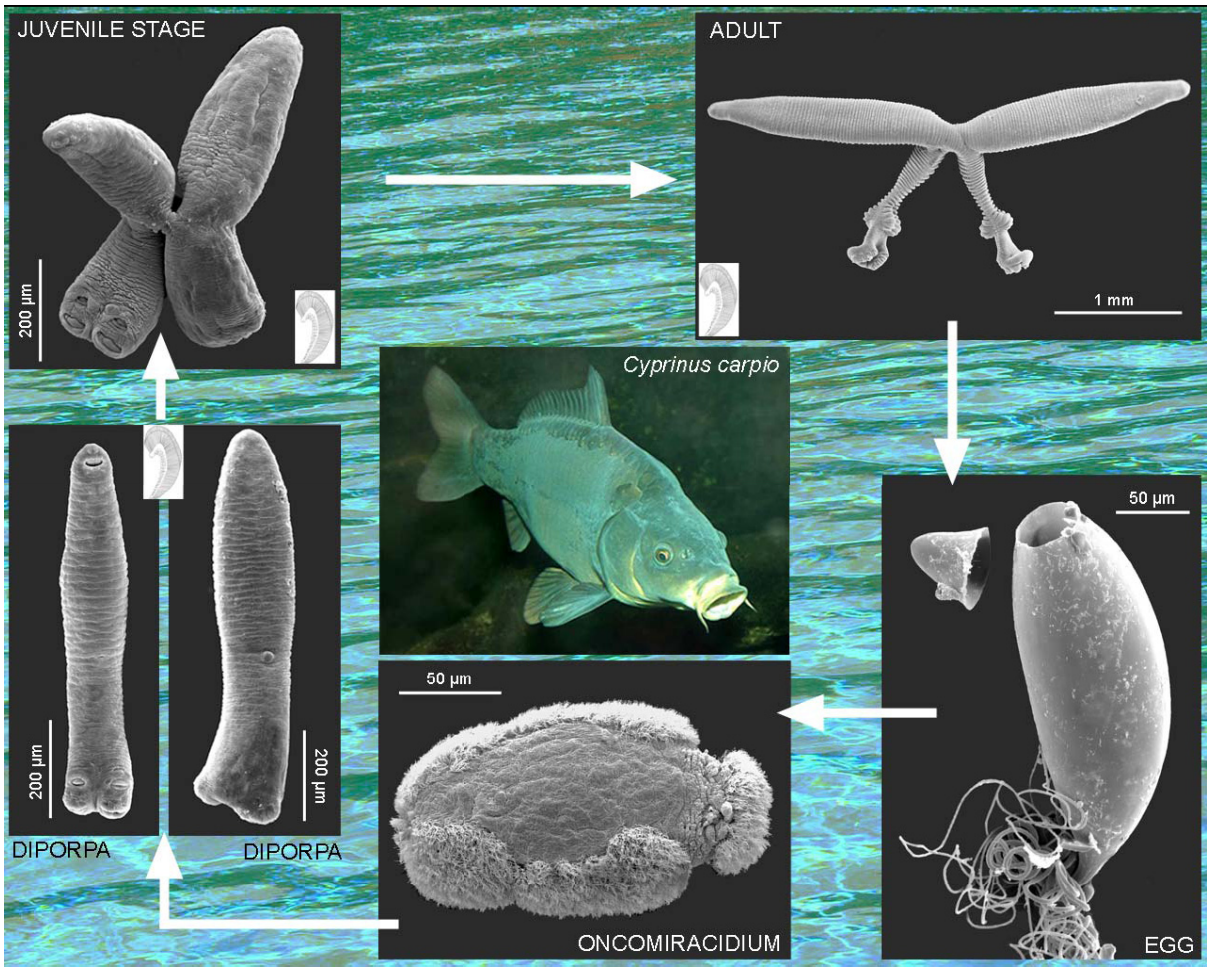

Figure 1. The life cycle of the parasite *Eudiplozoon nipponicum*.