Body Architecture of the Parasitic Worm

Visualization of Muscular Structures by LM, SEM and CLSM

Diplozoid monogenean *Eudiplozoon nipponicum*, with complex life cycle comprising oncomiracidium, diporpa, juvenile and adult stage, represents an ideal model for studies on morphological adaptations of metazoan parasites to the ectoparasitic life style. Combined morphological approach offers the advantage of more complex characterization and visualization of structures noticeable only when using specific microscopic method.

Crucial Questions to be Answered when Performing Scientific Research

Biology as a multidisciplinary science concerns with the study of living organisms, including their origin, evolution, distribution, development, structure, and function. Sub-disciplines of biology are further defined by studied organisms and methods used in the study. For example, functional morphology tries to answer how anatomical structures work or collaborate to allow the living organisms to undertake certain tasks and using this approach can be very conducive to examine the evolution and ecology of investigated species. Thus, understanding the functional morphology from cell to organ system is fundamental to all of the biological sciences.

There are several essential questions that need to be satisfactory answered when designing a scientific experiment. How to capture the audience’s attention? The scientist should come up with an attractive hypothesis and choose the right way how to get clear evidence for the drawn conclusions. What is the working hypothesis of the study? It is believed that process, how organisms developed during evolution and maintained themselves in changing conditions, is repeated in ontogeny of every creature living today, i.e. ontogeny recapitulates phylogeny.

Parasites represent an ideal model organism to validate this hypothesis. These creatures undergo significant changes during their life cycle, in which all functions and structures not needed for their survival are usually reduced, whereas features facilitating parasites’ fixation to host, nutrition as well as reproduction are further specialized and become even more prominent. It is presumed that parasitic organisms have undergone similar process during their evolution.
Similarly to parasites benefiting from their host, man reduces all biological functions and senses not needed nowadays.

An insight into how some of these organisms become parasitic and gradually adapted to the host environment could explain the lazy behavior of human being in a recent world full of convenience due to technical advance. Does selected method fit the research goal? Combined approach is an effective way to insure that all experiments conducted are accurate and the best possible outcomes may be found. For the needs of functional morphology of parasitic worms, microscopic methods are the best suited when using correct technique to visualize target structures. Therefore a combination of electron and light microscopy, including the fluorescence, gives reliable results by providing a more complex view insight parasite’s general biology. Our goal has been to develop combined approach that will, among other things, facilitate veracious multidimensional depiction of the parasite’s body organization.

The Need for a Combined Microscopic Approach

_Eudiplozoon nipponicum_ is an oviparous, blood-feeding monogenean ectoparasite of carp. This parasite is unique in its pairing strategy, in which two sexually immature stages (diporpae) fuse together with subsequent rearrangement and interconnection of their nervous systems. After somatic fusion, the two individuals grow together and survive only as a pair fused in permanent copula, i.e. they act as a single organism [1-4]. Such a reproductive strategy, in which two independent heterogenic individuals fuse into a single hermaphrodite organism without the need to search for mating partner, represents a high specialization to the parasitic life.

Combination of light microscopy (LM) and histological sectioning allows visualization of specific structures depending on the used staining procedure. To
study the interactions between the parasitized fish gills and attached parasite, the three-color Masson’s trichrome staining, suited for distinguishing cells from surrounding connective tissue, has been performed. The **scanning electron microscopy** (SEM) is based on using a focused beam of high-energy electrons in a raster scan pattern to generate a variety of signals at the specimen’s surface. For biological specimens, the emission of low-energy secondary electrons is detected to visualize their surface topography. The signal from the lower situated areas is less intensive than those from the higher sites and thus imagined structures appear darker. **Confocal laser scanning microscopy** (CLSM) is a microscopic technique for obtaining high-resolution images with depth selectivity by a process called optical sectioning. The basic principle is that the light from the focused plane is detected, whereas the scattered light from out-of-focus planes is excluded, producing remarkably detailed images. Images are acquired point-by-point and then can be three-dimensionally reconstructed using a specialized software. Using specifically binding fluorescent probes and direct localization of labeled structures by fluorescence, this approach facilitates 3D non-invasive examination of target object.

The muscle tissue is present in majority of body organs or structures. In muscles, actin is the major protein component of thin filaments. These are, together with the motor protein myosin, arranged into myofibrils comprising the mechanism of muscle contraction. Since the phalloidin from the death cap (commercially known as phalloidin) binds to filamentous actin, its conjugate with fluorophore allows the fluorescent visualization of musculature.

Microscopic analyses have confirmed our hypothesis that complicated structure of parasite’s tegument (fig. 2) supports its firm fixation to the host gills and represents an obvious evolutionary adaptation of previously free-living non-parasitic worm to the parasitism. During this process a complex mechanism dedicated to parasite’s attachment to the host and its localization on host body that is advantageous for feeding and reproduction has evolved. Attachment system comprise the prominent buccal suckers (fig. 4A, 4B and 5A) at the ventral site of the parasite’s forebody along with the two well-developed muscular haptors (fig. 2, 3A-C, 5B), each with four pairs of clamps in two rows and two central hooks, located on the hindbody. As shown by CLSM (fig. 3B, 5B) and confirmed by histological sectioning, each clamp is operated by groups of muscle bundles, ensuring sufficient mobility of the clamp skeletal jaws.

Mentioned buccal suckers seem to assist in the parasite's translocation while searching for an optimal niche and we speculate about their temporary attachment function during feeding as well. They appear to be located in a buccal capsule and
probably evert when needed (e.g. while feeding), resembling the fingers of gloves. Supporting attachment structures can be found at the surface of tegument, such as tegumental folds and prominent lobular extensions in the middle part of hindbody (fig. 2). The lobular extensions along with the tegumental folds facilitate the parasite's firm fixation to the host gills by locking to the gill lamellae.

In addition to mentioned adaptations, the parasite exhibits a complex digestive tract well equipped for hematophagous feeding, consisting of subterminal mouth opening (fig. 4C), eversible pharynx (fig. 4A and 5C) with adjacent glandular structures, and a blind-ending gut with cecal lining. The prominent musculature forming the pharynx supports the idea about its sucker function during feeding on host blood. Glandulo-muscular organs (fig. 4B), located apically and opened into the mouth corner, are considered to be a part of the digestive tract but their real function still remains enigmatic. Interestingly, the CLSM with phalloidin labeling has been the first method that has drawn attention to these previously unnoticed structures.

**Conclusion**

In conclusion, the combination of several microscopic techniques has been proved as a powerful tool to exactly map the parasite’s body architecture, because it allows visualization of structures noticeable only when using specific method, and thus offers a complex insight into the body organ morphology and arrangement.

**References**


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