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Electron Microscopic Studies on the Sclerites of *Dactylogyrus extensus* Mueller & Van Cleave, 1932 (Monogenea, Dactylogyridae) an ecto parasite from *Cyprinus carpio* L.

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Abstract

Dacylogyrus extensus is a highly pathogenic monogenea among cyprinid fish than *Dactylogyrus vastator.* Discrimination of the related parasite always carried out through light microscopy and electronmicroscopy. Therefore the release of sclerites free from attached musculature is vital.

Using sonication technique and subsequent scanning electron microscopy (SEM) the functional morphology of the attachment and copulatory sclerites of *Dactylogyrus extensus* extracted from *Cyprinus carpio* L. was studied. Ultrasonication technique to release the hamuli and marginals has also allowed for the description of several previously unrecorded structures. These included two specialized sites of attachment for the auxillary sclerite on the hamulus and the form of copulatory organ and marginal hook. The hamuli of immature dactylogyrid worms can be discriminated from those of adult worms on the basis of the extent of development of the auxillary sclerite attachment of the hamulus.

Key words: Copulatory sclerite, *Dactylogyrus*, Electronmicroscopy, Hamulus, Marginal, Sonication.

Introduction

Dacylogyrus extensus is a highly pathogenic monogenic trematode parasite on cyprinid fish than *Dactylogyrus vastator*. *D. extensus* causes high mortality at the fingerling and fry stages thereby causing economic losses to the carp farming industry. The identification of monogeneans relies on the size and shape of the sclerotised parts of the opisthaptor, as principally seen through the light microscope. However, the minute details can be seen through the confocal microscopy and electron microscopy. The sclerotised structures are surrounded by tissues and their proportions can be misinterpreted and distorted by fixation and slide preparation. There are relatively few published accounts of mongenean attachment mechanisms conducted at the scanning electronmicroscope level (SEM) [1,2,3,4,5,6,7,8,9,10,11,12]. This is the first study on the external morphology of dactylogyrid sclerites, since El_Naggar's paper concentrated on TEM examinations of *Cichlidogyrus* hooks [1]. Mo and Appleby used the enzymatic digestion of freshly collected worms to free the sclerotised parts of the opisthaptoral sclerites from live monogenan worms with main focus on *Gyrodactylus* sp [5]. In this technique, certain structures associated with the sclerites such as muscle caps on hamuli and marginal hooks are retained. The sonication technique applied to specimens of *Dacylogyrus extensus* allowed close examination of these sclerites free from surrounding tissue. In this study the details of the opisthaptor is studied with the main focus on the identification of the related worms with the reliability towards identification since some may cause detrimental impact for aquaculture.

Materials and Methods

Dactylogyrus extensus attached to individual gill filaments were removed from infected carp *Cyprinus carpio* placed in a 1:1500 solution of phenoxyethanol and the parasite allowed to detach. An ultrasonication technique was used to produce debris-free sclerites from active, live worms which were then viewed using scanning electronmicroscopy (SEM) following the procedures of Shinn *et al*. [12].

Results

The opisthaptor of *D. extensus* is having a pair of hamuli and 14 marginals. *Hamulus*

The opisthaptoral complement of 2 hamuli and 14 marginal hooks well documented in the literature were clearly evident (Fig 1).

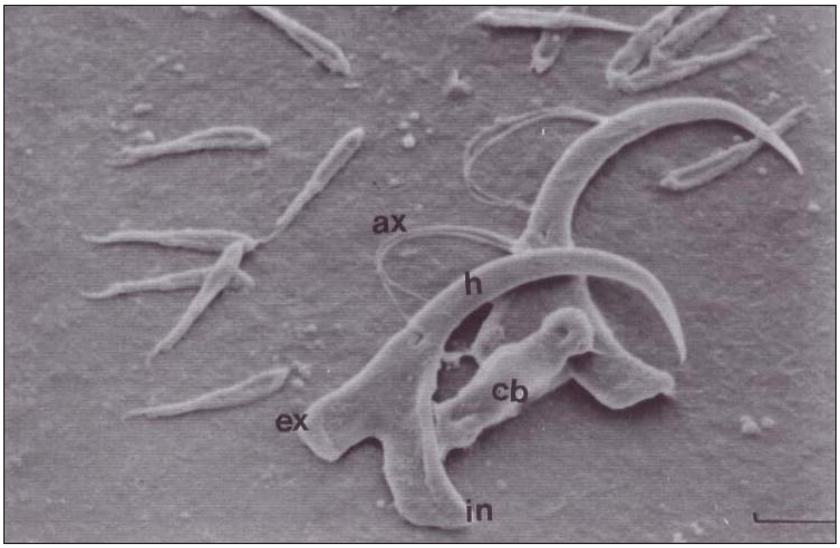

Figure 1: SEM of the opisthaptoral complement of *Dactylogyrus extensus* – Scanning electron micrograph (ax-auxiliary sclerite; cb-connecting bar; h-hamulus ex- external root process; in–internal root process) Scale bar = 10 μm

Each hamulus has a bifurcate root, with an internal and external root process which joins to form a curved shaft which tapers to a spike (Fig 1). The hamuli represent the main means of attachment, being deeply embedded in the gill tissue and penetrating through the primary lamella cartilage. The ventral surface of the internal root and the proximal part of the shaft of the hamulus has a thickened margin of 1.7 μm (Fig 2). The external and internal root processes of the hamuli are provided with attachment surfaces for muscles. The ends of both the external and internal root processes are provided with a thickened cap which is about 3.8 μm in thickness. The hamuli have longitudinal striations. The point of the hamulus is characterized by a 30˚ turn of its very end.

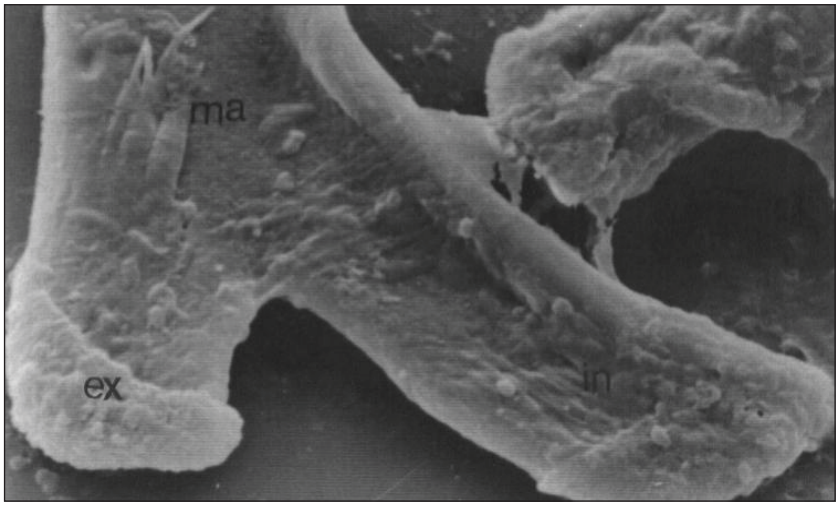

Figure 2: Scanning electron micrograph of the basal part of the hamulus showing the external and internal root processes and their muscular attachment surfaces. (ex - external root process; in- internal root process; ma- muscular attachment surface.) Scale bar = 10 μm.

Auxillary Sclerite

The auxillary sclerite is seen as a double filament extending from the dorsal surface of the hamulus shaft near its base to the region where the hamulus point begins to curve away from the hamulus shaft. In mature adults the auxillary sclerite has a double origin from the outer and inner surfaces of the hamulus shaft, apparently linked through a 7.5 μm diameter hole or pore in the shaft region, but terminates nearer to the origin of the hamulus point. In mature worms the two components of the sclerite remain separate throughout their length, although they attach to the same point on the dorsal surface of the hamulus. The auxiliary sclerite is slightly thickened at the point the sclerite emerges from the pore in the hamulus. The two filaments that make up the auxiliary sclerite narrow, but gradually thicken as they approach their site of attachment on the hamulus shaft. The two filaments of the auxillary sclerite are fused to produce a continuous structure in the adult which passes through a ring structure on the hamulus shaft. This ring structure can be clearly seen in Fig 1. The ring is highly thickened and spread out over the hamulus at its base to provide a secure structure.

Connecting Bar

Separating the two hamuli and presumably playing an important role in maintaining their overall position within the opisthaptor, is a connecting bar (Figs 1 and 3).

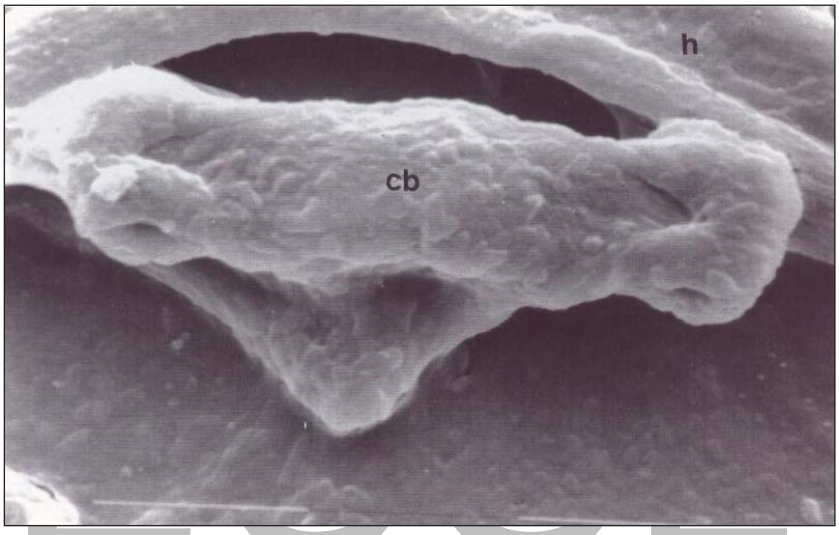

Figure 3: Scanning electron micrograph showing the connecting bar attached to the hamuli at point where the internal and external root processes join together. (cb – connecting bar; h- hamuli) Scale bar = 10 μm

This substantial structure is attached to the flattened base of the shaft. The bar consists of three main elements, a central bar, thickened bulbous ends and a flattened triangular process ventral to the main bar. The thickened process at either end must serve as the point of attachment for the articulation of the whole connecting bar with the hamuli. The connecting bar is apparently hinged with the hamuli by ligaments.

Marginal Hooks

The marginal hook consists of three elements; sickle or blade, shaft of the handle and the handle proper (Fig 4).

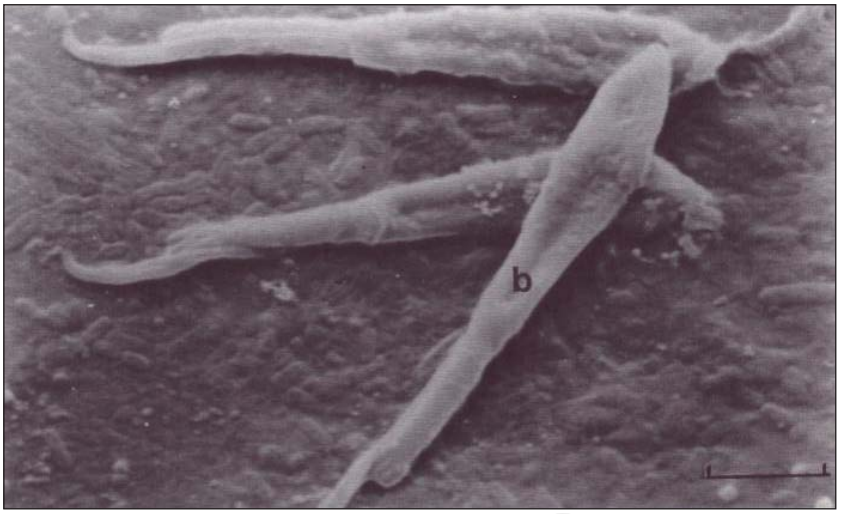

Figure 4: Scanning electron micrograph showing the marginals of *D. extensus.* (b= blade; sp = spike) Scale bar 10 µm

The marginal hook has a long, slender blade which turns through 90˚ and stops abruptly to give a sharp short point. The process of the blade or "heel" is unpronounced and represents a small elevation before turning into the shaft of the handle. These two features of the marginal hook give its inner curve a rectangular appearance. The shaft of the handle is uniform along its length and represents quarter of the total length of this sclerite. Proximally, there is a thickened but laterally flattened handle, with a depression running along each side. The handle makes up over half the total length of the marginal hook. A filament loop can be seen extending from the marginal hook blade.

Copulatory Organ

Extraction of this structure reveals an L-shaped copulatory tube proper and a straight supporting bar. The end of this supporting tube is curved with an apparent central groove. The inner curve of the supporting bar is marked by a toothed edge. The copulatory tube proper differs from Gussev's description and terminates with a bi-prolonged hooked structure [13].

Discussion

In *D. extensus* immediately prior to attachment to the host, the worm positions itself between gill filaments such that the entire ventral surface of its opisthaptor is in contact with one gill filament [14]. It was found that dactylogyrids required approximately 55 minutes to release their sclerites compared to 1-20 minutes for various species of *Gyrodactylus* (1 minute for *Gyrodactylus gasterostei* Gläser, 1974 and 20 minutes for *Gyrodactylus derjavini* Mikailov, 1975). This depends of the arrangement of the sclerites and the attached musculature.

The haptor then compresses allowing the hamuli to rotate outwards and flatten. The hamuli are then rapidly contracted causing the points to be drawn back together. It is essential to note that attachment into soft tissues only with the hamuli may not be durable enough. The hamuli will tend to tear the soft tissues of the gill filaments and the worms may then be easily washed off by the water current. This is avoided by penetration of the cartilage of the primary gill lamellae. The external and internal root processes of the hamuli of *D. extensus* are provided with muscular attachment surfaces which must allow the hook to move back and forth while achieving its function of anchoring the worm to the primary gill lamellar cartilage. The interlocking striations found on hamuli shaft presumably increase the strength of attachment by providing a non-smooth surface in contact with host tissue. Interlocking striations have also been reported on the hamuli of *Gyrodactylus salaris* Malamberg, 1957 such striations would allow the hamuli points to indirectly grip the cartilage on insertion [5].

Sonication has enabled sclerites to be freed from surrounding tissue and the retention of certain structures that would otherwise be lost when using proteolytic enzymes. SEM examination of the sclerites from *D. extensus* has revealed the association of the auxiliary sclerite with the hamulus. Although included in taxonomic drawings of *Dactylogyrus* sp., the function of the auxiliary sclerite attached to the hamulus is poorly understood. If this auxiliary sclerite is a complete loop passing through the hamulus then the scelerite is well suited to being thinkened in this region to prevent the sclerite snapping as the filaments abrade against the walls of the hamulus pore [15]. Although the benefits of the sonication

technique are clearly evident, there is however, one drawback to the sonication technique in that whole hook sets are not preserved but are separated during the sonication process. Such sclerite sets may provide vital information to the state of maturity of a parasite in question. The age or state of maturity of a parasite is usually gauged by the extent of development of the copulatory organ cirrus structure etc. However, the development of the auxiliary sclerite can also used to provide a measure of the parasites age. The precise function of the auxiliary sclerite is still poorly understood.

Marginal hooks, situated in the periphery of the haptor, are anchored by muscle to their blades allowing them to move independently of the hamuli. It is believed that the filament loop attached by some means to the marginal hook, like in *Gyrodactylus* [12] is closely associated with a groove running along the blade; however, high power SEM examination of this region has so far failed to confirm this.

The description of the copulatory organ of *D. extensus* in Gussev [13] given as "the tube of copulatory organ in the form of L and the supporting bar of the copulatory organ with expanded ends". However, following examination of the copulatory organ of *D. extensus* with the SEM, it appears that there are some irregularities with the drawings of Gussev [13]. The toothed edge was also recorded by Maillard *et al*., following the extraction of the copulatory sclerite of *Diplectanum aequanus* [15]. It is suggested that the curved end of the supporting bar achieves its function by looping over the lower portion on the copulatory tube proper. The serrations on the lower edge of the supporting bar effectively lock the copulatory tube in position. No such association or explanation of how the supporting bar achieves its function is given in Gussev's [13] account. The ends of both portions of the copulatory organ are sharp rather than rough which might be expected with deterioration through excessive sonication.

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