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# Occurrence of Cholinesterase and Ciliated Sensory Structures in a Fish Gill-Fluke, *Diclidophora merlangi* (Trematoda: Monogenea)

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*Summary.* Cholinesterase has been demonstrated histochemically in the nervous system of adult *Diclidophora merlangi*. Reactivity for the enzyme was strongest in the cerebral ganglia and commissure, main ventral nerve cords, and nerve plexuses of the suckers, pharynx and cirrus organ. Cholinergic components were found both in motor fibres innervating muscular tissue and in subtegumental nerve plexuses whose free endings may have a sensory function. A number of such endings were found in the tegument. Electron microscopic examination of the tegument revealed a sensory structure composed of a cilium which extends beyond the tegumental surface from a structure resembling a nerve terminal. The sensory structures occur singly and are believed to have a tactile function.

*Zusammenfassung.* Cholinesterase konnte histochemisch im Nervensystem adulter Würmer der Art *Diclidophora merlangi* nachgewiesen werden. Die stärksten Reaktionen zeigten die Cerebralganglien und Commissuren, vorwiegend im ventralen Nervenhauptstrang und im Nervenplexus der Saugnäpfe, des Pharynx und des Cirrus. Cholinergische Komponenten wurden sowohl in den motorischen Nervenfasern der Muskulatur wie im subtegumentalen Nervenplexus nachgewiesen, dessen freie Nervenenden sensorische Funktionen haben dürften. Eine Anzahl solcher Nervenendungen wurden im Tegument gefunden.

Die elektronenmikroskopischen Untersuchungen des Teguments deckte eine Struktur auf, die mit einem Cilium besetzt ist, das über die integumentale Oberfläche hinaus geht und der Struktur einer Nervenendung ähnlich ist. Die sensorischen Strukturen treten einzeln auf und haben vermutlich taktile Funktionen.

## Introduction

The nervous system of at least one monogenetic trematode has been shown histochemically to contain cholinesterase. Halton and Jennings (1964), using an indoxyl esterase method and selective inhibitor, demonstrated the enzyme in the nervous system of whole-mount preparations of a fresh-water gill fluke, *Diplozoon paradoxum*. Negative results were obtained, however, when the method was attempted with a related gill fluke, *Discocotyle sagittata*.

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The present investigation was made primarily to see if similar methods could be used to demonstrate nerve elements in a marine gill fluke, *Diclidophora merlangi*. Initial findings were positive and, in addition, revealed a number of cholinesterase-positive nerves ending apparently in the external tegument (cuticle) of the worm. Parts of the tegument were examined with the electron microscope in order to define more clearly the nature of these nerve endings.

## Materials and Methods

Live worms were collected from the gills of infected whiting (*Gadus merlangus*), caught in the Irish Sea, and immediately fixed in 10 per cent formalin buffered to pH 7.3 with 0.1 M phosphate. A number of specimens were lightly flattened between glass slides and fixed for subsequent examination as whole mounts. After fixation at 4° C for 3 hours specimens were washed in two 15 minute changes of phosphate buffer. Frozen sections (10—15 μ thick) were cut with a microtome fitted with a freezing stage, and air-dried on glass slides prior to incubation. Whole mounts were transferred directly to incubation media. Cholinesterase activity was demonstrated in sections and whole mounts using the indoxyl acetate or the acetyl — or butyryl-thiocholine iodide methods (Pearse, 1960). In all cases, incubation was for 12 hours at 20° C and controls were incubated without substrate. Eserine sulphate, used as an inhibitor of cholinesterase, was included in a test medium at a final concentration of 10 μM.

For electron microscopy, live worms were cut into thirds and fixed for 3 hours at 4° C in 3 per cent glutaraldehyde buffered to pH 7.3 with 0.2 M phosphate, containing 0.5 mM calcium chloride and 3 per cent sucrose. Tissue was then washed in three 20 minute changes of phosphate buffer containing 5 per cent sucrose, post-fixed in 1 per cent osmium tetroxide buffered to pH 7.3 with phosphate, dehydrated and embedded in Maraglas. Ultra-thin sections were cut with glass knives on an LKB Ultrotome, mounted on bare copper grids, and finally double-stained with uranyl acetate and lead citrate for examination in an AEI EM6B electron microscope at 60 kV.

## Results

### *Light Microscopy*

Cholinesterase activity was found throughout the nervous system of *D. merlangi*. The enzyme hydrolyzed acetylthiocholine iodide more readily than the butyryl derivative suggesting that some of the activity was due to the presence of acetylcholinesterase. In all cases, the enzyme was completely inhibited by eserine sulphate.

Fig. 1. *D. merlangi*. Anterior region of a whole mount showing staining for cholinesterase in the nervous system. Note intense reaction in the cerebral ganglia (*CG*) and commissure (*CO*), anterior and posterior ventral nerve cords (*VC*), buccal suckers (*BS*), pharyngeal lips (*PH*) and cirrus (*CI*). Arrows indicate the band of staining due to reactivity in the subtegumental musculature of that region. *BP* bipolar nerve cell, *UP* unipolar nerve cell, *DC* dorsal nerve cord, *LC* lateral nerve cord, *TC* transverse connections. Indoxyl acetate. ×75

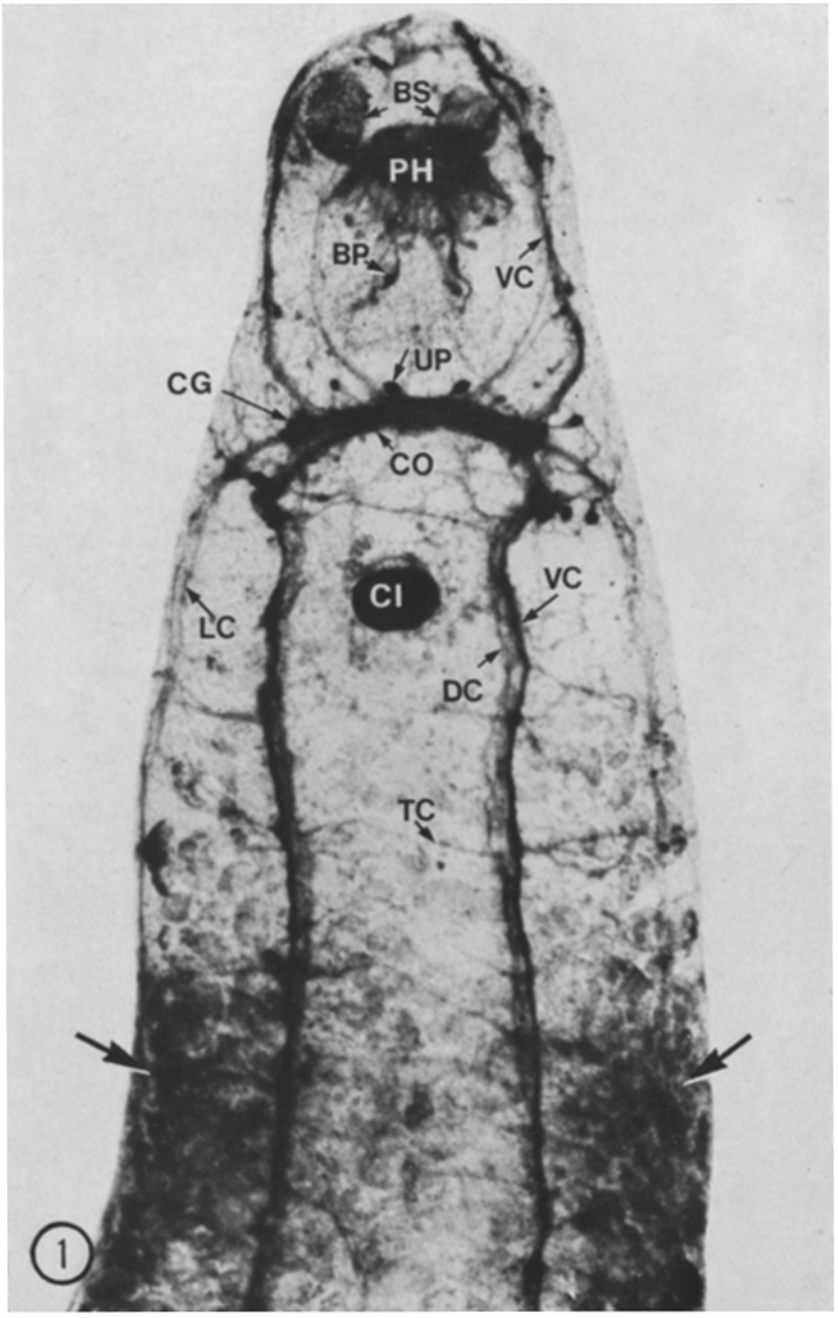



Fig. 1

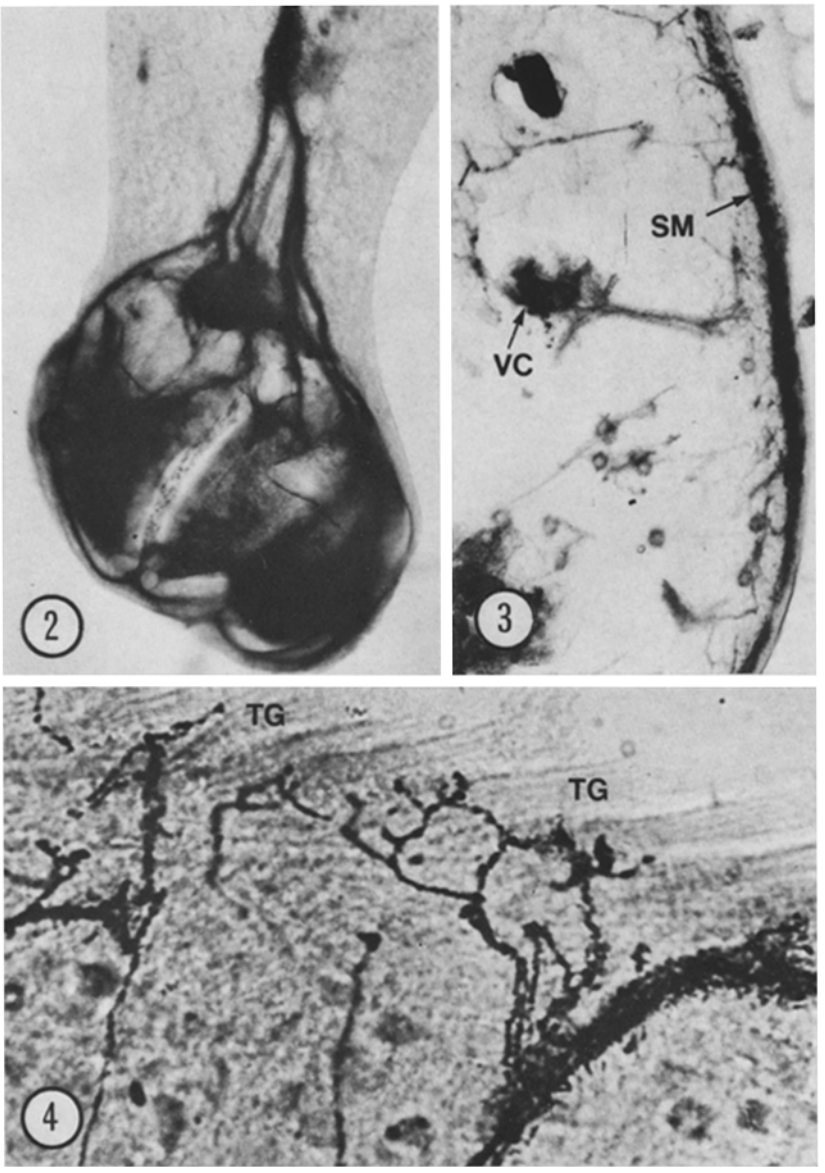



Fig. 2. *D. merlangi*. Whole mount of part of the opisthaptor showing staining for cholinesterase in the innervation of the clamp musculature. Indoxyl acetate. ×120

Fig. 3. *D. merlangi*. Transverse section showing cholinesterase activity in part of the subtegumental musculature (*SM*). Note staining in the ventral nerve cord (*VC*) and its branches. Acetylthiocholine iodide. ×150

Fig. 4. *D. merlangi*. Transverse section showing a subtegumental nerve plexus with free nerve endings both in and in close proximity to the tegument (*TG*). Acetylthiocholine iodide. ×520

Fig. 1 shows staining of the nerves in the anterior part of a whole mount of the worm. Regional differences in staining were observed and reactivity was strongest in the cellular components of the nervous system and at sites where there are large concentrations of nerve fibres. Thus an intense reaction was given by the cerebral ganglia which lie immediately posterior to the pharynx and comprise two fairly loose groups of 6—8 cells connected by a broad commissure of fibres (Fig. 1). No cellular elements were observed in the commissure but, anteriorly, fibres connect to a pair of intensely-staining unipolar nerve cells (Fig. 1).

Intense staining was also seen in the nerve plexus and bipolar nerve cells of the pharynx and in the nerve plexuses in the musculature of the two buccal suckers and cirrus organ (Fig. 1). A nerve ring encircles the mouth and this and associated nerve cells reacted strongly with the substrate.

The main anterior nerve cords and the ventral pair of posterior fibres and their connections stained strongly as did the fibres that innervate the clamps of the opisthaptor (Fig. 2). These latter fibres arise from the ventral nerve cords and run as single fibres to each of the eight clamps where they subdivide and anastomose as a plexus in the suckers. In contrast, only weak to moderate staining was displayed by the dorsal and lateral posterior nerve cords and their transverse connections.

Whole mounts that had been incubated in test media invariably showed a band of cholinesterase activity in a region approximately one third of the way down the body (Fig. 1). This reaction was particularly pronounced in the lateral margins and examination of sections showed that the subtegumental musculature in this region, in contrast to that elsewhere, stained strongly for the enzyme (Fig. 3). The significance of this is not clear, but both the tegument and subtegumental region in *D. merlangi* are well supplied with nerves arising as lateral branches from the main longitudinal cords, and innervation of the region in question is particularly rich (Fig. 3).

Numerous nerve plexuses were found with free endings in close proximity to the tegument (Fig. 4) and although specialized sensory structures were never resolved with the light microscope a number of cholinesterase-positive endings were seen in the tegument.

### *Electron Microscopy*

Electron microscopic examination of the tegument revealed a number of ciliated processes connected to structures resembling nerve endings. The processes occurred singly and although the majority observed were found in the mouth region a number were seen in other parts of the tegument.

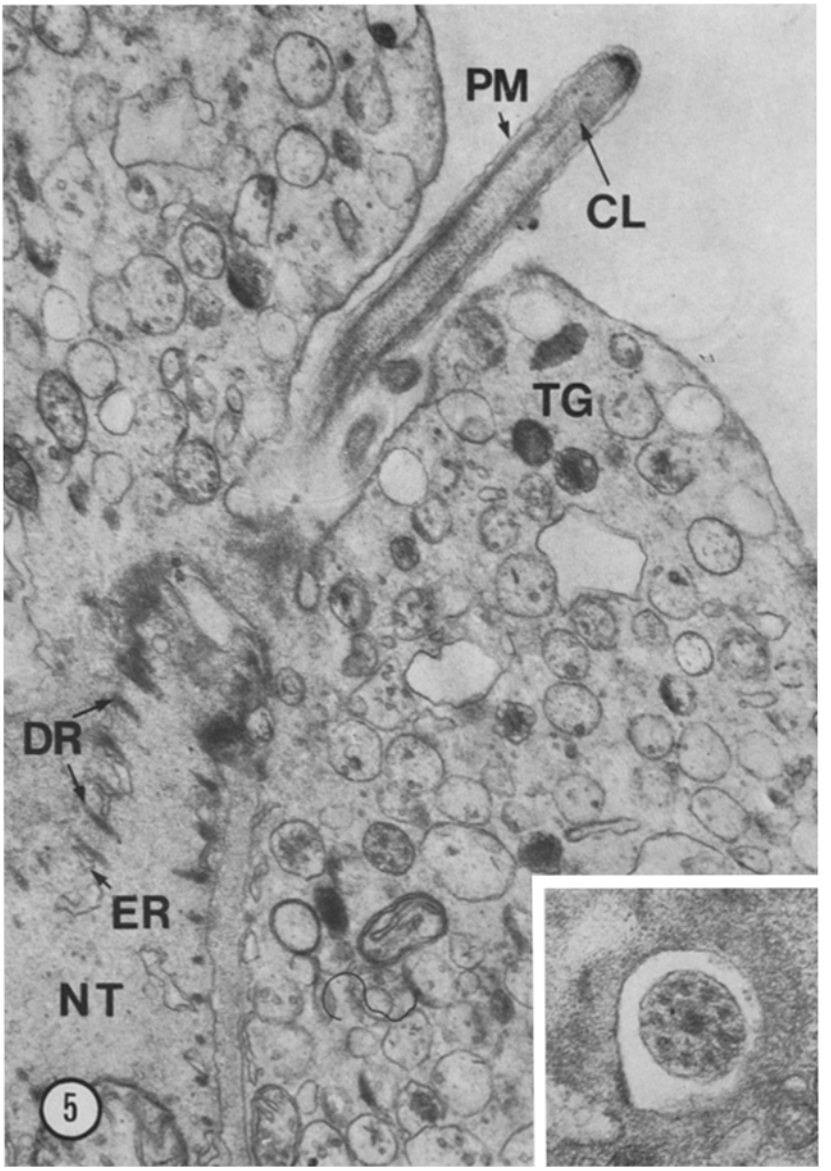
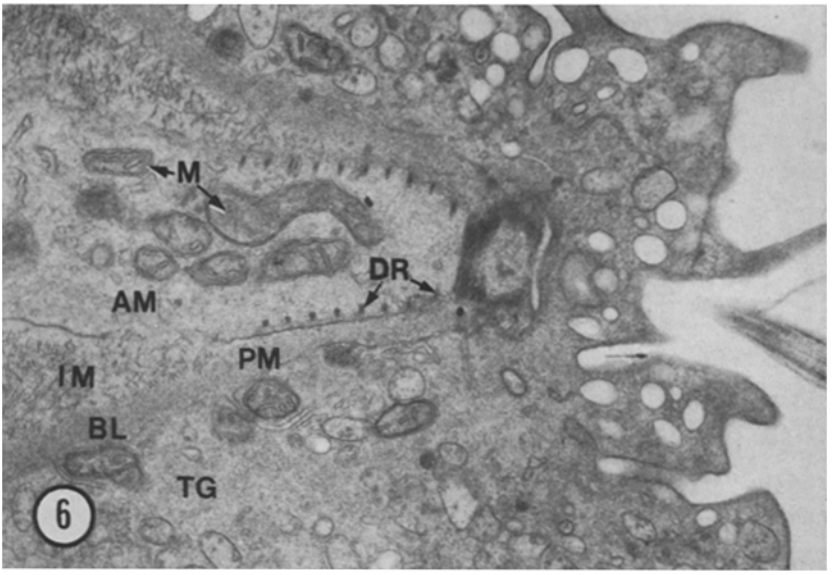



Fig. 5. *D. merlangi*. Electron micrograph of a section through part of a ciliated sensory structure showing the projecting cilium (*CL*), plasma membrane (*PM*), surrounding tegument (*TG*) and nerve terminal (*NT*). Note profiles of smooth endoplasmic reticulum (*ER*) between some of the dense rings (*DR*). ×28,000. Inset: Transverse section of a cilium showing nine outer fibre units. ×35,000



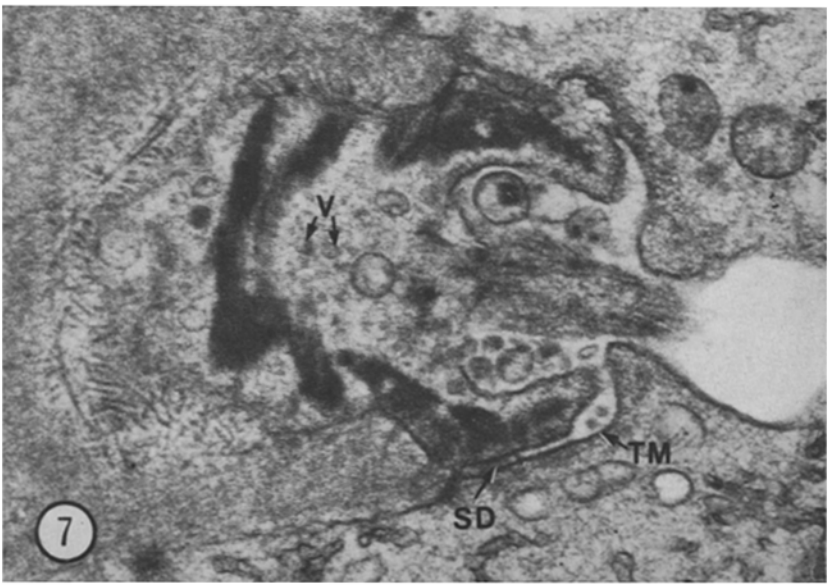




Fig. 6. *D. merlangi*. Electron micrograph of a section through the nerve terminal of a sensory structure. Note the sections of dense rings (*DR*) which lie just within the plasma membrane (*PM*), the amorphous matrix (*AM*) and numerous mitochondria (*M*). The terminal is surrounded by fibrous interstitial material (*IM*) and, distally, by the basal lamina (*BL*) of the tegument (*TG*). ×20,000

Fig. 7. *D. merlangi*. Electron micrograph of a section through part of a sensory structure showing the basal region of the cilium. Note the vesicles (*V*) in the matrix of the terminal and the septate desmosome (*SD*) between the tegument membrane (*TM*) and plasma membrane of the terminal. ×33,000

Each process (Fig. 5) comprises a single cilium covered only by a unit membrane and, when viewed transversely (Fig. 5, inset), shows nine outer units. The cilium has a normal basal body but no rootlet. Below the cilium the process broadens into a structure, presumed to be a nerve-ending, containing mitochondria (Fig. 6) and varying numbers of small vesicles with lightly-dense contents (Fig. 7). These organelles are embedded in an amorphous granular matrix enclosed by a plasma membrane which is continuous with that overlying the cilium. An apical septate desmosome which may be ring-like unites the plasma membrane of the nerve-ending with that of adjacent tegument (Fig. 7). Basally, a gap of about 800 Å separates the two membranes and is filled with an extension of the basal lamina, below which the nerve-ending is surrounded by fibrous interstitial material. At least 10 dense rings, measuring approximately 500 Å in diameter, lie within the matrix of the nerve terminal and run around its outer edge (Figs. 5—7). They are spaced along the sides of the structure at intervals of 800—1,000 Å being separated from the plasma membrane by a gap of 400—500 Å. In some sections (Fig. 5), short profiles of smooth membraned cisternae were observed between a number of rings.

## Discussion

The finding of cholinesterase in the nervous system of *D. merlangi* adds to the now substantial biochemical and histochemical evidence (reviewed by von Brand, 1966) for widespread occurrence of the enzyme in parasitic helminths. Histochemical data indicate that in most cases the enzyme is localized within nerve elements suggesting a functional role in neuromuscular activity is possible.

In *D. merlangi*, cholinergic components are apparently present not only in motor elements innervating muscular organs such as buccal suckers, pharynx and clamps, but also in subtegumental nerve plexuses whose free endings may have a sensory function. In this connection it is interesting to note that although it has not been conclusively shown that the sense receptors in *D. merlangi* contain cholinesterase, the enzyme has been demonstrated (Halton, 1967) in sensory bulbs that terminate nerve fibres in the tegument of adult *Fasciola hepatica*.

Light microscopic studies on the sense receptors of both larval and adult trematodes are well documented (Rohde, 1968). In addition, ultrastructural observations on tegumental receptors have been published for the cestode, *Echinococcus granulosus* by Morseth (1967) and digenetic trematodes, *Schistosoma mansoni* by Morris and Threadgold (1967), *Cyathocotyle bushiensis* by Erasmus (1967) and *F. hepatica* cercariae by Dixon and Mercer (1965). The finding of tegumental receptors in *D. merlangi* extends this list of ultrastructural observations to include a monogenetic trematode.

All the terminal cilia described, except that of *S. mansoni*, are covered only by a plasma membrane and those of *E. granulosus* and *C. bushiensis* have an extensive rootlet system at their bases. In *S. mansoni* both cilium and apical bulb are covered by tegment.

The above observations on parasitic helminths, including the results of this study, describe receptors which are of the same fundamental type: an apical bulb-like structure which possesses a dorsal cilium and is connected to a nerve trunk or dendron. However, Rohde (1966) has described a wide variety of sense receptor types in the tegument of the aspidogastrid trematode, *Multicotyle purvisi*. At least ten classes of sense receptor are characterized and although there appears to be considerable overlap between the classes there is no doubt that some variety does exist. For example, multiciliate or compound sensillae have been found in the tegument of the head region of a number of monopisthocotylean Monogenea (pers. comm. Dr. K. Lyons). Similar sensory receptors are present in other invertebrates and in the vertebrate ear (Bullock and Horridge, 1965).

A tactile function would seem to be the most likely role for a majority of the ciliated receptors found in trematodes, including those of *D. merlangi*. They are without pigment and lack the complexity of the photoreceptors which have been described for a number of larval trematodes by Isseroff and Cable (1968). In addition, ciliated receptors have been found around the adhesive organ of *C. bushiensis* by Erasmus (1967) and around the oral region of *F. hepatica* cercariae by Dixon and Mercer (1965) and together with the present finding of similar structures in the mouth region of *D. merlangi* shows that in a number of worms ciliated sense receptors occur at sites where the parasite would presumably require tactile organs (tangoreceptors).

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