

## Setae of the First Antennae of the Copepod *Cyclops scutifer* (Sars): Their Structure and Importance\*

(ciliary structure/mechanoreceptor/information flow/prey-predator relationship)

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**ABSTRACT** Ultrastructural studies of the setae of the first antennae of *Cyclops scutifer* (Sars) have revealed their sensory function. The setae are the extension of modified ciliary structures which function as mechanoreceptors. The setae apparently act to detect gravitational and inertial forces. This is of particular importance in sensing disturbances generated by prey or predators.

Any living animal receives a constant flow of information from its environment. This information is involved in adjusting the animal's metabolism, possibly through the endocrine system, changing its behavior so that the animal can optimally cope with the environment. During the flow of information through the animal, information is also released to the environment and to neighboring animals.

To perceive a given type of information adequately, an animal must have a sufficient number of sensors. An understanding of the operation of such sensors is necessary for one to comprehend the mechanics of animal behavior. Various studies have made the sensory-behavioral system of the honeybee *Apis mellifera* L. one of the best known (1-4). In contrast, little is known about the sensory-behavioral system of copepod species that comprise the zooplankton of lakes and seas. We know only that copepod eyes are very simple (5) and incapable of the sophisticated image-detection achieved by honeybee eyes. In water, electromagnetic waves are inefficient carriers of information because of water's high extinction coefficient, strong diffractive properties, and often high turbidity. On the other hand, its diffusivity, sound conductance, and incompressibility make water an ideal carrier of mechanical and chemical information. It seems reasonable, therefore, to hypothesize that the antennae are the most important sensory organs of planktonic copepods and that this is why their antennae exhibit more complex organization than their eyes.

In 1851 Leydig (ref. 6, p. 292 and Fig. 8) described the structure of crustacean setae and concluded that they must serve a sensory function because they are innervated, and because crustaceans are closely related to insects, which recognize their food by touching it with their antennae. This inference was neither challenged nor directly proven, though Claus (7) disagreed strongly with vom Rath (8, 9) on the structure of the innervation of the setae. In the absence of any further work on the structure of copepod antennae, it seemed desirable to

investigate their ultrastructural organization by using modern techniques of electron microscopy.

We collected individuals of the common widespread freshwater copepod *Cyclops scutifer* Sars from Hogans Pond on the Avalon Peninsula of Newfoundland, Canada. The animals were sorted and placed in filtered pond water. Each animal was then picked up in a small drop of water and placed in a fixative solution (10). The abdomen was cut off; this allowed fixative to enter the exoskeleton. After fixation, the specimen was either stained with osmium tetroxide and embedded in Epon for studies of fine structure with a Zeiss 9S Electron Microscope or freeze-dried (11) for observation in a Cambridge Stereoscan 2A scanning electron microscope. Some of the setae of the first antennae were slightly damaged before they were coated. Coating of the chitinous exoskeleton with gold resulted in charging problems and was less successful than the method of Jones and Fordy (12).

Fig. 1A is an electron micrograph of two setae at the apex of the antenna. The chitinous sheath is continuous along the antenna and seta. The connection between the seta and the antenna is constricted (arrow), which possibly renders some flexibility to the seta. A band of 100-200 microtubules can be seen (Fig. 1B) inside the seta. In the antenna, basal body-like structures showing 9+0 arrangement are found in the proximity of the seta (Fig. 2B). These structures are ensheathed in a microtubule complex, as seen in various sectioning planes (Fig. 2A-C). The electron micrographs suggest possible continuity between bands of microtubules (Fig. 1A), the basal body-like structures, and the microtubule complex sheath. Evidence for curvature of the microtubule bands can be seen in Fig. 1B, where distal upper parts of the microtubule filaments are cut tangentially. The interior of the antenna contains long, energized mitochondria (Fig. 1A), striated muscle fibers, glycogen granules in  $\beta$ -form, synaptic junctions, and synaptic vesicles. However, no nerve tissue could be detected in the setae. Fig. 3A is a scanning electron micrograph of a male *C. scutifer*. The first and second antennae display many setae projecting in different directions. The arrow on the antenna points to the damaged seta shown enlarged in Fig. 3B, where we can see the band of microtubules connecting the seta with the antenna. The other arrow points to an area on the rostrum where four thin hairs about 28  $\mu$ m long originate. (The lower left hair in Fig. 3C is the original length, with the end indicated by an arrow. The other three hairs were broken off at different lengths during the freezing process.) These hairs are about 10 times shorter and thinner than

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the setae of the antennae. They are parts of chemoreceptors of the type that Elofsson (13) described for other species of copepods.

The ultrastructure of the seta strongly suggests that it is a modified ciliary process. Thurm, studying honeybees (14), and Moran and Varela, studying cockroach legs (15), illustrated mechanoreceptors which are similar to the structures shown in our study. In contrast to the situation described by Thurm for honeybees (14), however, the large band of microtubules in *C. scutifer* probably does not move freely through the tight constriction at the junction of the seta and the antenna. Hence the band of microtubules cannot serve only to connect mechanically the bipolar nerve cell with the moving tip of the seta. The band could also function as a piezoelectric crystal, transmitting electrical signals derived from vibrations and movements of the seta.

From behavioral studies it appears that copepods routinely obtain information from their physical environment by use of the mechanoreceptors in the antennae. Some examples of the different functions that the setae may perform follow:

(i) In the well-known "hop and sink" pattern of swimming, the zooplankton may use, in the sinking phase, the information from the antennae to determine the direction of gravity (16). Schröder (17) used a sugar solution of the same specific density as the zooplankton to test his hypothesis that the animals sensed acceleration (see *ii* below). He found that the

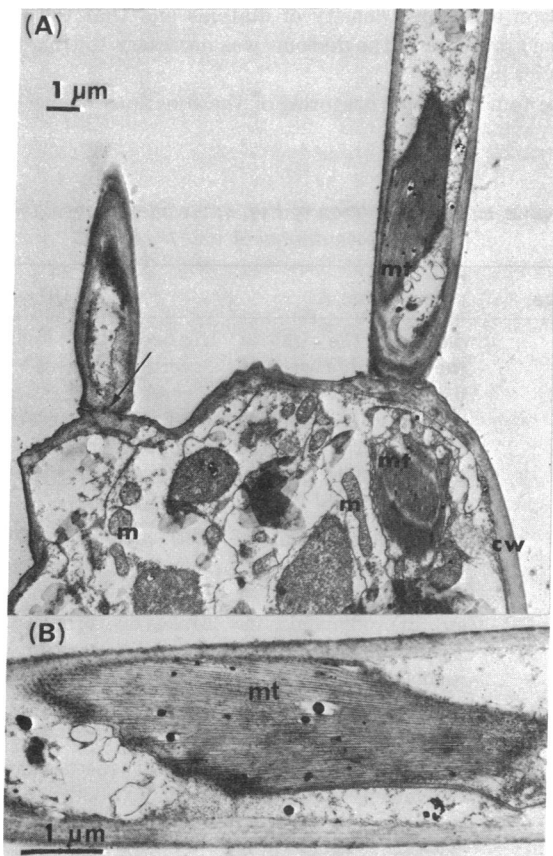


FIG. 1. (A) Longitudinal section of the first antenna showing the apical region with two setae. The chitinous sheath (*cw*) is continuous along the antenna and the seta; note the constriction (*arrow*) at the junction, bands of microtubules (*mt*) and mitochondria (*m*). (B) A band of microtubules (*mt*) shown in higher magnification.

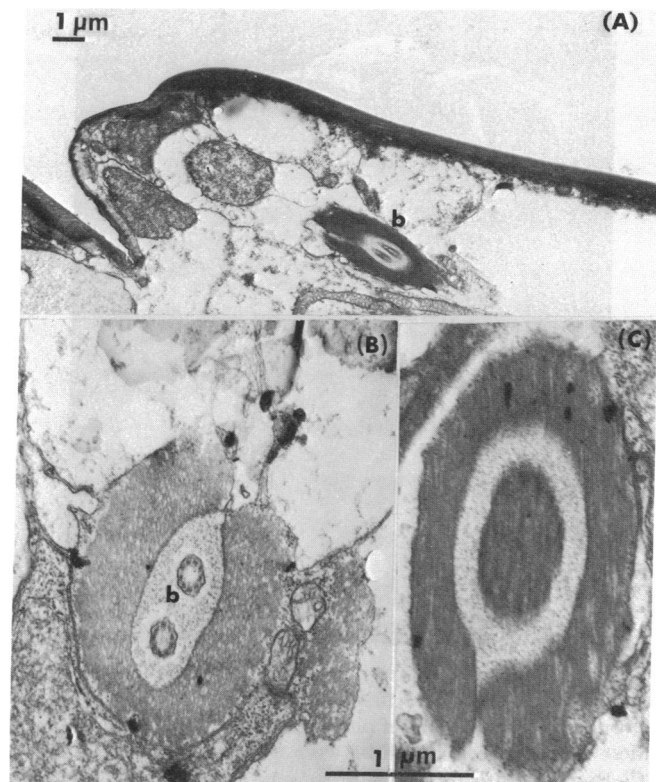


FIG. 2. Basal body-like structures (*b*) in various planes of sectioning: (A) Tangential; (B) somewhat transverse, showing 9+0 arrangement and the surrounding microtubules; (C) tangential, above the basal body-like structure.

animals, under conditions of neutral buoyancy, cannot sense acceleration, nor (unexpectedly) can they orient themselves. We used physiologically inert starch solutions to reproduce this observation, and found that instead of an organized "hop and sink" pattern, there is a disturbed pattern very similar to an escape reaction, in which the animal covers a great distance in a short time without any sinking phase. The above results confirm those of Grosser *et al.* (18) and Hantschmann (19). The question remains whether bending or vibrating of the setae produces the signal or whether the positions of the antennae are detected by means of setae, functioning as proprioceptors.

(ii) The animal can sense the net force of different accelerations of the surrounding water and its own body (17). Picking up a copepod with a pipette is, therefore, most challenging. The mechanism for sensing such a force should resemble that for sensing gravity, with the difference that it does not occur exclusively in the sinking phase.

(iii) Signals of a fluid mechanical nature may be generated by the filtering mechanism of the animal to sense fluid boundaries and other obstacles. Certain zooplankters appear to sense the presence of immobile particles at a distance of 1–5 mm (20, 21).

(iv) When swimming, zooplankters must produce fluid mechanical disturbances of the water. These signals should be characteristic of the shape of the animal and may be used by other animals of the planktonic community in establishing predator-prey relations (17, 22, 23) and in mating behavior. For example, a male is capable of avoiding other species in a highly diverse population and singling out a female of its own

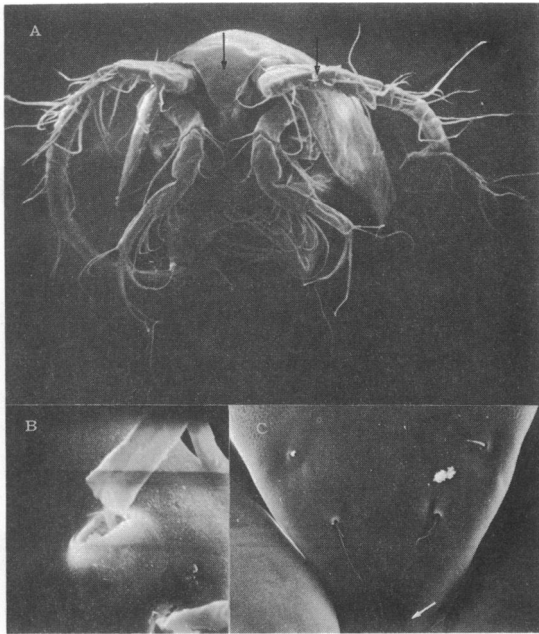


FIG. 3. Scanning electron micrograph of *C. scutifer*: (A) Frontal view of rostrum and first and second antennae with setae. Arrows indicate locations of pictures B and C ( $\times 56$ ). (B) Band of microtubules at connection between antenna and seta ( $\times 1400$ ). (C) Rostrum with four sites of sensory hairs (chemoreceptors). End of the lower left hair is indicated by an arrow ( $\times 620$ ).

species for copulation. Re-evaluation of movies made earlier (21) shows that every animal in a homogenous male population of *Cyclops abyssorum* tends, even in darkness, to maintain a living space about 40 mm in diameter. Maintenance of this space further supports the concept of detection and recognition by means of the disturbances generated by other animals. Chemical communications between animals would depend on diffusion rate of messengers through the water column, and this rate is slower than the velocity of swimming animals.

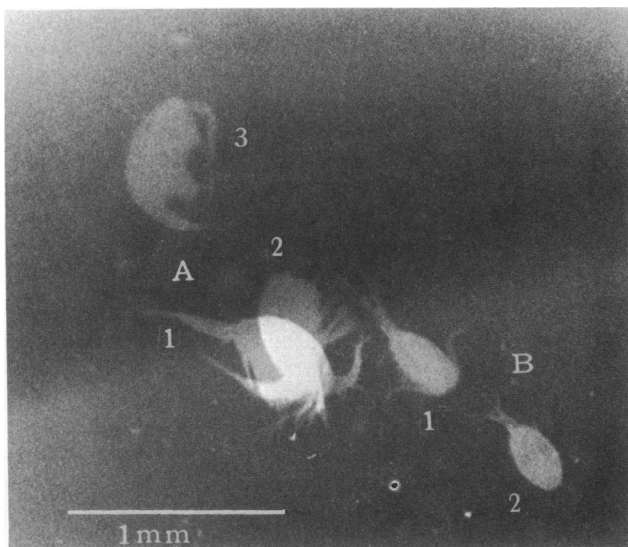


FIG. 4. Avoidance of physical encounter by an adult *C. scutifer* and a copepodite, showing that mechanoreception gives the animals the information necessary to avoid collisions.

In order to test this statement, we took pictures of swimming *C. scutifer* with a Bolex H16 camera, using a strobe-illumination (0.156 msec duration of the flashes at time intervals of 5 msec). Fig. 4 shows an encounter of a male with a copepodite in 10 msec (three flashes) and Table 1 is the interpretation of this picture. Numerous other pictures show the same phenomenon and demonstrate conclusively that information about an approaching zooplankter is transmitted by fluid-mechanical means. Furthermore, Szlauer (24) found that in darkness *Daphnia pulex* can detect and avoid an approaching glass tube.

Investigating this fourth category of setae function is most challenging. The question is whether a copepod detects a prey animal, predator, or sex partner only through disturbances of the water. These disturbances could be produced by displacement of water during swimming, or by acoustic signals. Acoustic signals could be of discrete frequencies for individual species (25, 26) and could be recognized by setae of characteristic length.

An additional problem is the question of whether zooplankters need signals from their neighbors in order to form patches actively. It is even possible that an individual animal might "hear" the cracking of diatom tests by others and then move in the direction of a concentration of phytoplankton. This would explain the results of Bainbridge (27). He introduced a species of zooplankton into a density gradient of phytoplankton and found that small copepods swam in the direction of highest density of diatoms and that the actual physical presence of the diatoms was necessary to trigger this behavior pattern.

The four possible categories of the functions of mechano-

TABLE 1. Interpretation of Fig. 4: Avoidance of collision between animal A and B.

Stage	Animal A	Animal B
	Swims from the right to the left with a speed of 0.3 cm/sec; leaves chemical information behind; drifts in the early sinking phase towards animal B.	Has been sitting 1 min on a vertical grass wall, spreading chemical information; senses the approaching animal A.
1	All four pairs of legs in front, abdomen straight. Gets hydrodynamic information from B's antennae movement; flips abdomen backwards to turn 90°.	With symmetrical backward flip of the first antennae starts to take off. Makes one stroke with antennae, legs and abdomen, giving a speed of 20 cm/sec.
2	Gains speed through movement of legs. Flips abdomen down to obtain a speed of 30 cm/sec.	In full speed, antennae against body. Leaves picture field.
3	In decelerating phase (dorsal surface foremost), flips all legs forward.	

reception described above suggest that zooplankters may resemble insects in sensory physiology (28). Dumont (29) described field observations on reverse vertical migration and came to the conclusions that zooplankters must sense their neighbors and that they must possess a sensory system like that described above. Certainly, further investigations of both chemo- and mechanoreception in zooplankton will be necessary if we are to comprehend the way of life of this group of animals which forms such an important link in aquatic food chains.

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