Ultrastructure of the mouthpart sensory setae in mesopelagic copepods of the family Scolecitrichidae

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Abstract: The ultrastructure of the two types of sensory setae (worm-like and brush-like setae) on the maxilla and maxilliped in the scolecitrichid copepods *Lophothrix frontalis* and *Scottocalanus securifrons* was studied. The worm-like seta ends in an essentially naked, tapered tip, while the tip of the brush-like seta is fringed with slender filaments. The worm-like setae on the endopod of the maxilla are longer and originate more distally than the brush-like setae. In *L. frontalis* both types of setae are innervated by ≤3 dendrites, with each dendrite giving rise to ca. 100–400 cilia. The number of cilia in the worm-like seta is ca. 100–150 at the setal base and decreases distally, while that in the brush-like seta is ca. 100–700 and does not change along its length. The cuticle in the distal 3/4 of the worm-like seta is thin and electron sparse, but with many electron-dense particles of unknown origin attached. The cilia in the brush-like seta project distally into the space surrounded by the brush-like filaments and are exposed directly to the ambient water. These observations suggest that both types of setae are chemosensory, and it is inferred that the worm-like setae are engaged in first-stage, more general detection, while the brush-like setae are involved in the second-stage, more specific, detection of chemicals in food particles.

Key words: maxilla, maxilliped, chemoreceptor, detritivory, Scolecitrichidae

Introduction

Pelagic copepods of the families Diaixidae, Parkiidae, Phaennidae, Scolecitrichidae and Tharybidae are distributed primarily in the meso-, bathy- and benthopelagic layers of the oceans. Recent investigations have revealed that several species in these families are consumers of detrital particles and play important roles in the oceanic food web, contributing to remineralization of organic particles and linking small organic particles to higher trophic levels (Gowing & Wishner 1986, 1992; Nishida et al. 1991; Ohtsuka 1991; Ohtsuka & Kubo 1991; Steinberg 1995).

These families are characterized by the possession of two types of specialized setae on their maxillae, maxillipeds and, rarely, maxillules (Fig. 1). Following Bradford (1973) these setae are termed here "worm-like" and "brush-like" setae. In terms of external morphology and lo-

Fig. 1. *Scottocalanus securifrons* female. **A.** Maxilla. **B.** Basis of maxilla with 2 worm-like setae (*w*), one of which has setules. **C.** Endopod of maxilla with 3 worm-like (*w*) and 5 brush-like setae (*b*). **D.** Maxilliped with 2 worm-like (*w*) and one brush-like setae (*b*) on syncoxa.

cation, these setae appear to be restricted to the above families within the Calanoida (see also Ferrari & Markhaseva 1996).

The correlation between the possession of these setae and the detritivory supposedly common in these copepods leads to the hypothesis that these setae are involved in the detection of detrital food particles (Ohtsuka & Kubo 1991; Steinberg et al. 1994; Steinberg 1995). However, while the gross morphology of these setae has been dealt with for taxonomic purposes (e.g. Fleminger 1957; Bradford 1973; Park 1983), their ultrastructure and function are mostly unknown.

This study examines the external and internal ultrastructure of these setae in *Lophothrix frontalis* Giesbrecht and *Scottocalanus securifrons* (T. Scott), common scolecitrichids in Japanese deep waters, and discusses their possible function.

Materials and Methods

Samples were collected in Sagami Bay and off Kii Peninsula, on the Pacific coast of central Japan, by oblique tows of an ORI-net (Omori 1965; mesh aperture: 0.69 mm) from ca. 1000-m depth to the surface during the cruises of the R. V. *Tansei Maru* in 1990–1992 and 1996.

Live adult females of *Lophothrix frontalis* and *Scottocalanus securifrons* were sorted from the original samples and fixed in 2% glutaraldehyde and 2.5% paraformaldehyde buffered with 0.1 M Millonig's phosphate buffer (pH 7.4) for 2 wk at 4°C. The maxillae and maxillipeds were then removed from the body with dissecting needles for better penetration of fixative and resin. These appendages were post-fixed in 1% OsO_4 in the same buffer at 4°C and dehydrated through a graded ethanol series from 50 to 100%. For scanning electron microscopy (SEM) the appendages were critical-point dried, coated with gold, and examined with an Akashi Alpha–25A SEM at an accelerating voltage of 15 kV. For transmission electron microscopy (TEM) the specimens, fixed and dehydrated as for SEM, were embedded in Epon–812 resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100–CX TEM at an accelerating voltage of 80 kV.

Results

External Morphology

The maxilla of *Lophothrix frontalis* (Fig. 2A) carries three worm-like setae on the terminal part of the endopod and five brush-like setae originating from the basal part. In addition, one worm-like seta originates from the basis. On the maxilliped one brush-like seta (Fig. 2D) originates from the inner mid-margin of the syncoxa. The number and arrangement of the setae in *Scottocalanus securifrons* (Fig. 1) is basically similar to those in *L. frontalis*, except that in the former, one additional worm-like seta with setules originates from the basis of the maxilla (Fig. 1B), and two additional worm-like setae originate from the 1st and 2nd syncoxal endites of the maxilliped (Fig. 1D). The worm-like setae are much longer (360–410 μm in *L. frontalis*, 180–260 μm in *S. securifrons*) than the brush-like setae (150–230 μm in *L. frontalis*, 80–150 μm in *S. securifrons*).

In both species the worm-like and brush-like setae are devoid of marked surface protuberances on the setal shaft such as setules (Fig. 2A–E), except for very minute, irregularly arranged setules occasionally found on representatives of both types of setae (Figs 1D, 2B), the brush-like seta on the maxilliped in *L. frontalis* in which the basal 2/3 is furnished with very thin setules (Fig. 2D), and the worm-like seta on the basis of the maxilla in *S. securifrons* which bears thin setules along its length (Fig. 1B). Both types of setae have almost uniform thickness throughout their length except in the distal regions. The distal region of the worm-like seta tapers to a more-or-less blunt tip (Fig. 2B), while the tip of the brush-like seta is fringed with slender filaments (Fig. 2C–E). The number of filaments is ca. 50 in *L. frontalis* (Fig. 2C) and ca. 15 in *S. securifrons* (Fig. 2E). The filaments in *S. securifrons* have well-developed longitudinal grooves on the outer surface (Fig. 2F).

Internal Ultrastructure

While the following descriptions refer to both *Lophothrix frontalis* and *Scottocalanus securifrons*, the measurements and numbers of dendrites and cilia are based solely on *L. frontalis*.

The numbers of dendrites innervating the examined setae are 1 (n=2) in worm-like setae and 1 or 3 (n=5) in brush-like setae. The dendrite (=dendritic inner segment) ends in the
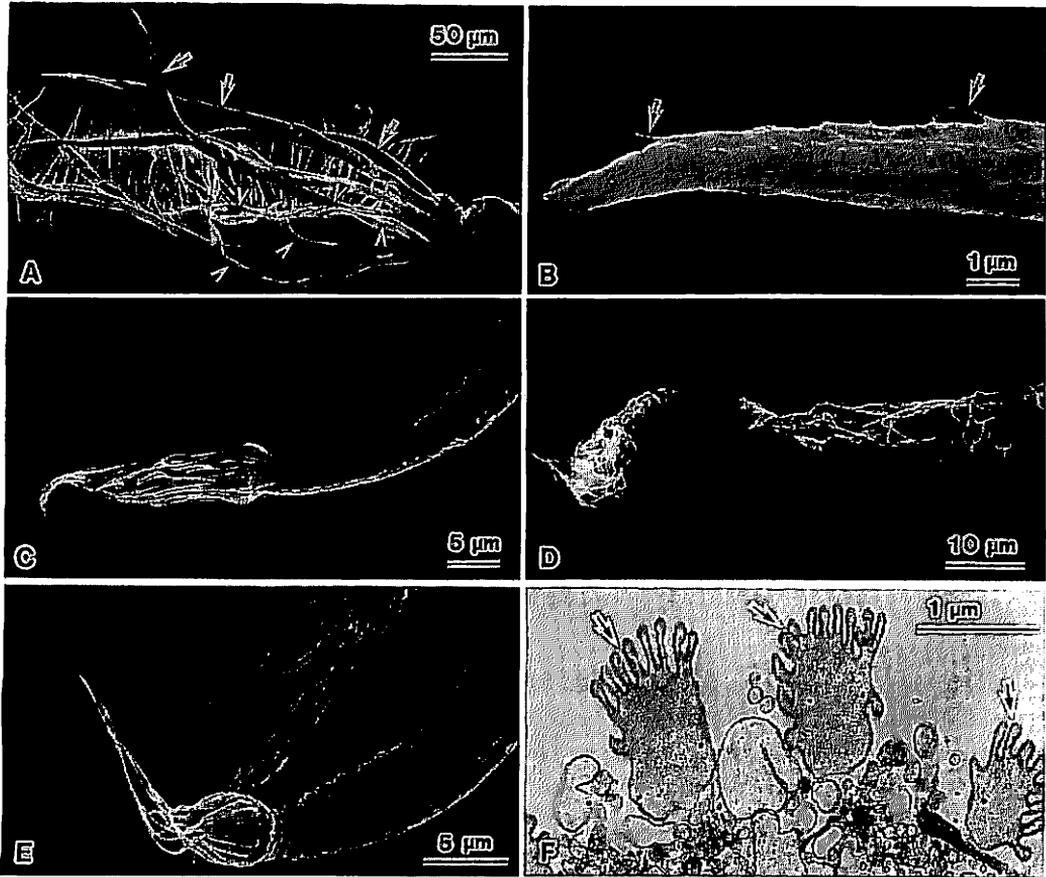
Fig. 2. SEMs (A–E) and TEM (F) from *Lophothrix frontalis* (A–D) and *Scottocalanus securifrons* (E–F) females. **A.** Worm-like (*arrows*) and brush-like setae (*arrowheads*) on the endopod of the maxilla. **B.** Distal region of worm-like seta on maxilla with minute setules (*arrows*). **C.** Distal region of brush-like seta on maxilla. **D.** Brush-like seta on maxilliped with setules on proximal surface. **E.** Distal region of brush-like seta on maxilla. **F.** Cross section of fringing filaments of brush-like seta showing grooves on outer surface (*arrows*).

lumen of the maxilla and maxilliped proximal to the base of the seta (Figs 3A–E, 5) and gives rise to numerous cilia (=dendritic outer segment, ca. 100–400/dendrite, mean=ca. 180, n=9) (Fig. 3D–F). The dendrite has basal bodies and rootlets corresponding to each cilium (Fig. 3A, B), which has a 9×2+0 pattern of microtubules at its base (Fig. 3C). The number of microtubules increases distally to 9×2+4 near the base but decreases again more distally (Fig. 3C, E). The dendrites are enclosed by enveloping cells (number undetermined), which extend distally into the lumen of the seta until the ordinary chitinous structure of the setal wall disappears (Figs 3A, B, D, F, 4A).

In worm-like setae the number of cilia at the basal region is ca. 100–150/seta (n=2) and decreases distally. The cuticle of the proximal 1/4 is thick (0.7–1.1 μm) and well-laminated, while that of the distal 3/4 is much thinner (0.1–0.3 μm), electron sparse, and lacks a laminated structure (Fig. 4E, F). In the distal region the boundary between the setal wall and the tissues in the lumen is obscure. Electron-dense particles of unknown origin accumulate on this electron-sparse tissue (Fig. 4E). The tips of different cilia often end close to the thin wall
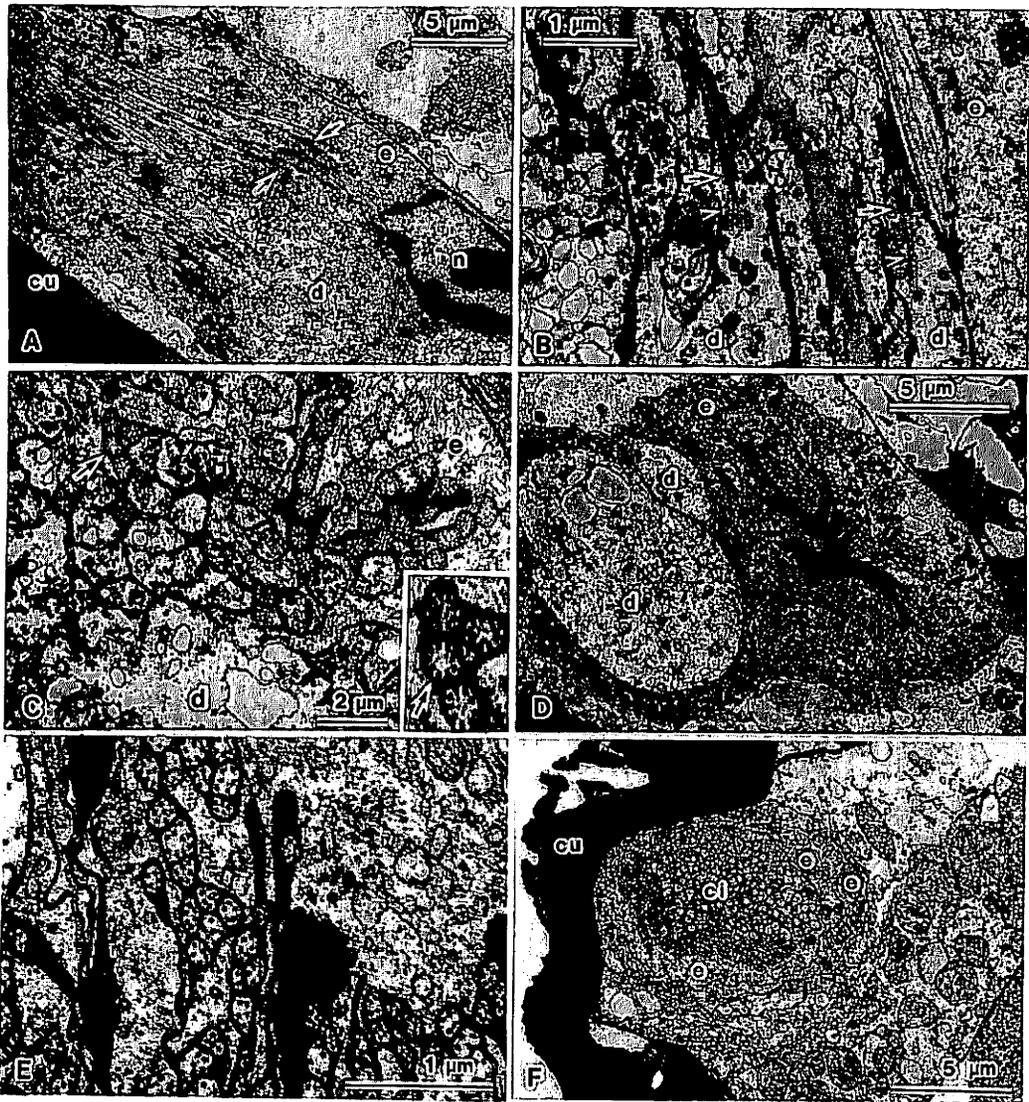
Fig. 3. TEMs from brush-like setae on the maxilliped of *Lophothrix frontalis* females at levels proximal to the maxilliped cuticle. **A.** Semi-longitudinal section of distal region of a dendrite (*d*) showing enveloping cell (*e*) with nucleus (*n*), maxilliped cuticle (*cu*), and bases of cilia (*arrows*). **B.** Basal region of cilia in (A) at higher magnification showing basal bodies (*arrows*), rootlets (*arrowheads*), enveloping cell (*e*), and dendrite (*d*). **C.** Cross section containing ciliary base with 9×2+0 pattern of microtubules (*arrows*, inset at higher magnification), enveloping cell (*e*), and distal region of dendrite (*d*). **D.** Cross section distal to (C) containing 2 dendrites (*d*), bundle of ca. 400 cilia from another dendrite, and enveloping cell (*e*). **E.** Cilia in (D) at higher magnification. **F.** Cross sention near maxilliped cuticle (*cu*) showing enveloping cells (*e*) and bundle of ca. 600 cilia (*ci*) appearing to originate from 3 dendrites as shown in (D).

at different levels along the seta (Figs 4F, 5).

In brush-like setae the number of cilia ranges from ca. 100 to 700/seta (mean=ca. 400, n=5). According to a series of sections from a single seta, the number of cilia is essentially the same at all levels of the seta, indicating that the cilia run throughout the entire setal length
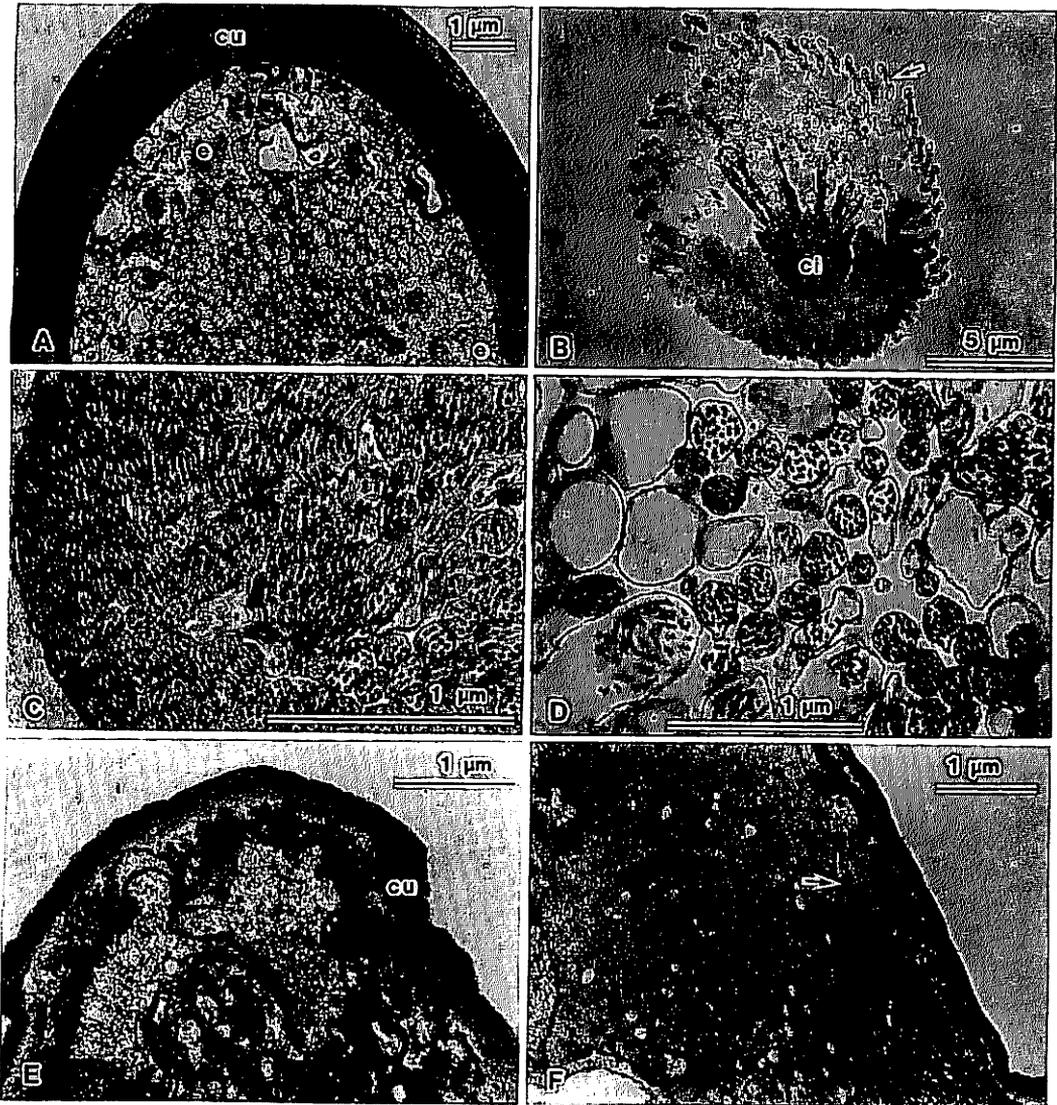
Fig. 4. TEMs from brush-like setae on the maxilliped of *Lophothrix frontalis* females (A–D) and from worm-like setae on the maxilla of a *Scottocalanus securifrons* female (E–F) at levels distal to setal base. A. Cross section near setal base containing well laminated cuticle (*cu*), bundle of cilia, and enveloping cells (*e*). B. Semi-cross section of distal region containing bundle of cilia (*ci*) and fringing filaments (*arrow*). C. Bundle of cilia in (B) at higher magnification. D. Cross section of cilia suspended in a space surrounded by fringing filaments, with each cilium containing different number of microtubules. E. Cross section of distal region showing thin cuticle (*cu*) and accumulation of dense particles on it. F. Semi-longitudinal section of medial region with cilium ending (*arrow*) at subcuticular position.

without branching. The cuticle of the basal 2/3 is thick (1–1.5 μm) and well laminated (Fig. 4A), but towards the tip it gradually changes into a less well structured, electron sparse wall ca. 1 μm thick, with accumulation of dense particles as in the worm-like seta, and the boundary with the inner tissue becomes obscure. The wall thickens at the base of the distal, fringing filaments to ca. 2 μm, and at this level the lumen contains only cilia (Fig. 4B, C). The cilia
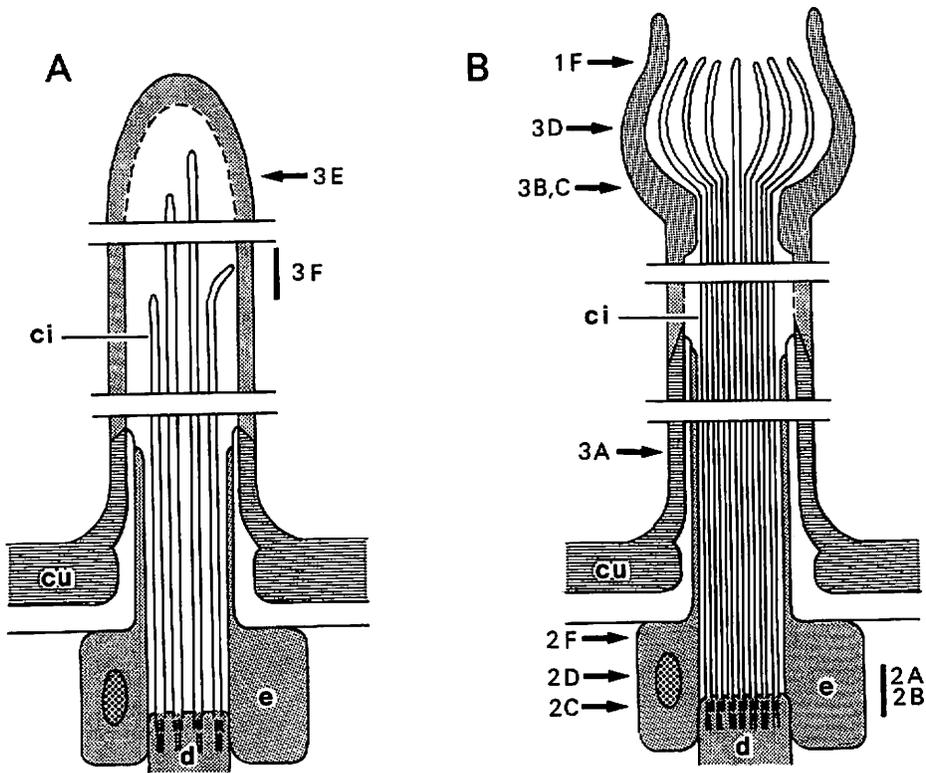
Fig. 5. Schematic drawing of worm-like (A) and brush-like setae (B). Only one dendrite (*d*), one enveloping cell (*e*), and a few cilia (*ci*) are shown for clarity. Dotted lines indicate the areas where boundary between cuticle and tissues of lumen is obscure. Arrows and vertical lines indicate the levels from which the cross sections and semi-longitudinal sections, respectively, of the denoted figures are derived. *cu*: cuticle.

project distally into a space surrounded by the brush-like filaments fringing the distal tip of the seta (Figs 4B, 5). Within this space the cilia are naked, the cell membrane being exposed to the environment. They contain varying number of microtubules (Fig. 4D).

Discussion

The worm-like and brush-like setae in the families Diaixidae, Parkiidae, Phaennidae, Scolecitrichidae and Tharybidae have often been referred to as "sensory" (e.g. Bradford 1973; Park 1983) without the support of structural and/or physiological evidence. The present observations demonstrate that they have sensory structures consisting of cilia originating from dendrites, although the cell perycaria have not yet been located.

The thin, electron-sparse cuticle covering the distal 3/4 of the worm-like seta and the abundant electron-dense particles on it suggest that the setal wall is permeable and adsorptive. The dendrite in the brush-like seta ends distally as naked cilia exposed directly to the ambient water, and the fringing brush-like filaments appear to protect these cilia from mechanical damage. These features would facilitate contact of the sensory elements with substances in the environment, strongly suggesting that these setae are chemosensory (see, e.g., Laverack 1968; Altner & Prillinger 1980). The number of cilia per dendrite (sensory cell) in both setae

is very large, ranging from 100 to 400, when compared with the aesthetascs on the antennule of calanoid copepods so far described to contain ≤4 cilia/dendrite (Gill 1986; Kurbjeweit & Buchholz 1991; Weatherby et al. 1994) and also with the aesthetascs in other crustacean orders which commonly carry 1–2 cilia/dendrite (see Table 1 in Hallberg et al. 1992) with the exception of an ostracod reported to have 8–25 cilia/dendrite (Heimann 1979). This large number of cilia/dendrite in both the worm- and brush-like setae results in a large number of cilia/sensillum, ca. 100–700, comparable to those in decapod aesthetascs which approach 700 cilia/sensillum (Hallberg et al. 1992), despite fewer innervating dendrites per sensillum (≤3) in the present setae than in the decapod aesthetascs (100–350/sensillum, see Hallberg et al. 1992). This appears to be an adaptation for increasing the surface area available for stimulus reception with only a restricted number of sensory cells. The presence of these setae, more or less in clusters, on the maxillae and maxillipeds, appendages which are directly used for the capture and manipulation of food particles (e.g. Paffenhöfer et al. 1982; Cowles & Strickler 1983; Price et al. 1983), suggests that they are involved in near-field discrimination of chemicals from food particles. This is in sharp contrast to the antennular aesthetascs, which are distributed along the anterior edge of an anterior, linear appendage (see, e.g., Griffiths & Frost 1976), a situation thought to be effective for remote chemoreception (Kubjeweit & Buchholz 1991), such as in the detection of sex pheromones from conspecific mates (Griffiths & Frost 1976).

The structural differences between worm-like and brush-like setae suggest that there are significant functional differences between them. First, the distal tips of the cilia in the worm-like seta appear to be scattered over different areas of the setal wall. Also, the worm-like setae on the endopod of the maxilla are located more distally and are longer than the brush-like setae. This provides the worm-like setae with a more exposed surface area than the brush-like setae. On the contrary, the brush-like setae have cilia with naked ends, concentrated at the distal tip, and exposed directly to the ambient water but enclosed by fringing filaments, suggesting a higher sensitivity but more restricted area of detection. These observations suggest that the worm-like setae are engaged in first-stage, more general detection, while the brush-like setae are involved in the second-stage, more specific, detection of chemicals. It is also hypothesized that brush-like setae, with their open tips, are sensitive to larger molecules that cannot diffuse across the membrane of the worm-like setae. This would provide the copepods with the ability to scan for a greater range of molecule sizes and possibly exploit differing food sources. Such putative functions are consistent with the food habits of the scolecitrichids, which appear to be detritivores in the open ocean and/or deep sea (Gowing & Wishner 1986; Nishida et al. 1991; Ohtsuka 1991; Ohtsuka & Kubo 1991; Gowing & Wishner 1992; Steinberg 1995). Detection and discrimination of chemicals from non-motile organic particles would be vital for survival in a food-limited environment.

The arrangement of sensory setae on the maxilla has been interpreted as diagnostic for each family (Fleminger 1957; Bradford 1973). For example, most taxa in the Scolecitrichidae carry 3 worm-like and 5 brush-like setae on the endopod while those of the Phaennidae have 1 worm-like and 7 brush-like setae. These differences may indicate that they differ in food preference and/or the ability to detect detrital matter. In fact the Phaennidae, in particular *Xanthocalanus*, is distributed mainly in hyperbenthic zones whereas *Amallothrix*, *Scaphocalanus*, and *Scolecithricella*, all belonging to the Scolecitrichidae, are distributed predominantly in the meso- and bathypelagic zones (cf. Ferrari & Markhaseva 1996), suggesting that the morphological and distributional differences between these two families might reflect different feeding strategies.

In addition to the above structural differences between the two types of setae, even more interesting is the high morphological diversity of the brush-like setae (e.g. Bradford 1973; Park 1983; Ohtsuka, unpublished data), as well as segmental and elemental modifications in the maxilla and maxilliped (e.g., well-developed hooks in *Cornucalanus* spp., suggesting strong carnivory; Arashkevich 1969) . The brush-like setae differ in length and thickness, number and shape of terminal filaments and presence or absence of accessory setules or spinules among species and higher taxa. For example, females of the mesopelagic scolecitrichid *Scopalatum vorax* (Esterly) carry a short, thickened brush-like seta with an expanded tip and four long, normal brush-like setae (Ferrari & Steinberg 1993), while *Scolecithricella* spp. are furnished with 5 brush-like setae of almost equal thickness and length (Park 1980).

Several species of these families have been suggested to be carnivores, and others to be omnivores or detritivores on the basis of gut-content analyses (see Table 4 in Gowing & Wishner 1992). Possible explanations as to the role of the chemoreceptors in carnivory may be: (1) the setae are sensitive to substances from live prey organisms as well, (2) the presumed predators feed on animals associated with detrital particles such as larvacean houses (Ohtsuka & Kubo 1991; Steinberg et al. 1994; Steinberg 1995), or (3) they are not used in carnivory at all. However, as to the functional differences among the highly diverse brush-like setae, still much remains to be discovered. The combination of gut-content analysis, examination of the fine structure of the brush-like setae, and observation of feeding behavior in different taxonomical and ecological groups is required for a better understanding of their function.

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