Ultrastructure of the Frontal Sensory Fields in the Lynceidae (Crustacea, Branchiopoda, Laevicaudata)

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ABSTRACT The clam shrimp family Lynceidae is unusual in possessing paired fields of short setae on either side of the rostral carina. We describe the position of these fields relative to the direction of water movement in live animals as well as the external and internal structure of these setae. The majority of morphological features support a presumed chemosensory role for these sensilla. These features include the lack of a setal socket and the relatively short length of each seta. The low number of enveloping cells (three or four) is uncharacteristic of chemosensory setae and is more typical of mechanoreceptors, as is the absence of any pores on the setae; these characteristics indicate that these fields may have both functions.

Branchiopod crustaceans of the family Lynceidae have long been recognized as differing markedly from the four other branchiopod families commonly referred to as "clam shrimp." Although five families were in the past united as the branchiopod order Conchostraca, a grouping that may be revived based on larval development (Clay Sassaman, personal communication; Martin, '92), morphological differences between lynceid clam shrimp and clam shrimp of the other four families (Cyclestheriidae, Cyzicidae, Leptestheriidae, and Limnadiidae) are striking (Fryer, '87). These differences have been used to argue for the separation of lynceids, either at the level of tribes within the Conchostraca (e.g., Linder, '45) or more recently as a separate order, the Laevicaudata, with other clam shrimp families recognized as the order Spinicaudata (Fryer, '87; see also Martin and Belk, '88; Martin, '92).

One of the many unusual morphological features exhibited by lynceids is the possession of paired, frontal fields of short setae, located on either side of the midrostral carina just anterior to the location of the eyes. These fields occur in all three genera of the family (*Lynceus, Lynceiopsis,* and *Paralimnetis;* Martin and Belk, '88), and in no other family of clam shrimp. The function of these fields of setae has never been determined; Martin et al. ('86), working with the genus *Lynceus,* referred to them as sensory fields, and this phraseology has been repeated by Martin and Belk ('88) and in a review of branchiopod microscopic anatomy by Martin ('92). However, ultrastructural details have never been provided.

Below, we describe the external and internal ultrastructure of these setae and comment briefly on their presumed sensory function.

MATERIALS AND METHODS

Lynceus gracilicornis were collected from a shallow ephemeral pond in Leon County, Florida, in April 1984 (see Martin et al., '86 for details of the habitat) and again in April 1990, from other ephemeral ponds in north Florida. Observations on live specimens were made in 1984 and again in 1990; observations made in 1990 were facilitated by the following method. Live animals were removed from water, and the dorsal region of the carapace valves was quickly dried with cotton or paper toweling. The dull end of an insect pin was then glued to the dorsal region of the valves using a fast-drying waterproof cement. Pins with clam shrimp attached were mounted in a small lump of modeling clay on the edge of a finger bowl containing fresh water, so that the clam shrimp were suspended in the water directly beneath a stereomicroscope with drawing tube attached. This arrangement allowed positioning the animals in almost any orientation, so that a clear view of the function of the head and thoracopods was possible.

Specimens prepared for scanning electron microscopy and transmission electron micros-

copy were fixed in 3% glutaraldehyde at room temperature in 0.1 M phosphate buffer, post-fixed in 1% osmium tetroxide an additional 2 h, and rinsed in cacodylate buffer before dehydration in a graded ethanol series. Specimens were infiltrated with unaccelerated EM-BED-812 embedding medium for 5 days to assure proper infiltration, after which samples were infiltrated and embedded with accelerated EM-BED-812. Gold and silver sections were cut using a Sorvall MT2-B ultramicrotome, stained with uranyl acetate and lead citrate, and examined and photographed using a JEOL 100CX at 80 kV.

RESULTS

Behavioral observations

In contrast to the filter-feeding scenario commonly attributed to all branchiopods and so often depicted in invertebrate texts, lynceids are active scrapers and scavengers (Fryer and Martin, unpublished observations; Martin, '92). Although the head is capable of fitting entirely within the confines of the closed valves, in life it is more often extended forward so that it extends beyond the edges of the carapace valves. This movement of the head is accomplished by rotating the head forward and upward, so that the head is bending at approximately the area just posterior to the occipital groove (see Fig. 1A). Whenever swimming forward, the clam shrimp's head is therefore positioned such that the fields of sensory setae are almost perpendicular to the direction of water coming into contact with the head (see arrow, Fig. 1A). When food (or food-bearing substrate such as a small stick or piece of algae) is encountered, the head bends ventrally back into the region between the valves, so that the labrum and mouthparts are placed in closer proximity to the feeding apparatus, which is composed of the maxilla and proximal endites of the thoracopods.

External morphology

The head of lynceid clam shrimp is large relative to the body size. In mature individuals it occupies approximately one third of the total body size and is therefore larger than that possessed by any other clam shrimp family. This large head bears two oval fields of short, simple setae on either side of the midrostral carina just anterior (although functionally ventral) to the naupliar eye and rostral pit (Fig. 1B,C). The fields are noticeable even at relatively low magnifications and have been commented on by many previous workers (see Martin and Belk, '88). As an example of the size of the fields, each field measured approximately 260 μm in width and 200 μm in length on one animal that had a head region measuring approximately 2.2 mm in length (measuring the distance from the occipital notch to the tip of the rostrum). These fields consist of numerous setae, the number of which can vary among individuals and even between fields on the same animal. The number of setae ranges from a low of approximately 50 to perhaps 90 setae per field. Among individuals (n = 8), setal counts were found to differ by as much as from 2 to 40 setae. Differences in the number of setae between the fields of a single individual were less substantial (2 to 15 setae).

Each seta consists of a long slender shaft, extending from a bulbous base and ending in a rather blunt tip. The base of each seta lacks a socket of any type, and is fused to the floor of the setal field (Figs. 2E, 3B). The length of each seta, measured from the base of the seta to the tip, can vary; longer setae may reach a length of approximately 75 μm while shorter ones may reach a length of only 25 μm. No pore was observed at any point along the length of the sensillum.

The cuticular surface underlying these fields is thinner than the adjacent cuticle of the head, and is convexly curved, causing the setae to point in various directions (anteriorly, ventrally, and posteriorly) (Fig. 2B–D).

Internal morphology

Serial cross-sections were cut through the frontal fields to determine if these simple setae were innervated. Each seta is innervated with four dendrites composed of modified cilia (Figs. 3A, 4C), therefore characterizing the structure as a sensillum. Each dendrite contains a 9 + 0 arrangement of microtubules housed within a liquor cavity (=scolopale space) (Fig. 4C). Three (Fig. 3A–D), possibly four (Fig. 4B), enveloping cells surround the ciliary region of the seta. The innermost enveloping cell (e1) (=scolopale cell) contains the supporting structure, the scolopale, of the ciliary region (Figs. 3A, 4C). The scolopale becomes densely packed distally along the sensillum shaft and produces a thin dendritic sheath, which completely encloses the liquor cavity containing the dendrites (Fig. 3B).

Within the outer segment of the sensillum (i.e., that portion above the cuticle), the four dendrites travel distally for a short distance,

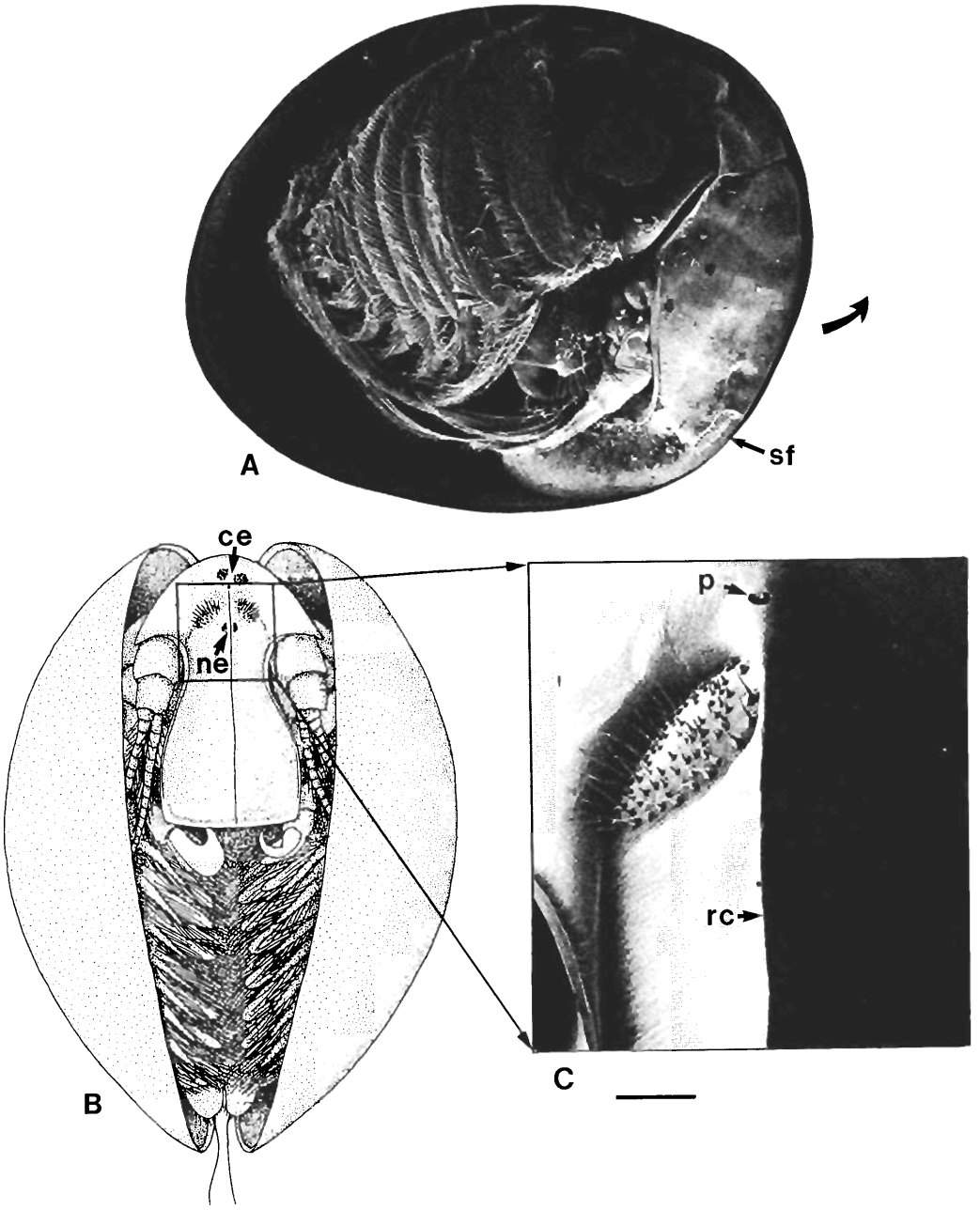

Fig. 1. *Lynceus gracilicornis*. **A:** Scanning electron micrograph (SEM) lateral view of entire animal, right valve removed. Arrow indicates range and direction of head movement. **B:** Diagram of entire animal, male, ventral view. Box encloses sensory fields (sf) further magnified in C. **C:** SEM of sensory field. ce, compound eye; ne, naupliar eye; p, rostral pit; rc, rostral carina. Scale bar in C = 100 μm.

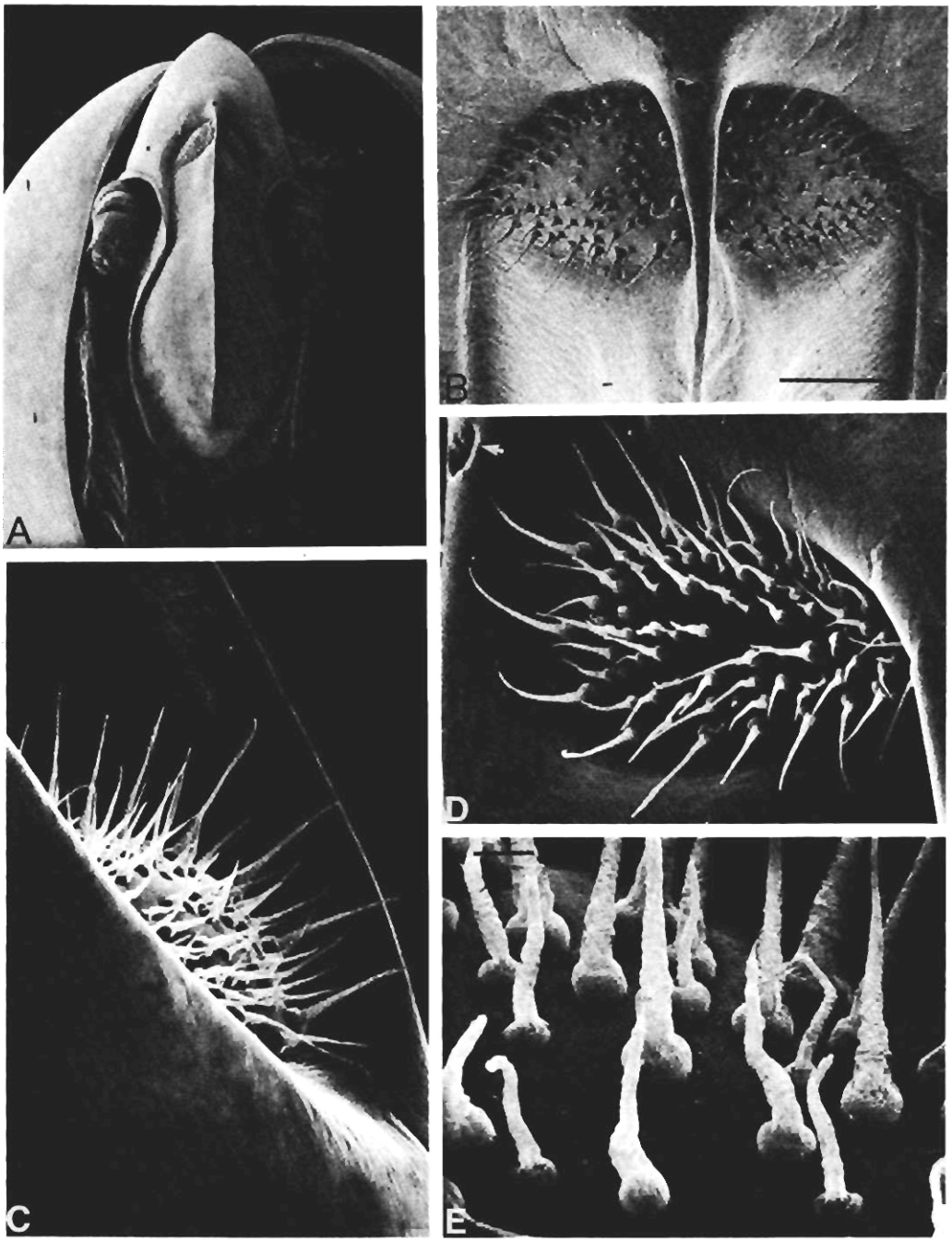

Fig. 2. *Lynceus gracilicornis*. SEM micrographs of external morphology of sensory fields. **A:** Frontal view of female. **B:** Sensory fields flanking rostral carina and rostral pit. Black arrow indicates rostral pit. Scale bar = 100 μm. **C:** Lateral view of sensory fields. **D:** Frontal view of left sensory field. White arrow indicates rostral pit. **E:** High magnification of setae within sensory fields. Scale bar = 10 μm.

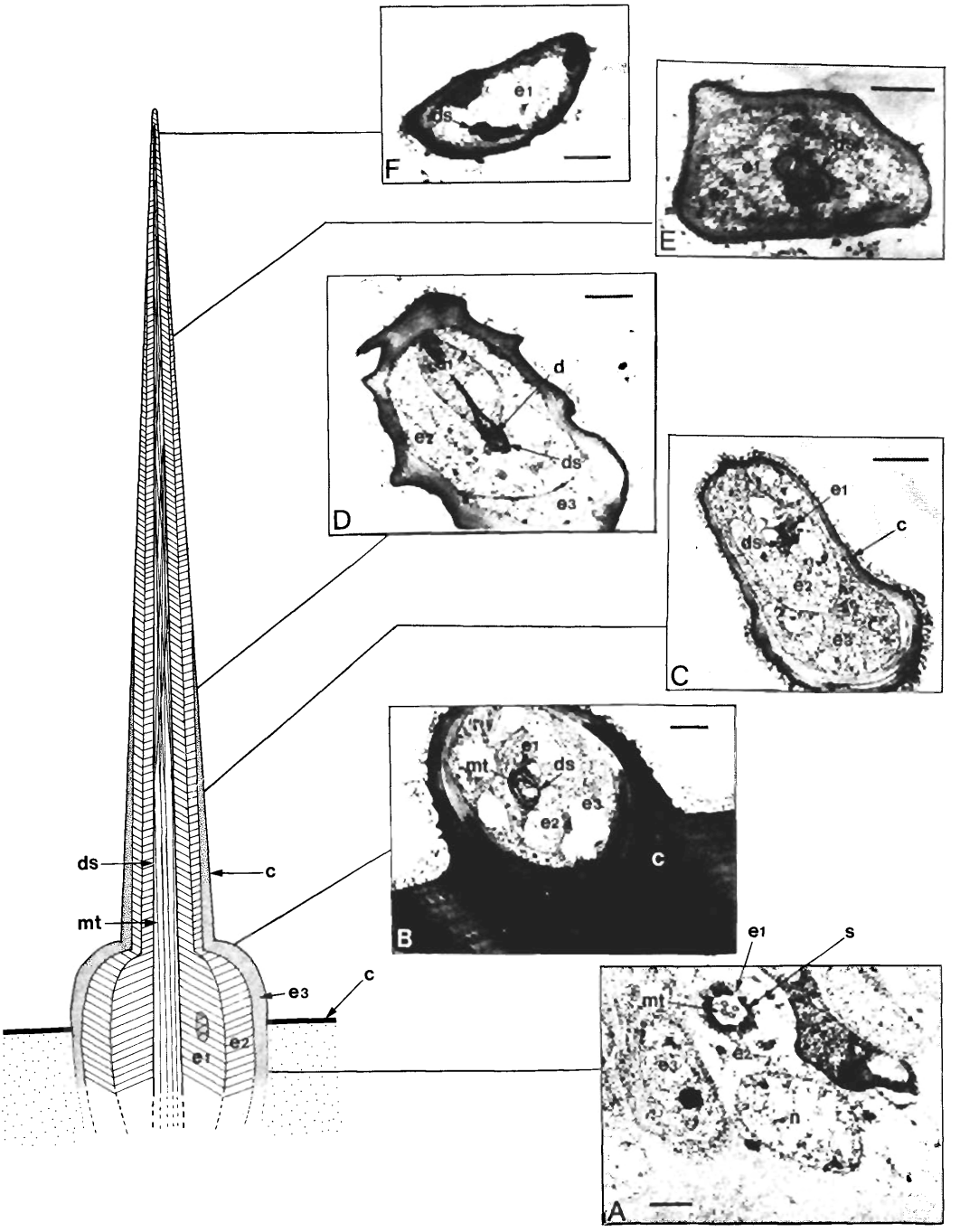

Fig. 3. *Lynceus gracilicornis*. Transmission electron micrographs (TEM) and diagram of single sensory seta illustrating internal anatomy. **A:** Cross section at a level below cuticle. Note the four groups of microtubules (mt) within the liquor cavity surrounded by scolopale (s). Scale bar = 1 μm. **B:** Cross section at level of bulbous setal base, where microtubules (mt) begin to adhere to the ciliary dendritic sheath (ds). Scale bar = 1 μm. **C:** Cross section at level where microtubules appear to disappear briefly by closely adhering to the dendritic sheath (ds). Scale bar = 2 μm. **D:** Cross section at approximately mid point of seta. Dendrites (d) becoming disorganized. Note three enveloping cells surrounding dendritic sheath (ds). Scale bar = 1 μm. **E:** Cross section at level where dendrites (d) are highly branched. Note only two enveloping cells at this level. Scale bar = 0.05 μm. **F:** Cross section near tip of seta. Note dendritic sheath and only one enveloping cell present at this level. c, cuticle; e1, enveloping cell 1; e2, enveloping cell 2; e3, enveloping cell 3; n, nucleus. Scale bar = 0.05 μm.

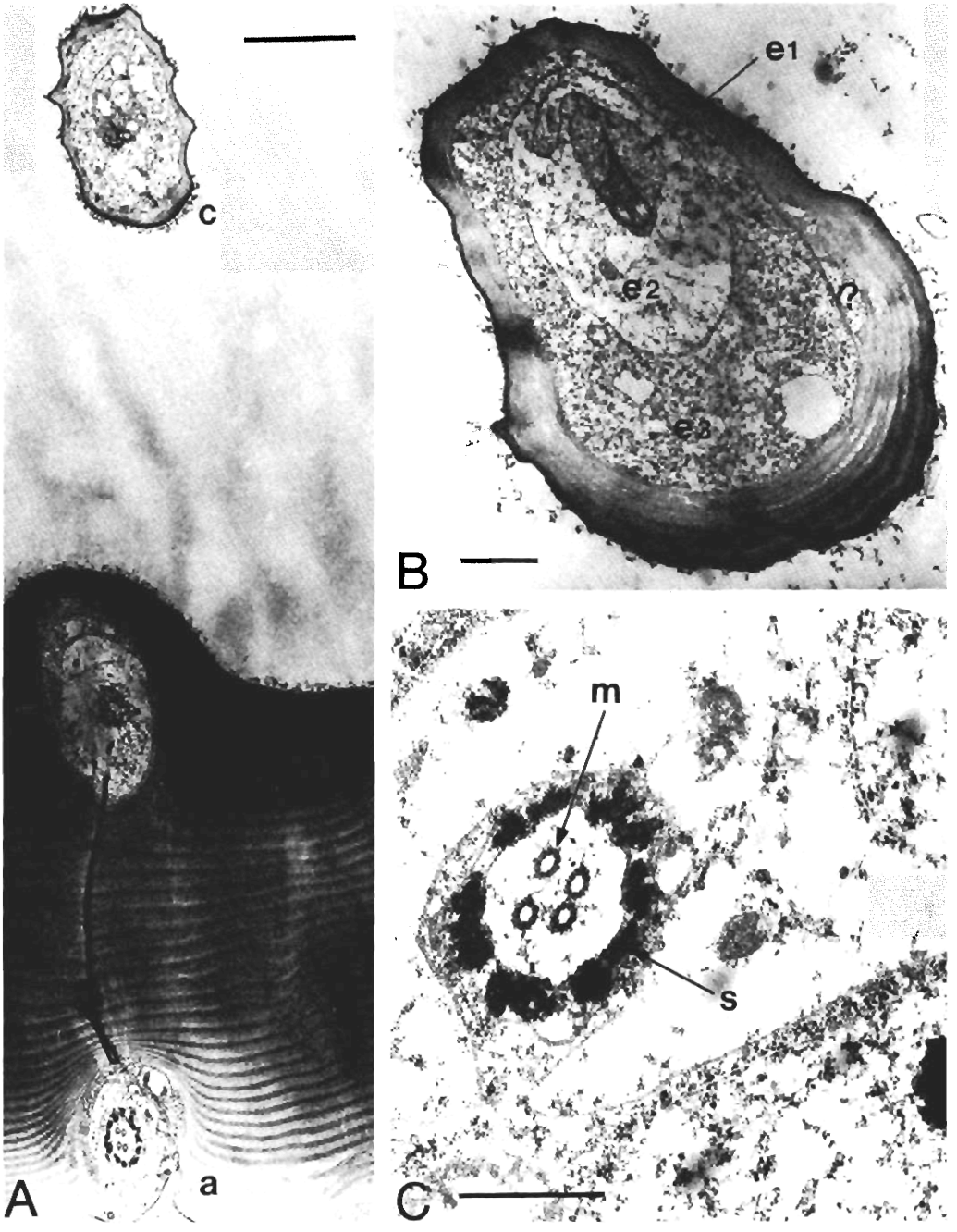

Fig. 4. *Lynceus gracilicornis.* TEM micrographs of sensory setae. **A:** Cross section of three different sensilla at various levels. Sensillum (a) shows the 9 + 0 arrangement of microtubules beneath the cuticular surface. Sensillum (b) at level where bulbous base fuses to the surface of the sensory field. Sensillum (c) sectioned near midpoint. Note position of microtubules. Scale bar = 5 μm. **B:** Cross section of a sensillum illustrating number of enveloping cells (3). Note (?) represents a possible forth enveloping cell. Scale bar = 1 μm. **C:** Cross section of a sensillum at inner segmental level with enlargement of microtubule (m) arrangement surrounded by scolopale (s). Scale bar = 1 μm. e1, enveloping cell 1; e2, enveloping cell 2; e3, enveloping cell 3.

then begin to adhere to the wall of the dendritic sheath (Fig. 3B) until they appear to disappear briefly (Fig. 3C) and then become highly branched or disorganized near the sensillum midpoint (Fig. 3D–F). This branching continues along the length of the setal shaft within the dendritic sheath and enveloping cell 1 (e1) (Fig. 3F). The ciliary region remains near the center of the sensillum at all levels, never coming into contact with the cuticular wall of the shaft (Fig. 3B–F). Enveloping cell 2 extends most of the way up the shaft but ends just proximal to the apex (Fig. 3E), while enveloping cell 3 (e3) appears to end near the midlength point of each seta (Fig. 3D,E). The cuticle covering the sensillum appears rather thin at the distal region of the sensillum, as opposed to the thicker proximal region.

Figure 4A illustrates a section cut through the convexly curved sensory field, which allows a detailed look at three different sensilla at various levels. Sensillum (a) was cut at a more proximal level beneath the cuticle (inner segment), as is recognizable by the four dendrites organized in the 9 + 0 arrangement surrounded by the scolopale (see also Fig. 4C). Sensillum (b) was cut at the level in which the cuticular floor of the setal field fuses to the cuticle of the setal shaft and clearly demonstrates the absence of any setal socket. Sensillum (c) was cut along the mid region as the microtubules appear to disappear while closely adhering to the dendritic sheath.

DISCUSSION

The setal fields of *Lynceus* have been described in previous papers as probably having a sensory function (Martin et al., '86; Martin and Belk, '88; Martin, '92). The present ultrastructural study supports the idea that these fields are sensory in nature and at least demonstrates the fact that they are innervated. Further classification of the setae as being either chemo- or mechano-receptors is more difficult.

According to Felgenhauer ('92), both mechanoreceptors and chemoreceptors have similar basic microanatomy, although morphological differences between the two may sometimes allow workers to elucidate their function. Mechanoreceptors usually feature a movable socket at the base of the sensillum (e.g., Altner et al., '83; Hamilton et al., '85; Felgenhauer and Abele, '83; Felgenhauer, '92; Laverack and Barrientos, '85). The dendrites of the outer segment of mechanoreceptors usually terminate at the base of the setal shaft and fuse to the inner cuticular wall of the shaft, usually at the level of the movable socket; they are thus responsive to movement and vibration. However, such dendrites also can occasionally extend up the canal of the shaft of the seta (Altner et al., '83; Derby, '89; Felgenhauer, '92). Setae in the sensory fields of *Lynceus* do not possess a socket at the base, and the dendrites continue distally to the tip of the sensillum and at no point fuse to the wall of the shaft. These characteristics would seem to argue for a chemoreceptive function. Additionally, the scolopale of mechanoreceptors is thought to produce a very thick dendritic sheath, whereas in chemoreceptors they produce a thin dendritic sheath (Felgenhauer, '92); the relatively thin dendritic sheath in *Lynceus* again would agree with the ultrastructure most typical of chemoreceptors.

However, some features are not in agreement with the usual depiction of the typical crustacean chemoreceptive sensillum. The salient exceptions are the number of enveloping cells that surrounds the dendrites and the absence of a pore. We observed three or four enveloping cells, as did Crouau ('89) when describing chemosensitive dendrites in mysidaceans, which is a number more typical of mechanoreceptors, although the number of enveloping cells is known to vary (Guse, '78, '80). The absence of a pore is usually a feature of mechanoreceptors, although, as Laverack and Barrientos ('85) note, the mode of entry of chemical stimulants may be by direct diffusion across the thin cuticle of the sensillum; absence of any obvious pore does not preclude the possibility of chemosensory function. Ache ('82) also found that chemosensory setae of decapod aesthetascs possess a permeable tip rather than a pore. Both studies (Ache, '82; Laverack and Barrientos, '85) found that stimulants reached the internal dendrites, despite the absence of any obvious pore in the integument of the setal shaft, and this may be the mode of chemical entry into the sensory setae of *Lynceus* as well.

External morphological characteristics of crustacean sensilla are also in support of a predominantly chemosensory role of these setae in *Lynceus gracilicornis*. Typical crustacean chemoreceptive sensilla are short, not robust or thick, often occur in numerous quantities, and are unlikely to be sensitive to mechanical disturbance (Laverack and Barrientos, '85). Scanning electron microscopy il-

lustrates these features for the frontal sensory fields of *Lynceus* and further argues for their primary function being one of chemoreception, although duality of function (chemo- and mechano-reception) is not unprecedented in crustaceans and may be occurring here as well.

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