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ORIGINAL ARTICLE

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Structure and formation of the uncini in *Pectinaria koreni*, *Pectinaria auricoma* (Terebellida) and *Spirorbis spirorbis* (Sabellida): implications for annelid phylogeny and the position of the Pogonophora

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Abstract Setation is an important taxonomic character of the Annelida. Within this taxon, Terebellida and Sabellida both have transverse rows of short, apically toothed setae which are situated inside the neuropodial rim. The apical spines are curved and their tips point anteriorly. These setae are termed uncini. In the terebellidans *Pectinaria koreni, Pectinaria auricoma* and in the sabellidan *Spirorbis spirorbis*, these setae arise from a follicle which consists of a chaetoblast and two follicle cells. The special structure of the uncini is a result of temporal modifications of the actin-filament system of the chaetoblast and changing spatial interactions between the chaetoblast and the follicle cells during the formation of these setae. Once the uncinus is formed, the microvilli are withdrawn and electron-dense material is deposited in the remaining canals. The microvilli are replaced by short processes of the chaetoblast, and the actin-filament system is replaced by a system of intermediate filaments which help to mechanically attach the uncinus to the follicle. Such uncini are also described in both pogonophoran groups, the Perviata and the Obturata (Vestimentifera). In several structural details they correspond to those of the species investigated in this paper, so that the hypothesis of a homology of the uncini seems to be justified. This hypothesis leads to the conclusion that uncini evolved in the common stem lineage of Pogonophora, Terebellida and Sabellida, implying a monophyletic origin of these three taxa. The uncini are compared to the hooked setae of the Arenicolida, Maldanida and Psammodrilida, which are also aligned in transverse rows inside the neurophodial rim. Hooked setae and uncini are hypothesized to be homologous. It, therefore, can be concluded that Arenicolida, Maldanida and Psammodrilida are closely related to the monophylum consisting of Terebellida, Sabellida and Pogonophora, and that these six taxa share a common ancestor, which evolved transverse rows of setae with apically curved spines and a formative site lateral to the edge of the neuropodial rim. According to the phylogenetic relationships proposed here, the Pogonophora are a subordinate taxon within the Annelida.

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A. Introduction

Setae can be found in several bilaterian taxa (Annelida: Gardiner 1992; Specht 1988; Westheide and Watson Russell 1992 – Mollusca: Brocco et al. 1974 – Brachiopoda: Orrhage 1973; Storch and Welsch 1972 – Pogonophora: George and Southward 1973; Gupta and Little 1970 – Echiura: Storch 1984). The special kind of setation in annelids, however, is regarded as a strong argument for the monophyly of the Annelida. The symmetrical arrangement of a dorsal and a ventral group of setae in each segment is hypothesized to be an autapomorphy of the Annelida (Bartolomaeus 1994a). Each seta is formed by a chaetoblast which lies at the base of an ectodermal pouch or setal follicle. Apical microvilli of this cell extend into longitudinally oriented canals inside the seta (Bouligand 1967). Differences in the number, size and orientation of the canals within one seta, therefore, reflect dynamic processes on the cell surface of the chaetoblast. Studies on the formation of the compound setae in *Nereis vexillosa* Grube, 1851, reveal that the size and shape of the setae result from modulations of the microvillar pattern during chaetogenesis (O'Clair and Cloney 1974).

Within the Annelida, setation is an important taxonomic characteristic (Doyle 1991; Fauchald 1977; Holthe 1986; Knight-Jones and Fordy 1979; Nilsen and Holthe 1985; Rosenfeld 1982; Thomassin and Picard 1972). Whereas in most polychaete annelids bundles of several long setae originate from both neuropodia and notopodia, among certain annelid taxa, such as the Terebellida, Sabellida, Oweniida, Maldanida and Arenicolida, only the notopodia bear such structures, while the neuropodium possesses a transverse row of several specialized setae. These are situated in a dorsoventrally oriented rim of the neuropodium and each seta consists of apically curved spines which arise from the wide setal shaft

which tapers off towards its base. Such setae are called hooked setae. If the length of the shaft is reduced and their spines point anteriorly and the setae are level with the surface of the epidermis, as in the Terebellida and Sabellida, these hooked setae are called uncini. Generally, they are arranged in transverse rows.

This paper describes the ultrastructure and the formation of the uncini of the terebellidan annelids *Pectinaria koreni* (Malmgren, 1866), and *Pectinaria auricoma* (Müller, 1776) and of the sabellidan annelid *Spirorbis spirorbis* (Linné, 1758). The observations will be compared with structural and developmental features of the capillary setae and with hooked setae of other annelids in order to find arguments for a possible homology between the uncini of serpulid and terebellid annelids. On the basis of these results, the following hypotheses can be proved:

1. Uncini are apomorphic structures of a subordinate taxon within the Annelida.
2. Due to morphological correspondences, several authors hypothesize that perviate and obturate (vestimentiferan) Pogonophora are annelids (Flügel and Callsen-Cencic 1994; George and Southward 1973; Nielsen 1995; Southward 1988; van der Land and Nørrevang 1975). As in the above-mentioned annelids, setae with a toothed apical margin have also been described in perviate and obturate Pogonophora (see George and Southward 1973; Southward 1988). These seta can help to precisely determine the sister group of the Pogonophora within the Annelida.
3. If a homology between hooked setae and uncini can be substantiated, the Maldanida, Arenicolida and Psammodrilida would be closely related to the above-mentioned three taxa.

These hypotheses also demonstrate the specific value of comparative studies of annelid setae, their formation and their evolutionary evaluation in a discussion on annelid phylogeny. This investigation is part of a larger attempt towards a comparative analysis of chaetogenesis in annelids.

B. Material and methods

Trochophores and benthic stages of *Pectinaria auricoma*, and older tubiculous larvae and benthic juveniles of *Pentinaria koreni* were collected in summer 1989, either from plankton samples taken off the island of Helgoland or from bottom stages dredged from the "Phoronis-Grund" off Helgoland, and were fixed for light and electron microscopy. In summer 1989, *Spirorbis spirorbis*, which lives in calcareous tubes on *Fucus serratus*, was collected at Helgoland and removed from the tubes to obtain the spawn to rear it in petri dishes. The hatching larvae and juvenile animals were fixed in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.2, 4° C) for 60–90 min, rinsed in the same buffer, postfixed in 1% OsO_4 buffered in 0.1 M sodium cacodylate for 60 min at 4° C and dehydrated in an acetone series.

For transmission electron microscopy, the animals were embedded in Araldite and cut into a complete series of silver-interference coloured sections (65–75 nm) with a diamond knife (Reichert Ultracut microtome). The sections were kept on formvar-covered single slot copper grids, automatically stained with uranyl acetate and lead citrate in an LKB Ultrostainer and examined (Zeiss EM 10B and EM 900 electron microscopes). Two larvae of each species, one cross-sectioned and one sectioned longitudinally have been investigated. The position and ultrastructure of the setae were reconstructed according to a complete series of sections. The course of the formation of the uncini was redrawn from the investigation of six developing neuropodia in *P. koreni* and three in *S. spirorbis*.

For scanning electron microscopy, dehydrated juvenile and adult animals were dried in CO_2 according to the critical-point method (critical point drier, Balzers), sputtered with gold (sputter coater, Balzers) and examined (Novoscan scanning electron microscope).

For light microscopical investigations, *Pomatoceros triqueter* (Linné, 1758) from the island of Helgoland and *Clymenura clypeata* (Saint Joseph, 1894) from the bay of Arcachon were fixed in Bouin's fluid, dehydrated in an alcohol series; methylbenzoate and butanol; and embedded in Paraplast. Sections 10 µm thick, were stained with Masson-Goldener's trichrome and investigated (Zeiss Axioscop) with Normarsky contrast.

Individuals of the species investigated are deposited at the Zoological Museum, Göttingen. The names for the different higher taxa of the Annelida are based on the classification presented George and Hartmann-Schröder (1985).

C. Results

I. Terminology

Some remarks must be made on the terminology of the seta and especially of certain parts of the uncini, because the description of the formation of the uncini will depend on these terms. In the literature on annelid setae, various names have been applied to different parts of the uncinus. Holthe (1986) gives an excellent description and terminology for the uncini. This will generally be adopted, although in this paper the term uncini is restricted to those setae which possess anteriorly curved apical spines, while the others will be termed hooked setae. Both share the same structural components: the apical group of curved spines is termed the capitium and a single, large tooth in front of the capitium is called the rostrum. The tips of the spines or the rostrum may face a compact or sometimes toothed structure, the subrostral process. The shaft will be termed the manubrium (Fig. 1).

II. General

In the trochophore larvae of *P. auricoma*, all setae are still developing and are hiddein in epidermal pouches. Although they are almost invisible externally, in transverse sections two capillary setae and at least two uncini per segment could be counted on each body side, so that the prospective number of notopodia and neuropodia and, thus, the species could be determined. In tubicolous pelagic larval stages of *P. koreni* its setigerous segments can be distinguished externally. On each body side, the first segment has two capillary setae, the second and third bear one uncinus and two capillary setae each, and segments 4–15 have two capillary setae and transverse rows of three to five uncini each. In adults, rows of uncini are restricted to the setigerous segments

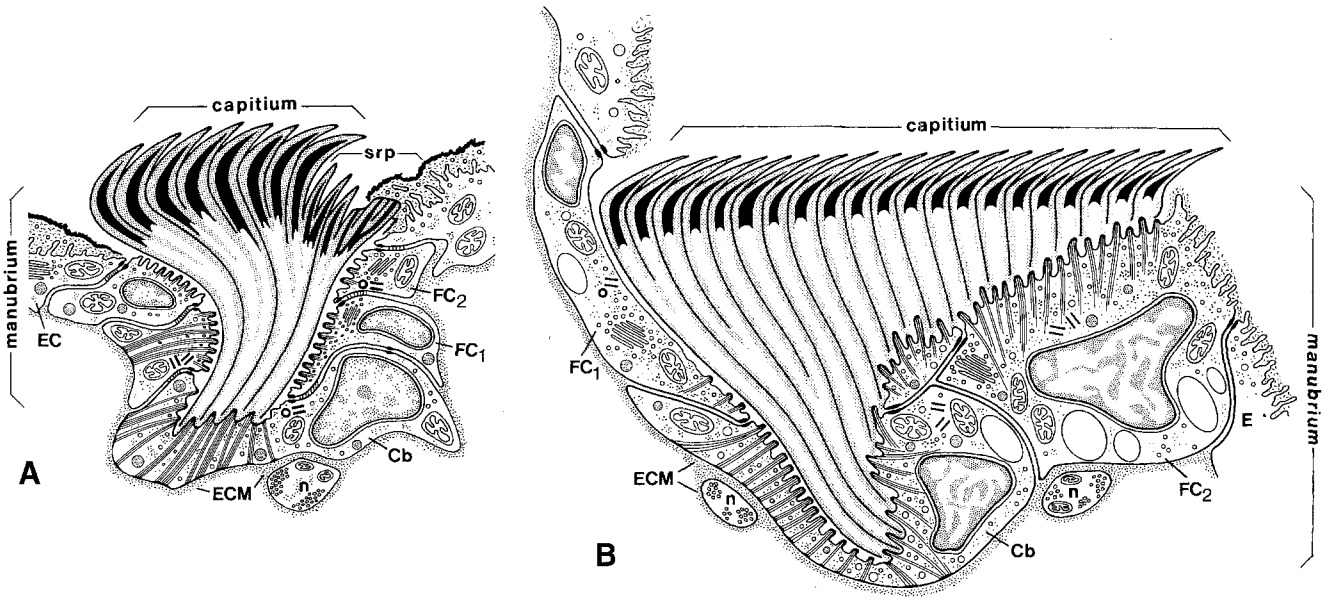


Fig. 1A, B Uncini in *Pectinaria koreni* (**A**) and *Spirorbis spirorbis* (**B**). **A** Solitary uncinus of the third setigerous segment, **B** Ventralmost uncinus of second setigerous segment. *Cb* chaetoblast, *E* epidermis, *EC* epidermal cell, *ECM* extracellular matrix, $FC_{1,2}$ follicle cells, *n* neurite, *srp* subrostral process

4–15. The surface spines of the uncini always curve anteriorly and their axis is parallel with that of the body. In the earliest stages of setal development, both capillary setae and uncini are situated close to each other and are only separated by a little extracellular matrix (*ECM*). Later, muscle cells invade this ECM and completely separate notopodial and neuropodial setae (Fig. 7A). The individual uncini follicles lie side by side and are never separated from each other by an ECM. The perifollicular ECM, which is continuous with the subepidermal ECM (basal membrane), surrounds all follicles as a whole. The independent movement of only one seta, thus, seems unlikely in the neuropodia and the entire row of hooked setae must move as one unit. The new uncini are always formed adjacent to the capillary seta, so that the youngest uncini are found midlaterally and the oldest ventrally (Fig. 2A). Structurally the unicini of both species differ in the number, length and density of the apical spines (Hartmann-Schröder 1971, Figs. 156, 157).

Only the first chaetigerous segment of the larva of *S. spirorbis* bears a pair of dorsolaterally situated capillary setae. The second, third and fourth segments bear capillary setae and two or three uncini, which are aligned with their neighbours in a lateroventral transverse row. As in the *Pectinaria* species, perifollicular ECM surrounds all follicles as a whole and new uncini are formed close to the capillary setae. Although the topographical relation between capillary setae and uncini is switched in the abdominal segments of adults, the formative site of the uncini still lies adjacent to the bundle of capillary setae in these segments.

III. Uncini

In all three species investigated, each uncinus arises from a setal follicle which consists of three cells, two follicle cells and one basally situated chaetoblast (Fig. 1A). In the *Pectinaria* species, both follicle cells subsequently surround the uncinus, which is thus percellular, whereas each uncinus of *S. spirorbis* lies intercellularly between both follicle cells (Fig. 1B). In this species, both follicle cells also shed a small amount of cuticular material. In any case, apical adhaerens and septate junctions link the cells of the follicle and almost no differences can be found between them on the subcellular level. Besides from the nucleus, each cell contains a diplosomal pair of centrioles, rough endoplasmatic reticulum, a few dictyosomes and vesicles of different sizes and mitochondria, the number of which is much higher in the follicle cells than in the chaetoblast.

In *P. koreni* and *P. auricoma*, each uncinus consists of a broad capitium with several anteriorly curved spines. Their tips face a group of short, straight spines. Anterior to this group, the cuticle covers a blunt process of the uncinus. Spines and process represent the subrostral process of the uncinus (Fig. 2B, D). Underneath these structures, the manubrium of the unicinus gradually tapers off towards its base. The proximal section of the manubrium has an almost constant diameter and is very short in the solitary uncini of the second and third setigerous segments (Fig. 1A). Inside the uncini, canals run from the spines to the base and join on their way downwards so that the total number of canals decreases from the apex to the base. Generally, the peripheral canals are smaller in diameter than the central ones and seem to be slightly twisted around them (Fig. 3H, I). An electron-dense material almost completely fills the canals of the spines and the upper part of the manubrium (Figs. 2C–E, 3A, H).

In *S. spirorbis*, the capitium consists of numerous parallel rows of apical spines which are bent towards the manubrium and face an anterior crest. The crest does not

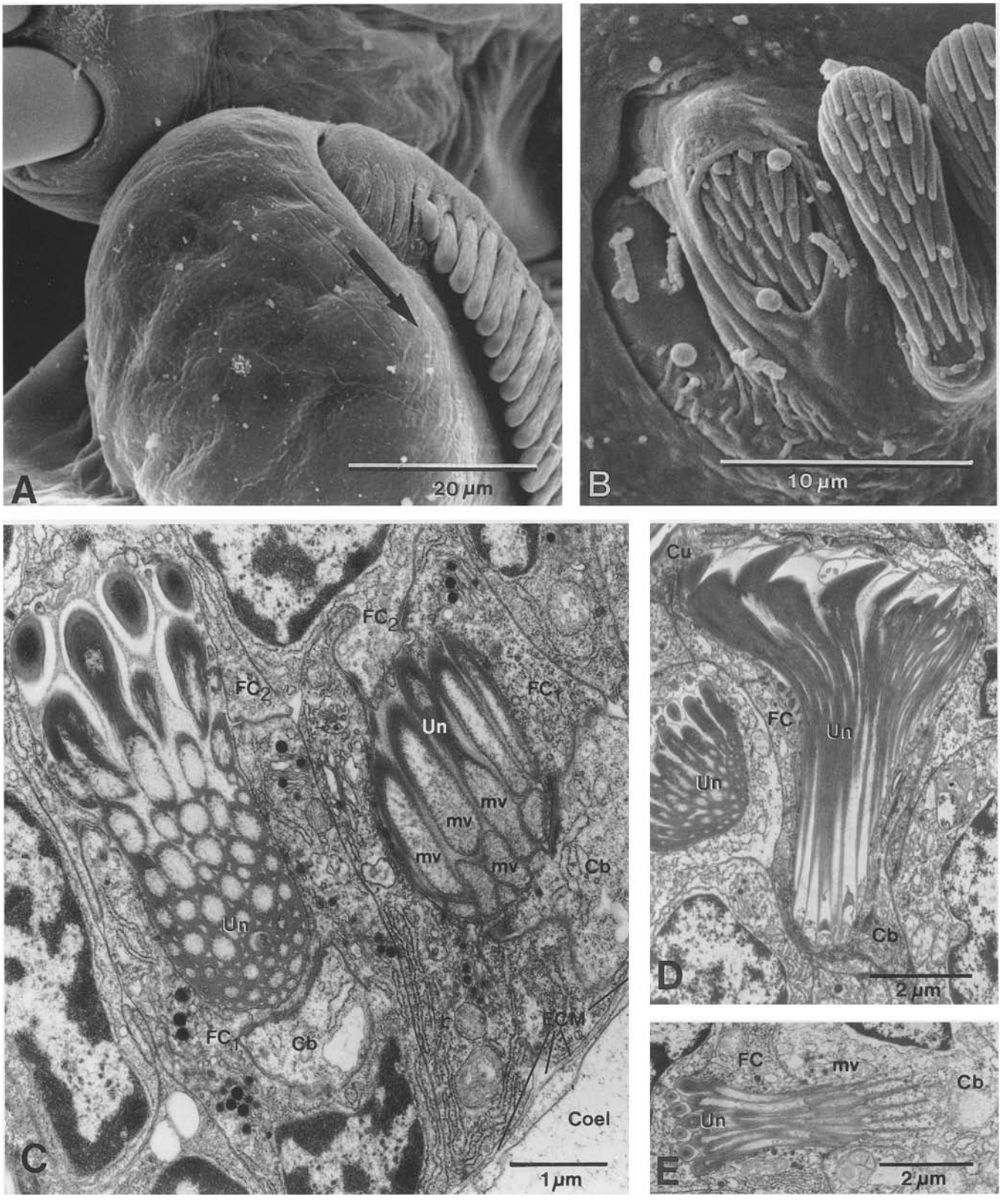
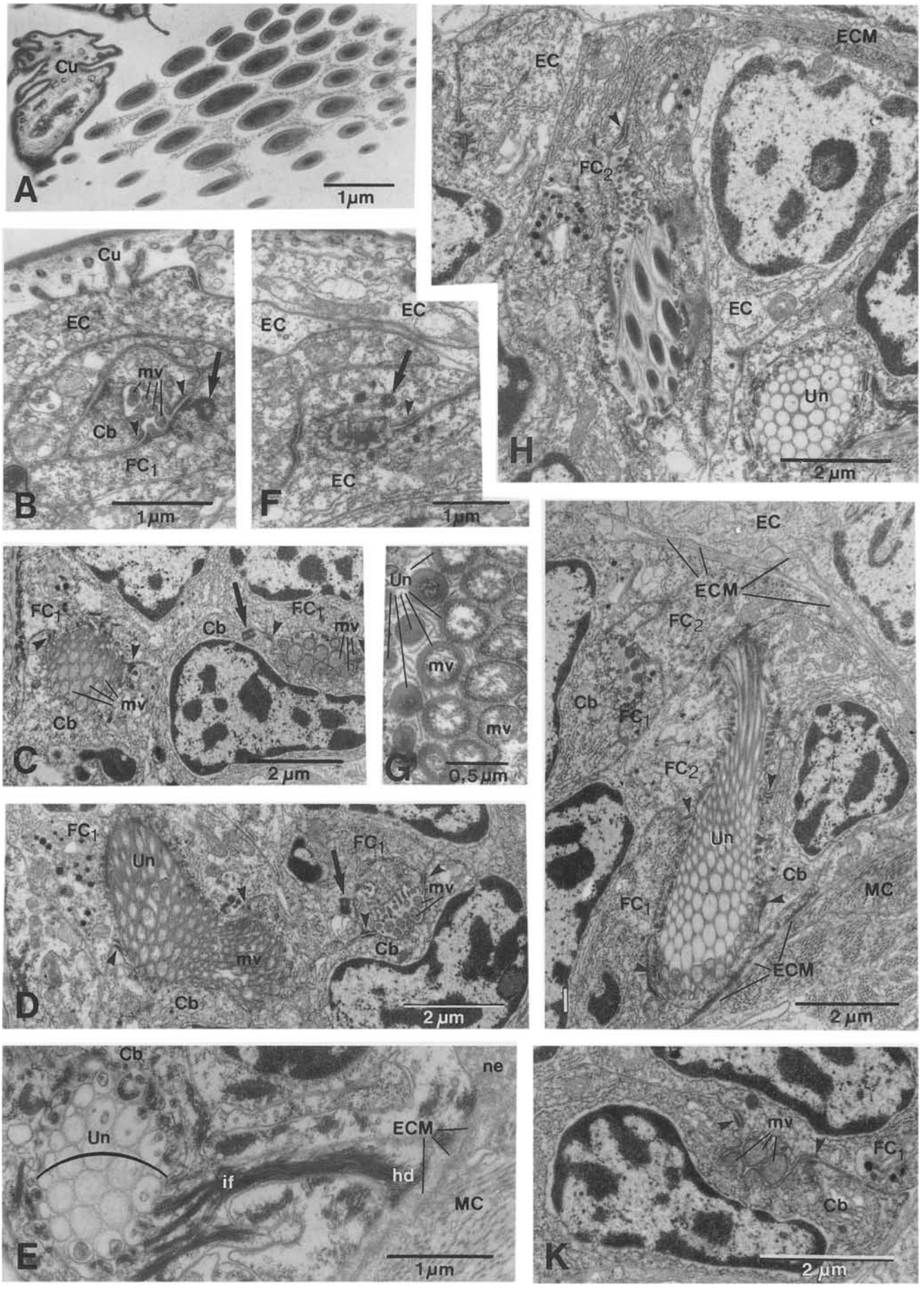


Fig. 2A–E Uncini and their formation in *P. koreni* (**A, B**) and *P. auricoma* (**C–E**). **A** Formative site lies medially and close to the capillary setae (*arrow* points ventrally). **B** Fully differentiated uncinus pierces the cuticle. **C** Two adjacent follicles at different developmental stage. **D** Complete uncinus (*Un*) hidden beneath the epidermal surface, note the spines facing the subrostral process. **E** Actin filaments withdraw the microvilli (*mv*) from the uncinus. *Cb* chaetoblast, *Coel* coelum, *Cu* cuticle, *ECM* extracellular matrix, $FC_{1,2}$ follicle cells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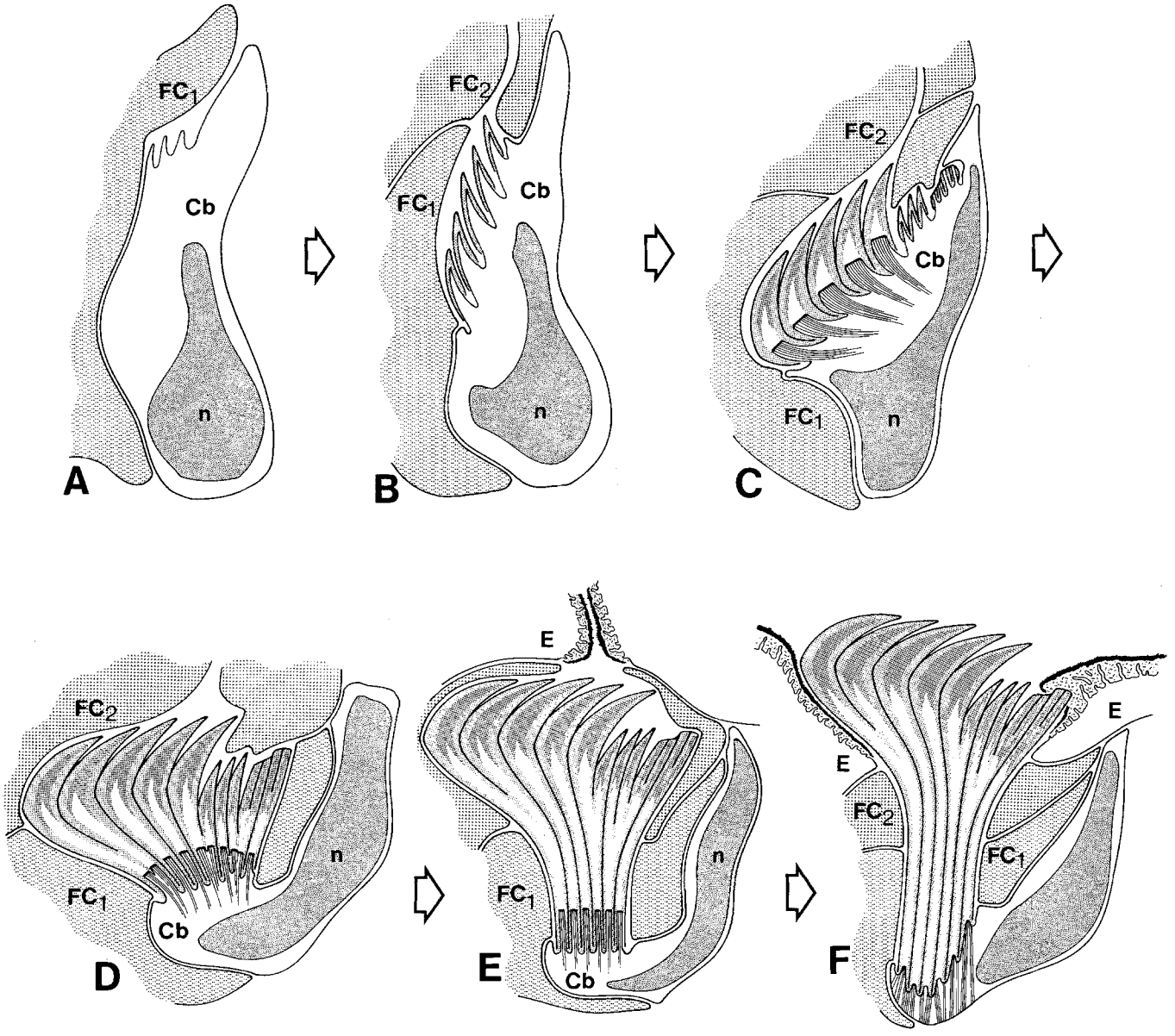


Fig. 4A–F Formation of the uncini in *P. koreni* and *P. auricoma*. **A** The chaetoblast (*Cb*) generates microvilli along an apicobasal axis. **B** Setal material surrounds the stout microvilli. **C** Microvilli are withdrawn by actin filaments and their orientation determines the curving of the spines; additional microvilli are formed where chaetoblast and first follicle cell (FC_1) have met. **D, E** Tapering of the manubrium is caused by merging of microvilli. **F** Actin filaments are replaced by intermediate filaments which attach the uncinus to the follicle. *E* epidermis, *n* nucleus of chaetoblast

Fig. 3A–I, K *P. koreni. Large arrows* point to centrioles and *arrowheads* to the adhaerens junctions between the cells of a follicle. **A** Sagittal section of the capitium. Note the dense core inside the spines. **B** Earliest stage of a developing follicle, prospective chaetoblast (*Cb*) with microvilli (*mv*). **C** Adjacent developing uncini. **D** Left follicle: formation of the subrostral process. Right follicle: formation of the spines. **E** Truncated microvilli and intermediate filaments (*if*) inside the chaetoblast of a completely formed uncinus (*Un*). **F** Cuticularized duct leads into the follicle. **G** Setal material surrounds the microvilli to form the apical spines. **H** Sagittal section of the capitium of an uncinus which lies inside the follicle. **I** Microvilli are withdrawn from the uncinus. **K** Stout microvilli determine the structure of the apical spines. *Cu* cuticle, *EC* epidermal cell, *ECM* extracellular matrix, $FC_{1,2}$ follicle cells, *hd* hemidesmosome, *MC* muscle cell, *ne* neurite

correspond to the subrostral process in other species because it is formed in a different manner. In contrast to the *Pectinaria* species, the manubrium does not taper proximally but resembles a triangle instead, because the caudalmost part of the uncinus, which emanates from the surface of the animal, is much higher than the frontal part which is level with the epidermal surface (Fig. 8A). The canals left inside the uncinus mostly run in a parallel orientation from the apex to the base and merging of adjacent canals was only observed in the caudalmost part of the uncinus (Fig. 1B). As in both *Pectinaria* species, an electron-dense material completely fills the canals of the capitium (Fig. 5B, C).

In the two *Pectinaria* species, short processes of the chaetoblast extend into the canals of each uncinus. The surface of the follicle cells which face the manubrium also bears such processes (Fig. 3E, I). In *S. spirorbis*, the processes of the chaetoblast only extend into the most posterior canals, whereas the anterior canals are occupied by microvilli of the first and second follicle cells (Figs. 1B, 5C). In all species, hemidesmosomes connect these processes to the uncinus. Intracellularly they are attached to strong bundles of intermediate filaments which cross the cytoplasm and adhere to hemidesmosomes on the matrix side of the cells (Figs. 3E, 5G). In this way, the chaetoblast and the follicle cells anchor the uncinus to the follicle and mechanically connect it to the perifollicular muscular system.

IV Development of the uncini

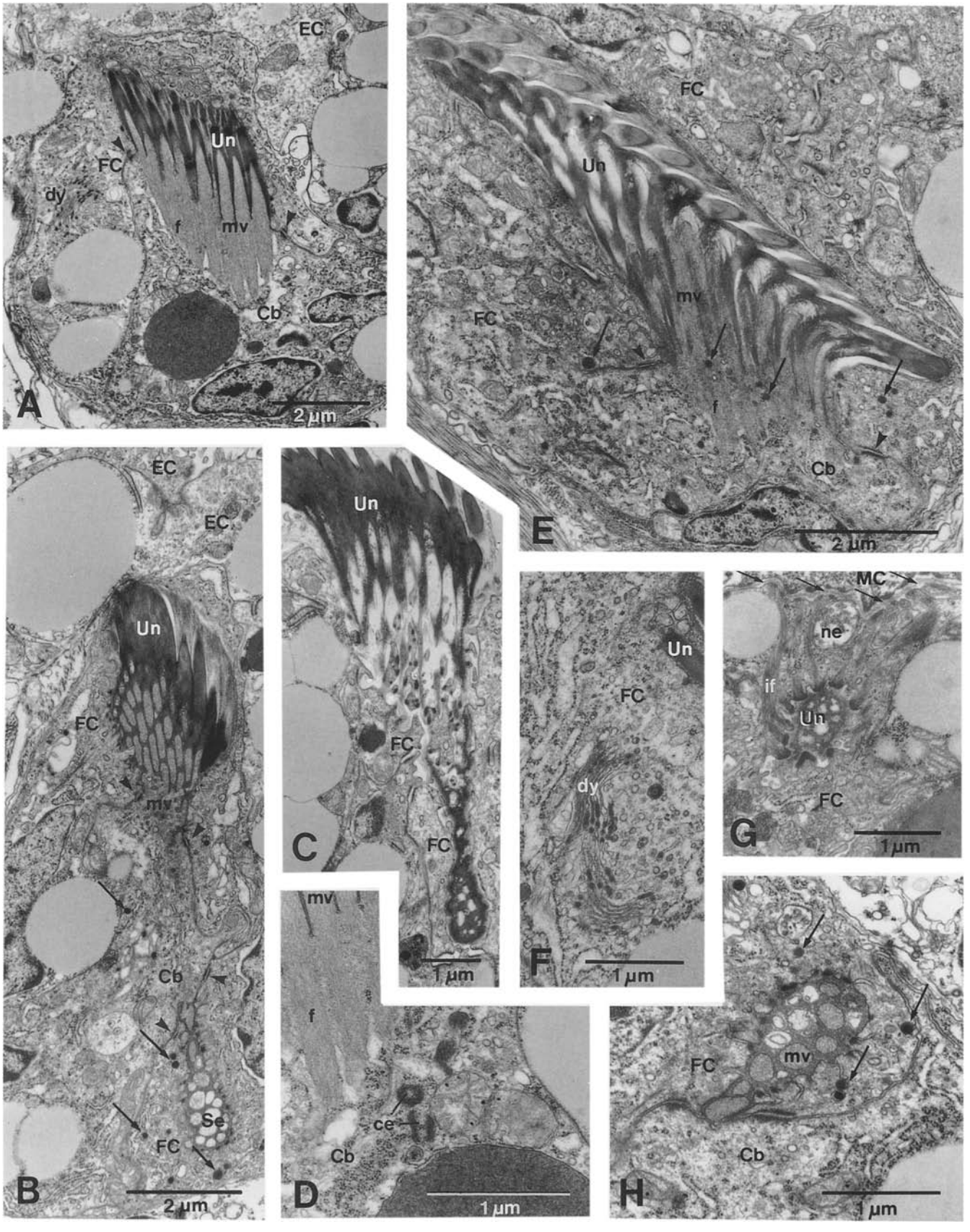
In *P. koreni* and *P. auricoma*, four cells form the *anlage* of each uncinus, i.e. one epidermal cell, which produces a cuticle, two follicle cells and one prospective chaetoblast (Fig. 4). The earliest stage observed consists of an epidermal invagination which is cuticularized except for a small, basally located cavity (Fig. 3B, F). This is lined by two adjacent cells. One, the prospective chaetoblast, continuously generates conical microvilli along an apicobasal axis (Figs. 3K, 4A). The other cell, the proximal or first follicle cell, contains several vesicles with electron-black material referred to as setal material in this paper. This material seems to be released into the space. In several early developmental stages, this material can be seen to surround the tips of the microvilli (Figs. 2C, 3G). In later stages, the microvilli become longer and thicker and their bases nearly touch each other. The deposition of setal material creates the prospective spines of the uncinus and the microvilli form their core (Figs. 2C, 4B).

During this first developmental phase, the proximal follicle cell, which was initially separated from the apical region of the chaetoblast, has continuously changed its position, so that it finally encloses the spines of the prospective uncinus and comes into contact with the apical edge of the chaetoblast. At this stage, the orientation of the newly formed spines begins to change. This is initiated by the appearance of actin filaments inside those microvilli which formed the spines (Fig. 4C). The filaments adhere to the plate-like, electron-densely stained tips of the microvilli and extend deep into the cell body, almost perpendicular to the original orientation of the microvilli. During further development, the actin filaments withdraw the microvilli from the tips of the spines, while the manubrium is being formed parallel to the orientation of the actin filaments. Therefore, the spines are curved towards the manubrium (Figs. 2D, 4D). At the same stage, several smaller microvilli can be seen on the surface of the chaetoblast, apical to the primarily formed ones and exactly in that region, where the proximal follicle cell and the chaetoblast had met (Figs. 3D, 4C). Setal material of the proximal follicle cells surrounds these microvilli to form the subrostral process of the uncinus. Because the orientation of the actin filaments inside the primarily formed microvilli had been changed before the formation of the subrostral process began, the spines of the uncinus face this process. Electron-dense material fills the small canals which are left when the microvilli are retracted (Fig. 2C).

Both groups of microvilli, i.e., the one which generated the capitium and the one which formed the subrostral process, converge during the development of the manubrium. Tiny vesicles containing an electron-dense material are released from the chaetoblast and both follicle cells. Those released by the chaetoblast fuse with the cell membrane between the bases of the microvilli and elongate the manubrium, while those released by the follicle cells form the compact and strengthened peripheral wall or cortex of the uncinus. The fusion of adjacent microvilli and the reduction of their diameter lead to a basal tapering, which is characteristic of the manubrium of both *Pectinaria* species (Figs. 2D, 4E). Finally, the microvilli are almost completely withdrawn from the uncinus and modified into small cytoplasmic processes. The actin filaments are replaced by intermediate filaments. At almost the same time, the first follicle cell also aquires a system of intermediate filaments to attach the uncinus to the follicle (Fig. 4E). The development of the uncinus has now been completed, but generally it still remains hidden inside the setal pouch (Figs. 2D, 3H). It is important to mention this observation because the uncinus does not appear on the animal's surface by growing; instead, cellular movement, caused by the continuous formation of subsequent uncini, is responsible for the definitive position of the uncinus within the neuropodium.

The continuous generation of new uncini in all three species has another effect on the structure of each individual uncinus (Figs. 2C, 3C, D) because the relationship between the cells of the zone of differentiation, and especially between the developing uncinus, the chaetoblast and the follicle cells, are constantly changing. The apical spines, which during formation were initially oriented perpendicular to their definitive position within the neuropodium, are turned during formation until they finally align with their older neighbours in transverse rows (Fig. 2A). In both *Pectinaria* species, the course of the hollow canals inside the uncinus reflects this process, because in each uncinus the peripheral canals are twisted along their way from the apex to the base, whereas the central one remains in its primary orientation (Fig. 3I).

In *S. spirorbis,* the *anlage* consists of four cells: one epidermal cell and three follicular cells (Fig. 6A). A narrow duct pierces the epidermal cell and leads to a cavity which is lined laterally by two follicular cells and basally by the chaetoblast. The latter bears numerous microvilli which arise in several longitudinal rows (Fig. 5A). These rows of microvilli are oriented oblique to the epidermal surface. Setal material covers the microvilli like lancets and forms the prospective spines of the uncinus. Electron-dense vesicles, generated by dictyosomes, release their contents into the narrow space between the micro-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villi (Fig. 5A, E). In follicle cells, vesicles with electron-dense contents are produced by dictyosomes. These vesicles empty into the perisetal space and enlarge and strengthen the cortex of the uncinus (Fig. 5F, H).

Each microvillus of the chaetoblast contains a strong bundle of actin filaments which extends into the cytoplasm of the chaetoblast (Fig. 5D). These actin filaments retract the microvilli from the developing uncinus, leaving hollow canals inside. As in the *Pectinaria* species, actin filaments and spines have initially the same orientation, but during further development the orientation of the microvilli changes drastically, so that the long axis of the spines and that of the microvilli form an angle of about 90° (Fig. 5E). The formation of the capitium of the uncinus is almost identical in *S. spirorbis* and the *Pectinaria* species. Deposition of setal material between and around an anterior group of microvilli leads to the formation of a crest. Its formation differs from that of the subrostral process in the *Pectinaria* species.

During the formation of the manubrium, the microvilli are withdrawn more rapidly in the prospective caudal part of the uncinus and the microvilli merge more often with each other than in the caudal part (Fig. 5B, C). In a fully developed uncinus, the caudal edge is, therefore, much longer than the frontal one and causes the triangular shape of the uncini of *S. spirorbis*. During the formation of the manubrium, the chaetoblast changes its shape and leaves only a small cytoplasmic seam beneath the prospective frontal part. The nucleus and most of the cytoplasm are concentrated underneath the caudal edge (Fig. 6B). At the end of chaetogenesis, the chaetoblast completely removes its microvilli from the frontal edge of the uncinus and leaves hollow canals which are occupied by short processes of the first and second follicle cells. These processes contain intermediate filaments and apical hemidesmosomes that attach the uncinus to the follicle. Truncated microvilli of the chaetoblast only remain in the canals of the caudal part (Figs. 5G, 6C). As in the *Pectinaria* species, their actin filaments are replaced by intermediate filaments which fasten the uncinus to the follicle.

V. Capillary setae

A few capillary setae indicate the position of the notopodium in larval *Pectinaria* species and *S. spirorbis*. Two capillary setae always appear simultaneously at the beginning of development, while additional setae are formed later. Each seta has its own follicle which in both *Pectinaria* species consists of three cells, the chaetoblast and two follicle cells (Fig. 7F). All cells are linked by adhaerens and septate junctions. ECM surrounds the

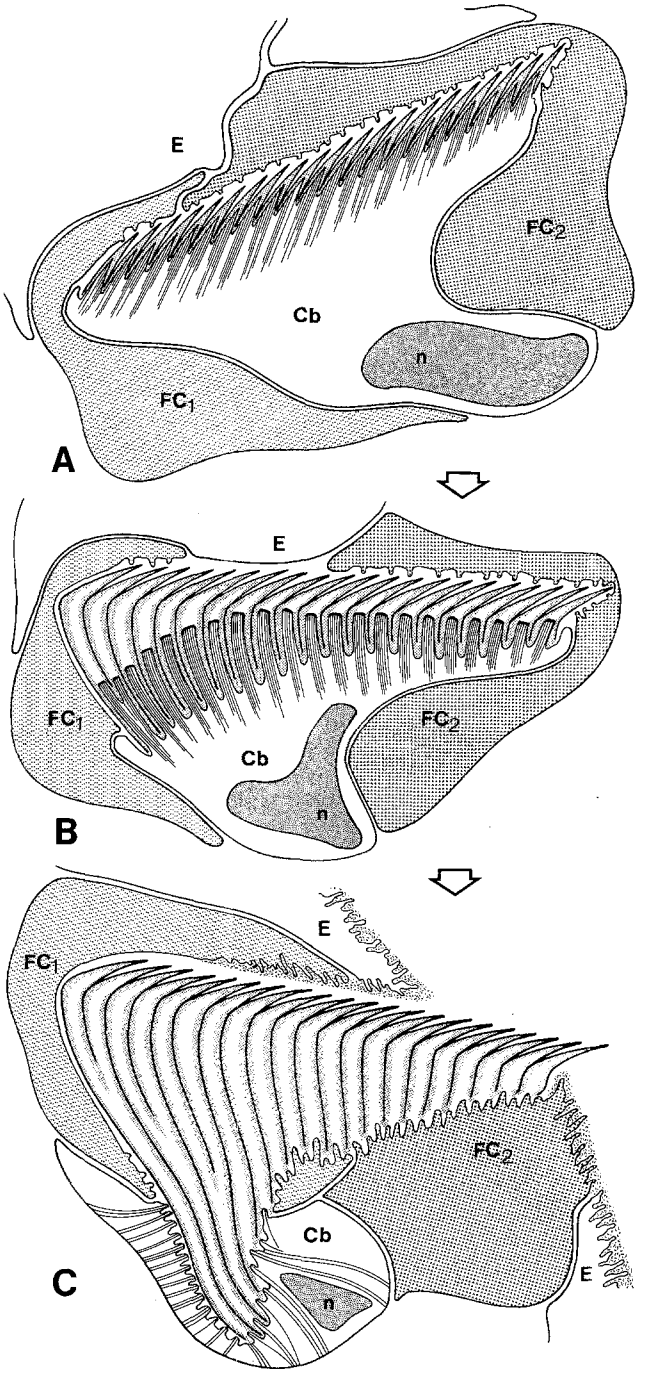
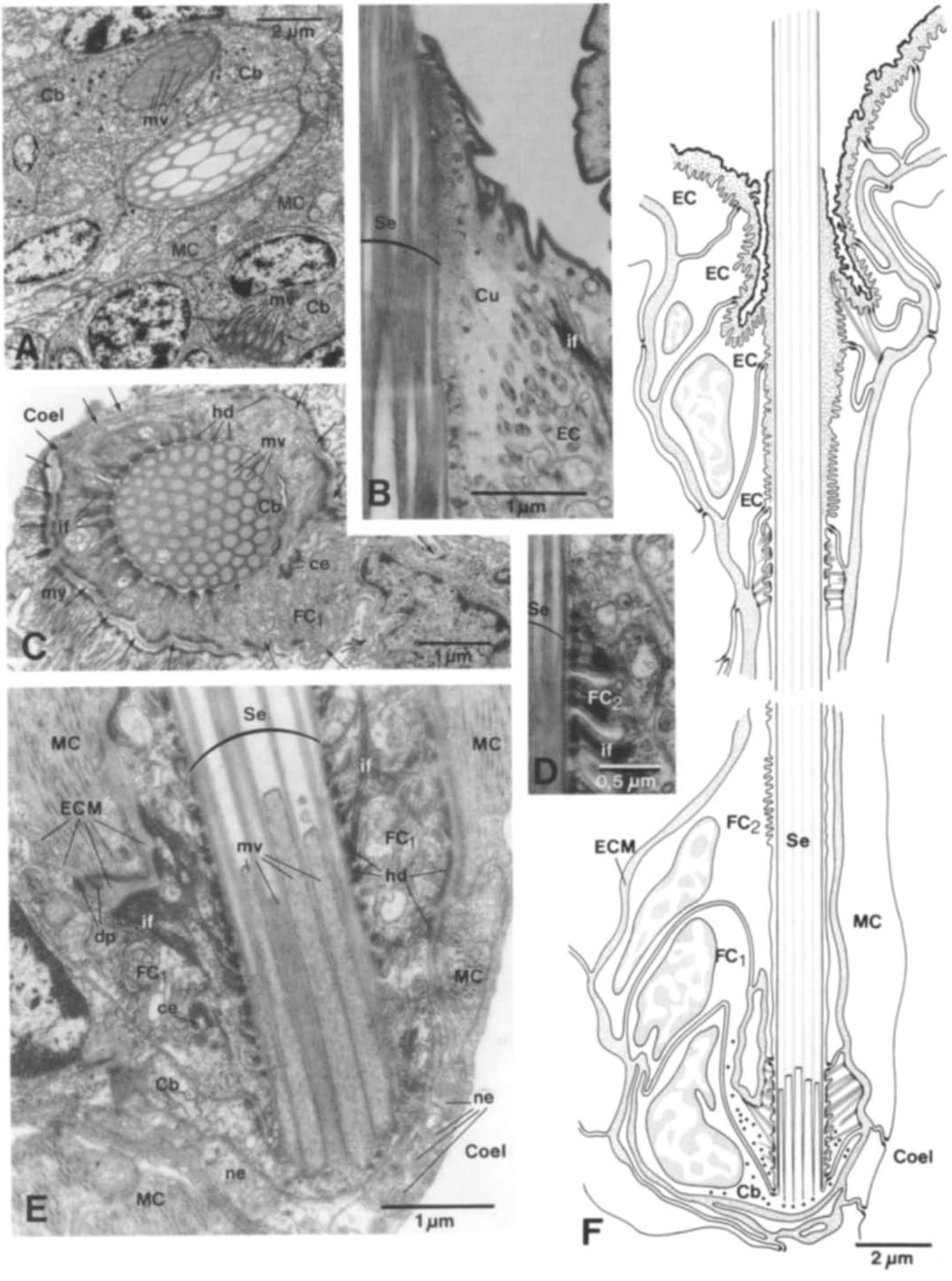


Fig. 6A–C Formation of the uncinus in *S. spirorbis* (scheme). **A** Apical microvilli of the chaetoblast (*Cb*) form a core inside the prospective spines of the uncinus. **B** Retraction of the posterior microvilli preceeds that of the anterior ones, orientation of the actin filaments determines the structure of the uncinus. **C** Microvilli of the chaetoblast are truncated and completely withdrawn from the anterior part of the uncinus. Canals left by them are occupied by processes of the follicle cells (*FC*). Actin filaments are replaced by intermediate filaments which attach the uncinus to the follicle. *E* epidermis, *n* nucleus

Fig. 5A–H Formation of the uncini in *S. spirorbis*. *Arrowheads* mark adhaerens junctions between the cells of the follicle. **A** Spines of the uncinus are formed. Note the actin filaments (*f*) inside the microvilli (*mv*). **B** Posterior part of the uncinus (*Se*) is formed more rapidly than the frontal part (*Un*), (*arrows* mark electron-dense vesicles). **C** Truncated microvilli of the follicle cells extend into the canals of a fully developed uncinus. **D** Chaetoblast, actin filaments and pair of centrioles. **E** Orientation of the actin filaments determines the curvature of the spines (*arrows* mark vesicles, some are inside the microvilli). **F** Electron-dense material of the vesicles is produced by a dictyosome (*dy*). **G** Posterior base of the uncinus is attached by hemidesmosomes and intermediate filaments (*if*), (*arrows* mark ECM). **H** Vesicles release their electron-dense contents (*arrows*) into the follicular lumen. *EC* epidermal cell, *MC* muscle cell, *ne* neurite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whole group of notopodial follicles, but there is no ECM separating individual follicles. Because muscle cells attach to the perifollicular ECM, single setae cannot be moved independently within one notopodium.

The large nucleus of the chaetoblast lies laterally to the seta and is surrounded by little perinuclear cytoplasm in all three species (Fig. 7A, C, E). The actual site of formation is connected to the perikaryon by a small cytoplasmic bridge and bears several microvilli in a staggered arrangement. The peripheral and shorter microvilli have a slightly smaller diameter than the inner and longer microvilli (Fig. 7C). The tips of the microvilli are blunt and each consists of an electron-dense plug to which the actin filaments adhere (Fig. 7E). Vesicles with electron-dense contents fuse with the basal intermicrovillar cell membrane and release material to elongate the seta. In this way the medulla of the seta is formed. Apart from these vesicles, the cytoplasm of the chaetoblast contains rough endoplasmic reticulum, a pair of centrioles and, compared to adjacent cells, only a few mitochondria.

The perikaryon of the first follicle cell lies in a lateral pouch and a small cytoplasmic bridge connects it to that part of the cell which enwraps the seta. Here, bundles of intermediate filaments cross the cytoplasm and are attached to hemidesmosomes on either side of the cell, connecting it to the perifollicular ECM and to the seta (Fig. 7C, E). Apart from this and a greater number of mitochondria, the follicle cell contains the same subcellular structures as the chaetoblast. The material of the electron-dense vesicles is presumed to contribute to the formation of the cortex.

In *P. koreni*, the second follicle cell is extremely long (28 µm) and surrounds the main part of the seta (Fig. 7F). Due to the length, the wall of the second follicle cell may be very thin (120–240 nm). The nucleus lies basally in a small lateral pouch of the cell. The cytoplasm of this cell is generally electron-bright and, with the exception of the electron-dense vesicles, contains almost the same organelles as the two other cells. Distally, strong bundles of intermediate filaments cross the cytoplasm and attach the seta to the follicle as in the first follicle cell (Fig. 7D). Distal to this region, epidermal cuticular material surrounds the seta and adheres to it (Fig. 7B). This is a remarkable observation, because it clearly demonstrates that the setae cannot be moved inside their follicles. The organisation of the perisetal muscular systems supports this observation.

Both notopodial setae in the tubicolous stages of *P. koreni* are accompanied by one large basoapically oriented muscle cell (38 µm in length) which partly envelops both follicles. Proximally, this muscle cell is attached to the first follicle cell by the perifollicular ECM and distally, it is connected to the perisetal epidermal cells by the subepidermal ECM (Fig. 7F). Other muscle cells extend mediolaterally. Contraction of these muscle cells retract the setae into the notopodium, whereas contractions of the large muscle cell protract the setae. Because the setae cannot be moved within the follicle, the entire perisetal epidermis and cuticle must be infolded during retraction. Within the perifollicular ECM, several neurites are found which are presumed to co-ordinate this setal movement (Fig. 7E).

Fig. 7A–F Capillary setae. **A** *P. auricoma*, larva, **B, D–F** *P. koreni* and **C** *S. spirorbis*. **A** Early anlage. Muscle cells (*MC*) invade the extracellular matrix between notopodial and neuropodial setae. **B** Cuticle (*Cu*) adheres to the seta (*Se*). **C** Intermediate filaments (*if*) of the first follicle cell (FC_1), (*arrows* mark ECM). **D** Apical attachment area of the second follicle cell (FC_2). **E** Microvilli (*mv*) of the chaetoblast (*cb*) and intermediate filaments of the first follicle cell. **F** Follicle as redrawn from electron micrographs. *ce* centrioles, *Coel* coelom, *dp* dense plaques, *EC* epidermal cell, *hd* hemidesmosomes

D. Discussion

Since O'Clair and Cloney (1974) detected the main principle behind the formation of annelid setae, i.e., that temporal modulations of the microvillar pattern on the surface of the chaetoblast determine the structure of the setae, no further studies on the mode of formation of setae have been undertaken. Nevertheless, considering the large number of different setal types in annelids, it seems fruitful to compare their development because this may be a good source of characteristics for when the evolution of different setae and the phylogeny of annelids is studied.

I. Setation in the Terebellida and Sabellida

The monophyly of the Sabellida is strongly supported by numerous autapomorphies (number 8 in Fig. 9), (Knight-Jones 1981; Pettibone 1982). Their perioral funnel-like tentacular crown consists of a pair of semicircular lobes. Each lobe primarily bears three ciliad bipinnate filaments used for respiration and suspension filter feeding. The distal ducts of the single pair of nephridia fuse and have a common dorsomedian nephropore. The larval protonephridium has a single multiciliated terminal cell whose cilia form a central flame (Bartolomaeus 1995).

This assumption of a monophyletic origin seems problematic especially for the Terebellida, but the existence of primarily non-retractible oral tentacles which are used in feeding and tube building (Pettibone 1982; Holthe 1986) is regarded as an autapomorphy of this taxon (number 4 in Fig. 9). In agreement with Holthe (1986) and Knight-Jones (1981), at least the Sabellariidae are excluded from the Terebellida. In order to determine the position of the sabellariids, the structure and development of the uncini of *Sabellaria alveolata* (Linné, 1767) is being investigated at present. Because the position of the notopodial and neuropodial setae in the abdominal region is the reverse of that of the thorax in sabellarids and sabellidans, it has to be tested whether sabellidans and sabellariids are sister groups (see also Hartmann-Schröder 1971; Knight-Jones 1981).

The setigerous segments 2 and 3 of the tubicolous larva of *P. koreni* each possess a single uncinus, which is lost during further development. This is an important observation, because in other terebellidan taxa, such as the Terebellidae and Trichobranchidae, biramous parapodia are described in the first setigerous segment (Pettibone 1982). The absence of neuropodial setae in the first setigerous segment is regarded as an apomorphic feature and the transitory uncini, found in *P. koreni*, may be reminiscent of the ancestral state of setal arrangement. This also coincides with data on the fate of the hooked setae in larval Maldanida. Initially, in the larvae and early juveniles, hooked setae are found in the first setigerous segment, but later they are lost (Bookhout and Horn 1949 for *Axiothella mucosa* Andrews, 1891). Arenicolids have a transverse row of hooked setae inside the neuropodial rim from the first setigerous segment onwards (Wells 1959).

In the three species investigated, the canals inside the spines, which remain after the retraction of the microvilli, are filled with an electron-dense material. George and Southward (1973) made a comparable observation when they studied some terebellidan and sabellidan uncini. This material is presumably released by vesicles which can sometimes be seen inside the microvilli of the chaetoblast. Although the exact origin of this material is uncertain, it seems to strengthen the spines and is characteristic for the uncini investigated so far. Its deposition may have a functional reason. Whereas the manubrium seems to be elastic, due to the hollow canals inside, the spines which must anchor the animal inside the tube have to be very stiff and this would be impossible if there were hollow canals inside the spines.

Although the uncini of *Pectinaria* species are presumed to be derived within the Terebellida (Holthe 1986), the formation of those structural components which are characteristic of all terebellidan uncini could be studied. In the *Pectinaria* species and in *S. spirorbis*, modifications in the orientation of the intramicrovillar actin filaments have an enormous impact on the structure, because these modifications are exclusively responsible for the characteristic curving of the apical spines. The mechanisms which are responsible for the re-orientation of the actin filaments, however, remain unknown. No spatial interactions between the cells of the follicle and other cells of the anlage are involved in this process, because the spines of the solitary uncini in the second and third setigerous segment of *P. koreni* are curved in the same way, as are those of the subsequent segments. Nevertheless, a modification of the spatial arrangement of the cells during chaetogenesis has some effect on the structure of the manubrium in the *Pectinaria* species.

The proximal follicle cell, which initially lies alongside the chaetoblast, enwraps the primarily formed conical microvilli during the formation of the spines and surrounds the developing uncinus like a ring after the capitium has been formed. It is supposed that a circular constriction of the first follicle cell forces the enclosed microvilli of the chaetoblast to converge and, thus, leads to a basal tapering of the manubrium. The triangular structure of the manubrium of the *S. spirorbis* uncinus corroborates this interpretation, because here each uncinus lies between both follicle cells and neither of them enwraps the uncinus. A subcellular structure which must be correlated with such a supposed constriction of the first follicle has, however, not yet been found in *Pectinaria* species.

The neuropodial growth zone lies almost perpendicular to the row of fully developed uncini in these species. During the course of chaetogenesis and the generation of new uncini, the whole follicle is forced to continuously change it position, so that the relationship between the follicle cells and the uncinus is constantly modified, and the uncinus, which at first is oriented perpendicular to its definitive position within the neuropodium, shifts during formation until it is finally aligned with older uncini in a transverse row. The arrangement of the canals inside the uncini reflects this process, because the peripheral ones are wound around the central ones which retain their primary orientation (not considered in Fig. 4). Such a twisting of the outer canals is lacking in the uncini of *S. spirorbis* and *Pomatoceros triqueter* (Linné, 1758) (Fig. 8B), because the neuropodial growth zone is aligned with the older uncini.

The capillary setae of the three species are still being formed when they project above the surface of the animal. This can be inferred from the structure of the chaetoblast, because it still has electron-dense vesicles and its apical microvilli still contain actin filaments and extend into the setae. In contrast to this, the formation of the uncinus and the final differentiation of its chaetoblast to an anchoring cell have already been completed while the uncinus still lies below the surface. Such an early ending of synthesis seems to be characteristic for the uncini.

II. Uncini in perviate and obturate Pogonophora

The detection of a transient gut during development (Gardiner and Jones 1993; Jones and Gardiner 1988, 1989; Southward 1988), a prototrochal ciliary girdle in the larva (Gardinger and Jones 1994; Nielsen 1987), a metameric segmentation of the opisthosoma and a terminal growth zone clearly indicates a close relationship between the Pogonophora and the Annelida. Such an assumption has been proposed by several authors (Flügel and Callsen-Cencic 1994; Gardiner and Jones 1994; George and Southward 1973; Hartmann 1954; Jones 1985; van der Land and Nørrevang 1975).

According to the currently available morphological data, both pogonophoran taxa, Perviata and Obturata must be closely related even though it is evident from 18 S ribisomal DNA that there is a considerable evolutionary distance between the two groups (Williams et al.1993). Based on the morphological data (Gardiner and Jones 1993, 1994; Southward 1993), the hypothesis of a sister-group relationship between the Obturata and Pervi-

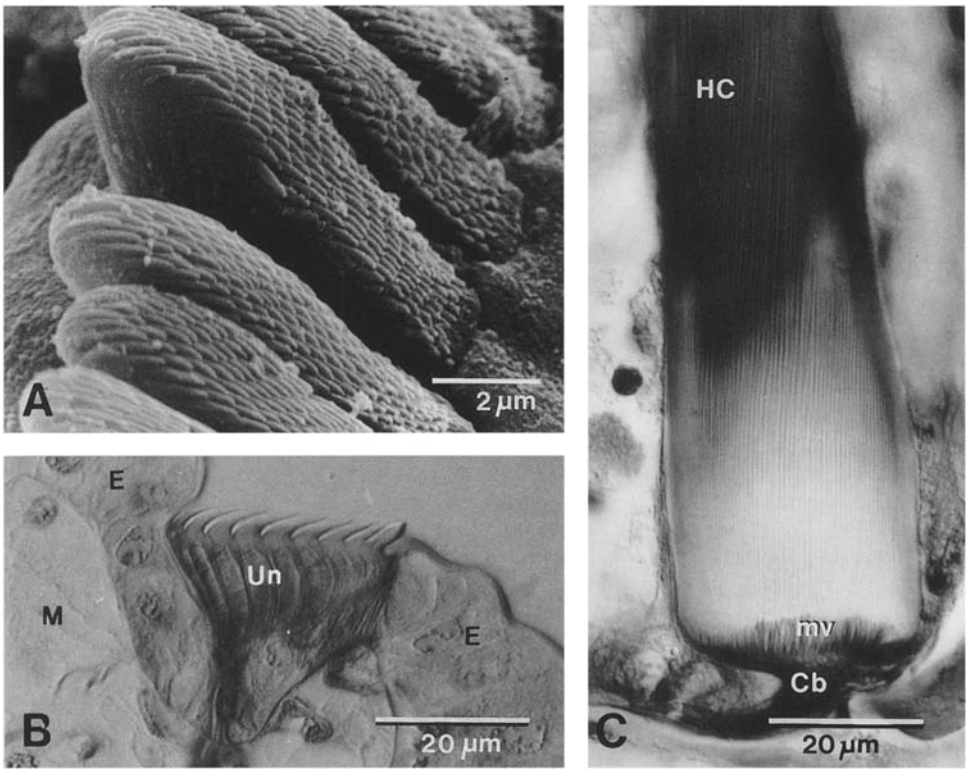

Fig. 8 **A** Abdominal row of uncini in *S. spirorbis*. **B** Abdominal uncinus (*Un*) of *Pomatoceros triqueter* (10 µm section, Normarsky contrast). **C** *Clymenura clypeata*. Base of an hooked seta (*HC*) of the second setigerous segment, note the wound canals (10 µm section, Normarsky contrast). *Cb* chaetoblast, *E* epidermis, *M* muscles, *mv* microvilli

ata can be substantiated by the following synapomorphies (number 5 in Fig.9): the reduction of the gut and anus, the trophosome, the chitinous tube and the large multicellular glands (pyriform glands) which secrete it, an intraepidermal nervous system and the extremely prolonged second body segment, the trunk (see Southward (1988) for further correspondences in the larval development). Because of the proposed sister-group relationship, in this paper the name Obturata is preferred to Vestimentifera (Fig. 9), (for nomenclatural problems see Gardiner and Jones 1993, 1994). Reduction of the mesenteries in the opisthosomal segments, annuli and spermatophores sustain the hypothesis of a monophyletic origin of the Perviata (number 6 in Fig. 9), whereas the obturaculum, muscularized vestimental wings and the larval siphon (Southward 1988) support the assumption of a monophyletic origin of the Obturata (number 7 in Fig. 9). Opisthosomal mesenteries and the absence of spermatophores, however, are regarded as plesiomorphic characteristics in the Obturata.

As in terebellidan and sabellidan annelids, transverse rows of toothed setae, which have been termed uncini by Jägersten (1956), have also been described in the Perviata and Obturata (Orrhage 1973; Jones 1985). As in the case of the Sabellida and Terebellida, these uncini are aligned in transverse rows and the apical spines point anteriorly. In the Obturata, transverse rows of uncini are restricted to the anterior opisthosomal segments. These rows are bands with up to five rows of uncini in *Riftia pachyptila* Jones, 1981, whereas in other Obturata the uncini are aligned in a single row. These rows or bands of uncini almost encircle each segment and are only interrupted by a dorsal and a ventral line (Gardiner and Jones 1993). In early developmental stages, these rows of uncini seem to be restricted to the ventral half of each segment (Gardiner and Jones 1993, Figs. 35, 36; Southward 1988, Figs. 3, 5); an anterior group of setae, which lies close to the distal margin of the trunk, is lost during development (Southward 1988). The position of the growth zone and the course of development of the uncini are unknown.

In Perviata, the uncini are restricted to the annuli of the trunk region where they mark the border between the anterior or preannular and the posterior or postannular regions. Here they form two semicircular transverse bands, the anterior and the posterior girdles. Each girdle consists of a single or a multiple row of uncini. If they are arranged in multiple rows, they resemble the large semicircular fields of hooked setae in Oweniida (George and Southward 1973, Nilsen and Holthe 1985; Southward 1993). During the growth of the trunk, the bands may become modified, so that they either appear to be four rings or they spiral around the trunk. The mode of formation can be inferred from observations on the formation of the girdles (George and Southward 1973, Fig. 2). The larva has two dorsal and two ventral uncini on each side of the body. George and Southward suggested that the anterior girdle is formed by the dorsal pair of uncini of the larva and the posterior girdle by the ventral pair. Provided that the site of formation of new uncini lies medially, the special pattern of the posterior and anterior girdle could be explained as follows. In the ventral row the youngest uncini lie more dorsally than the older ones, so that, during development, the whole posterior

girdle can only expand dorsally. In the anterior row of the youngest uncini lie more ventrally than the older ones and, during further development, the anterior girdle can expand only ventrally. According to this interpretation, the growth zone of the ventral or posterior row of uncini has an identical position to that in the Sabellida and Terebellida.

Structural details of the uncini have been mainly investigated in perviate pogonophorans. The uncini consist of a capitium with numerous rows of curved spines, a subrostral process and a manubrium (Gupta and Little 1970; Orrhage 1973). The subrostral process may be composed of a few spines which are also curved and face the spines of the capitium. If present, the number of rostral spines is reported to be much lower than that of the opposed, anteriorly directed spines of the capitium in perviate pogonophorans (Gardiner and Jones 1993; George and Southward 1973; Southward 1993). Electron-dense material strengthens the spines and is deposited in the canals after the microvilli have been withdrawn. The follicle of each uncinus consists of two follicle cells and one chaetoblast. Hemidesmosomes on the tips of short adluminal microvilli of these cells connect the uncinus to an intracellular system of intermediate filaments which adhere to hemidesmosomes on the matrix side of the follicle cells and the chaetoblast (George and Southward 1973; Gupta and Little 1970). During formation, microvilli of the chaetoblast seem to extend into the canals inside the uncinus. These microvilli are withdrawn at the end of formation and replaced by short projections of the apical cell membrane (George and Southward 1973), which contain intermediate filaments that attach the uncinus in the same manner in both *Pectinaria* species and *S. spirorbis*.

III. Homology of the uncini

The following characteristics strongly support the hypothesis of a homology of the uncini in Sabellida, Terebellida and Pogonophora:

- Uncini consist of a capitium, a subrostral process and a manubrium.
- Apically, the capitium has several rows of anteriorly cuved spines.
- The spines contain an electron-dense material which is deposited in the canals left by the retraction of the microvilli.
- The spines face the subrostral process which consists of spines that are posteriorly curved in pogonophorans and straight in terebellidans; in contrast to other Sabellida it is lost in *S. spirorbis*.
- The manubrium is short and tapers basally in terebellidans and pogonophorans; in sabellidans it is triangular, which is regarded as derived.
- Uncini are arranged in long transverse rows inside the neuropodial rim;
- their transverse axis is parallel to the long axis of the body; and
- each uncinus has it own follicle which consists of three cells, i.e., a chaetoblast and two follicle cells.
- Uncini are formed very rapidly compared to capillary setae.
- In contrast to capillary setae, the microvilli are completely withdrawn from the uncinus at the end of the synthetic phase of the chaetoblast and
- are replaced by short processes of the chaetoblast that attach the uncinus to the follicle, while
- the actin-filament system is replaced by a system of intermediate filaments.

The formative site always lies close to the capillary setae, so that the youngest uncini are found midlaterally and the oldest ventrally in Terebellida and in the thoracic segments of Sabellida (Pettibone 1982), or dorsally in the abdominal segments of adult Sabellida. A comparable position of the formative site is assumed for the Perviata (see above). Transverse rows of uncini on the opisthosomal segments in Obturata are regarded as plesiomorphic and their absence in perviate pogonophores must be apomorphic. In the Terebellida, the formative site of the neuropodium always lies laterally to the row of fully differentiated uncini, so that the uncini are twisted around their own axis during development. In *S. spirorbis* and other Sabellida (own unpublished observations), the growth zone is secondarily aligned with the neuropodial row of uncini.

IV. Phylogenetic implications

Within the Annelida, also species of the Maldanida, Arenicolida and Psammodrilida possess hooked setae which are aligned in transverse rows inside the neuropodial rim. These hooked setae are composed of a capitium with curved spines and a very long, basally tapering manubrium (Ashworth 1912; Gamble and Ashworth 1900; Holthe 1986; Kristensen and Norrevang 1982; Swedmark 1955, 1958). A subrostral process is lacking in the Arenicolida and in those members of the Maldanida that have several long hairs which are attached to the manubrium opposite the tips of the spines. The spines of the hooked setae in Arenicolida and Maldanida consist of one main tooth (rostrum) and several marginal teeth, which are all curved towards the manubrium; the axis of the rostrum and of the manubrium form an angle of 70–90° (Gamble and Ashworth 1900, unpublished data). In juvenile and adult *Arenicola marina* (Linné, 1758), however, the main tooth of the neuropodial setae is not curved towards the manubrium, but extends apically, and its axis meets the longitudinal axis of the manubrium at an angle of about 135° (Ashworth 1912, for *A. marina*). These setae have often been referred to as acicular hooks. Nevertheless, they are preceded by hooked setae which are characteristic of their early postlarval stages.

Although the hooked setae of species of the Maldanida and Arenicolida are aligned transversely in the neuropodial rim, they never lie side by side like the uncini. Large gaps can be seen between the adjacent hooked

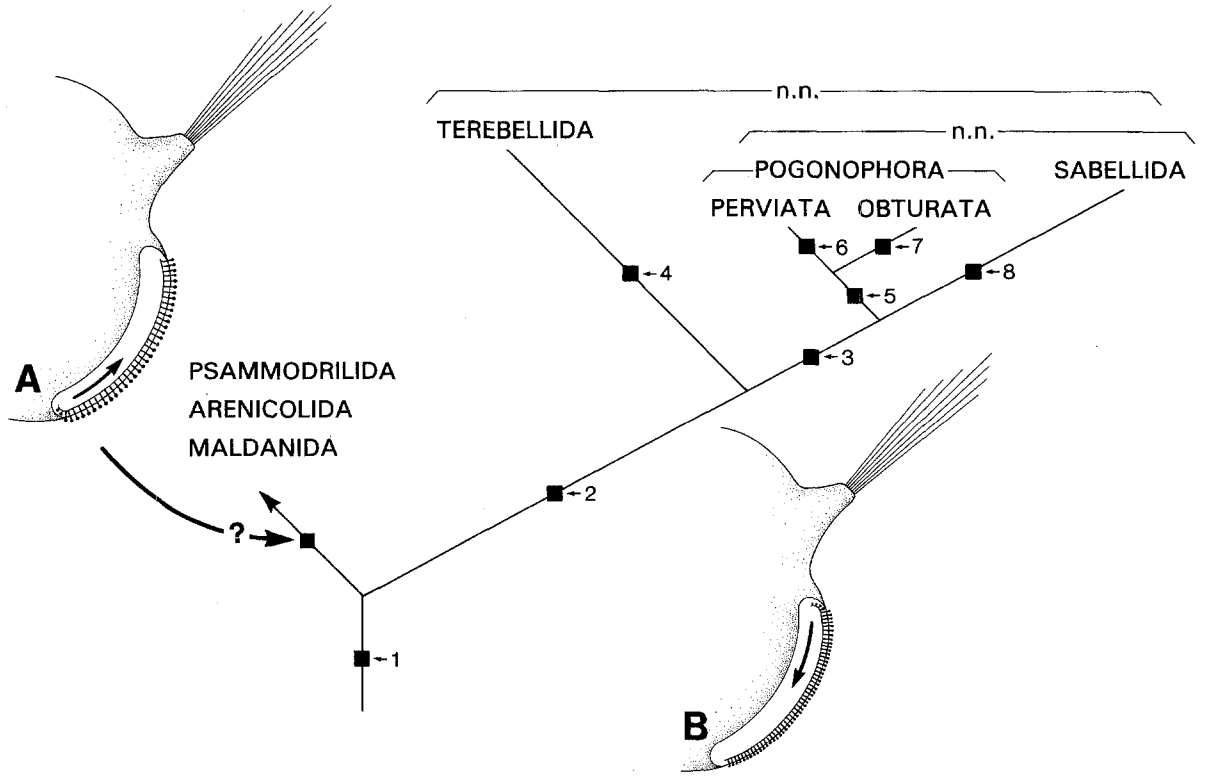

Fig. 9 Proposed phylogenetic relationships between the Sabellida, Pogonophora and Terebellida. Within the Annelida, their closest relatives are the Psammodrilida, Arenicolida and Maldanida. The monophyletic origin of these six taxa is substantiated by a transverse row of hooked setae inside the neuropodial rim of each segment and the lateral position of the growth zone (*1*). If this growth zone was primarily medial (**B**), Psammodrilida, Arenicolida and Maldanida would share a common ancestor which reversed the position of the growth zone (**A**). A medial growth zone (**B**) would then be plesiomorphic. Autapomorphies of the taxa: *2* Uncini with a reduced manubrial length, anteriorly curved spines are aligned in transverse rows. *3* Only one pair of nephridia draining the first segment, nephropori dorsal. *4* Non-retractile perioral feeding tentacles. *5* Trophosome, prolonged second segment, intraepidermal nervous system, multicellular pyriform glands secrete a chitinous tube. *6* Spermatophores, reduction of the mesenteries from segment three onwards, modification of the opistosomal rows of uncini, two rows of uncini in the second segment. *7* Larval siphon, obturaculum, muscularized vestimental wings, giant axons, reduction of the setae in the second segment, reduction of notopodial setae. *8* Setal inversion, perioral funnel-like tentacular crown consisting of a pair of semicircular lobes, each lobe with three bipinnate filaments, nephridia with a common dorsomedian nephridiopore, larval protonephridia with multiciliated flame cells

setae because their spines point ventrally and never anteriorly as in the Terebellida, Sabellida and in the pogonophoran groups. In species of both taxa, the hooked setae are formed ventrally, so that the oldest setae are found at the dorsal and the youngest at the ventral edge of the neuropodium (personal communication K. Meyer and M. Schweigkofler, Göttingen), (Fig. 9). In *Clymenura clypeata* (Saint Joseph, 1894) [Maldanidae], the outer canals are twisted (Fig. 8C). This hints at a mode of development comparable to that in *Pectinaria* species. According to Bobin (1944), the hooked setae of *C. clypeata* are actually formed laterally to the ventral edge of the neuropodial rim. Her drawings also indicate that the setae shift into their definitive position during formation. The hooked setae of Psammodrilida are restricted to certain abdominal segments and are arranged in transverse rows each consisting of at least three uncini (Kristensen and Nørrevang 1982).

Hooked setae and uncini are always aligned in a transverse row inside the neuropodial rim and their formative site primarily lies laterally to this rim. Both setae have curved apical spines and twisted outer intrasetal canals. These characteristics support the hypothesis that hooked setae and uncini are homologous.

The proposed homology between hooked setae and uncini includes the assumption that Psammodrilida, Arenicolida, Maldanida, Terebellida, Pogonophora and Sabellida share a common ancestor which evolved hooked setae (number 1 in Fig. 9). Thus, this characteristic, i.e. a transverse row of hooked setae inside the neuropodial rim, indicates the existence of a monophyletic taxon within the Annelida, which includes the mentioned taxa (Fig. 9).

It is presumed that transverse rows of uncini with anteriorly curved apical spines facing a subrostral process evolved in the common stem lineage of the Pogonophora, Terebellida and Sabellida. This hypothesis leads one to expect that the uncini of the Pogonophora are formed along a dorsoventral line in the same way as in the terebellidan and sabellidan annelids and can thus be tested. If the three taxa share a common ancestor, as presumed here, the single pair of nephridia in the first segment and the dorsal opening indicates a sister-group relationship between Sabellida and Pogonophorana (Bartolomaeus 1994b; number 3 in Fig. 9).

The formative site of the setae lies at the ventral edge of the neuropodium in species of the Arenicolida and Maldanida. If this condition should be plesiomorphic, the reversed medial position in the Terebellida, Sabellida and, presumably, the Pogonophora would be apomorphic and could be another characteristic used to substantiate the hypothesis for a common ancestor of these three groups (Fig. 9). But, if a ventral position should turn out to be apomorphic, it would clearly indicate a common ancestor of at least the Arenicolida and Maldanida.

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