

# Functional and evolutionary implications of the anterior sensory anatomy of species of root-lesion nematode (genus *Pratylenchus*)

Marcus W. TRETT\* and Roland N. PERRY

*Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, England and \*School of Biological Sciences, Queen Mary College, University of London, Mile End Road, London E1 4NS, England.*

## SUMMARY

The ultrastructure of the anterior sensilla of adult *Pratylenchus crenatus*, *P. penetrans* and *P. thornei* was examined using scanning and transmission electron microscopy. Each species possesses a complement of six inner labial, six outer labial and four cephalic sensilla augmented by a pair of lateral amphids. One or more sensory dendrites innervate each sensillum and arise from either the papillary or lateral ganglia of the nerve ring. Processes of non-neuronal sheath and socket cells were also identified. The six inner labial sensilla are each innervated by two dendrites and are believed to be combined chemo- and mechanosensory units. Single dendrites, whose processes terminate within the cephalic and labial cuticle, innervate the cephalic and outer labial sensilla which are thought to be mechanoreceptive. The four cephalic processes are enlarged and, characteristically, enclosed by the processes of the sub-median outer labial sensilla. Structurally, the amphids are the most complex of the anterior sensilla and are considered to be the primary chemosensory organs. Each contains seven dendritic processes within a cuticle-lined canal that opens to the exterior via an oblique, elongated pore on either side of the prestoma. The processes of two further dendrites terminate within an enlarged amphidial sheath cell process at the base of each canal. In addition to the above sensilla, lamellate terminals of eight « accessory neurons » that do not associate with the cuticle have also been identified amongst the labial tissue. The rôle of these structures is uncertain. These findings are compared with those for other species and their evolutionary significance discussed.

## RÉSUMÉ

*Implications fonctionnelles et évolutives de l'anatomie des organes sensoriels antérieurs chez les nématodes du genre Pratylenchus*

Les auteurs ont étudié l'ultrastructure des sensilles antérieures de *Pratylenchus crenatus*, *P. penetrans* et *P. thornei* adultes en microscopie électronique à balayage et à transmission. Chaque espèce possède une série de six organes labiaux internes, six organes labiaux externes et quatre organes céphaliques, ainsi qu'une paire d'amphides, latérales. Chaque sensille est innervée par une ou plusieurs dendrites sensorielles provenant d'un ganglion papillaire ou d'un ganglion latéral de l'anneau nerveux. Des procès liés aux cellules d'accompagnement et aux cellules de soutien non neuronales ont été également observés. Chaque organe labial interne est innervé par deux dendrites et paraît constituer une unité chémo- et mécanosensorielle. Les organes céphaliques et labiaux externes sont innervés par des dendrites simples dont les procès se terminent à l'intérieur de la cuticule céphalique et labiale. Ils sont probablement mécanorécepteurs. Les quatre procès céphaliques sont élargis et entourés par les procès des sensilles labiales externes sub-médianes. Parmi les sensilles antérieures, les amphides montrent la structure la plus complexe et paraissent être les organes chémosensoriels primaires. Chacune contient sept procès dendritiques à l'intérieur d'un canal cuticularisé s'ouvrant à l'extérieur par l'intermédiaire d'un pore oblique et allongé situé de chaque côté du prestome. Les procès de deux autres dendrites se terminent à l'intérieur du procès élargi d'une cellule de soutien amphidienne, à la base de chaque canal. En sus des sensilles décrites ci-dessus, ont été également localisées, dans le tissu labial, les terminaisons lamelleuses, non associées à la cuticule, de huit « neurones accessoires ». Le rôle de ces structures est obscur. Ces observations sont comparées à celles faites sur d'autres espèces et leur signification concernant l'évolution est discutée.

Despite the fundamental similarities in unit structure of nematode sensilla so far examined, there exists a broad spectrum of variations that arises from differences in the number, form and arrangement of receptive cell processes and their inferred functions (McLaren, 1976; Wright, 1980). This variety appears to reflect the wide range of environments inhabited by these species and their differing feeding methods (Trett, 1984). Studies of

the sensilla of plant-parasitic tylenchids have revealed a high degree of structural uniformity within this group (De Grisse, 1975; Coomans & De Grisse, 1981) but recent computer-based analyses (Trett, 1984) have demonstrated that even small variations in the sensory anatomy may have taxonomic significance. Corbett and Clark (1983) described the surface features of eighteen species of *Pratylenchus* and concluded that head shape

and annulation were valuable taxonomic features enabling species to be divided into three groups on the basis of the pattern formed by the first head annule and oral disc. The somatic or cephalic and labial cuticle is an integral part of most nematode sensilla so far described (= "cuticular sense organs"; Wright, 1980) and may play an important rôle in the transduction of certain stimuli (Trett & Lee, 1980; Trett, 1984).

The present study describes and compares the anterior sensilla of representative species of this genus to determine whether the observed cuticular variation reflects specific differences in the sensory anatomy. The three species examined, *Pratylenchus crenatus* Loof, 1960, *P. penetrans* (Cobb, 1917) and *P. thornei* Sher & Allen, 1953, represent each of the three groups defined by Corbett and Clark (1983).

### Materials and methods

Mature adult *P. crenatus*, *P. penetrans* and *P. thornei* were collected from sterile excised maize roots grown on Moutain's (1955) agar. Specimens selected for scanning electron microscopy were fixed in 2.5 % glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2, 280 mOs kg<sup>-1</sup>) for 24 h at 4°. Occasionally specimens were prefixed for 1h in 1 % osmium tetroxide in cacodylate buffer containing 0.05 % ruthenium-red (Trett, 1984). Dehydration through an acetone : water series preceded CO<sub>2</sub>-critical point drying in velin tissue envelopes (Trett, 1981). Coated and uncoated specimens were examined in a Hitachi S-450 scanning electron microscope at between 5 and 20 kV accelerating voltage.

Specimens for transmission electron microscopy were embedded in molten agar (< 37°), cut and fixed in glutaraldehyde fixative (280 mOs kg<sup>-1</sup>) for 24 h at 4°.

Post-fixation was in 1 % osmium tetroxide in 0.13 M phosphate buffer (pH 7.2, 280 mOs kg<sup>-1</sup>) for 1h at 4°. Blocks containing specimens were infiltrated with low viscosity epoxy resin (Spurr, 1969), polymerised and then sectioned on a Reichert UM2 ultramicrotome. Serial sections were collected on 1 × 2 mm, coated slot grids (Trett & Crouch, 1984) and stained in 2 % aqueous uranyl acetate or 1 % potassium permanganate followed by 0.2 % lead citrate (Venable & Coggeshall, 1965). Grids were examined in a Phillips EM 201 microscope at 100 kV accelerating voltage. Three-dimensional reconstruction techniques were modified from Bang and Bang (1957), Fuscaldò and Jones (1959) and Yamada and Yoshida (1972).

### Results

#### THE CUTICULAR SENSILLA

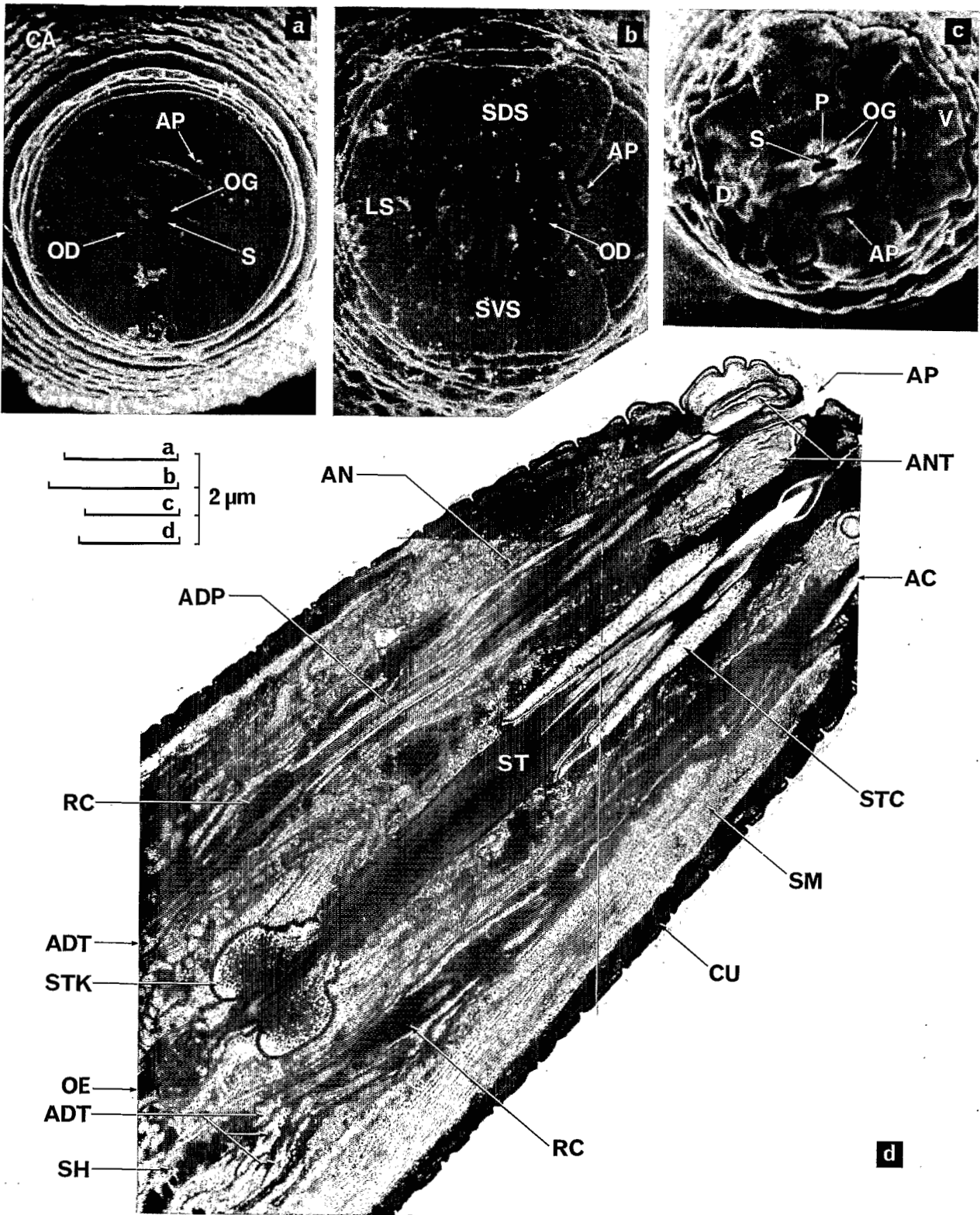
##### External Features

The somatic cuticle of the three species examined is elaborated into cuticular annuli which cover most of the body. The cephalic and labial cuticle is offset from the body by two, or occasionally, in the case of *P. crenatus*, three annules of approximately 4 to 6 µm in diameter compared with 9 + µm immediately posterior to the cephalic region (Fig. 1 a, b, c). Each species possesses a characteristic « cuticular mask » which incorporates the terminals of the cephalic and labial sensilla. In *P. crenatus* the mask consists of a central, dorso-ventral ridge of cuticle approximately 0.6 µm wide and 2.2 µm in length which includes the opening of the prestoma and is delimited on either side by an oral groove (Fig. 1 a). The six pores of the inner labial sensilla are located within this groove, three on either side of the prestoma

#### KEY TO ABBREVIATIONS USED IN FIGURES

AC : amphidial canal. ACP : accessory cell process(es). AD : amphidial dendrite(s). ADP : amphidial dendritic process(es). ADT : amphidial dendrite terminals. AN : accessory neuron. ANT : accessory neuron terminal(s). Ant : anterior. ASH : amphidial sheath cell process. ASO : amphidial socket cell process. BR : basal ring (plate) of cephalic framework. CA : cuticular annuli. CCU : cephalic cuticle. CF : cephalic framework. CP : dendritic process of cephalic sensillum. CS : cephalic sensillum(-a). CU : cuticle. D : dorsal. DA : dense apical cuticle of stylet. DN : dendrite(s). DP : dendritic process(es). EDG : electron-dense granule(s). EDP : expanded dendritic process. ELG : electron-lucent granules. H : hypodermis. ID : dendrite giving rise to processes that remain within the amphidial sheath cell process. IDP : dendritic process that remains enclosed within the amphidial sheath cell process. IDT : terminal of an internal dendrite. ILD : inner labial dendrite. ILP : dendritic process(es) of an inner labial sensillum. ILS : inner labial sensillum. L : lateral. LCU : labial cuticle. LPN : lateral papillary nerve. LS : lateral sector of cephalic/labial cuticle. LU : lumen. MI : mitochondria. MS : matrix substance within receptor cavity. MV(P) : microvillus-like processes arising from internal dendrite terminal. OD : oral disc. OE : oesophagus. OG : dorso-ventral oral groove. OLD : outer labial dendrite. OLP : dendritic process of outer labial sensillum. P : pore of inner labial sensillum. P1a-d : anterior attachment process of stylet protractor muscle cell 1. P2a-c : anterior attachment process of stylet protractor muscle cell 2. P3a-c : anterior attachment process of stylet protractor muscle cell 3. PM : protractor muscle cell. PN : papillary nerve. Post : posterior. RC : receptor cavity. S : stoma/prestoma. SDS : sub-dorsal sector of cephalic/labial cuticle. SH : sheath cell process. SM : somatic muscle cell. SO : socket cell process. SOLS : sub-median outer labial sensillum. ST : stylet. STK : stylet knob. SVS : sub-ventral sector of cephalic/labial cuticle. V : ventral.

Fig. 1. a, b and c. Scanning electron micrographs of the head ends of the three species of *Pratylenchus* illustrating the morphology of the labial and cephalic cuticle. a : *P. crenatus*; b : *P. thornei*; c : *P. penetrans*. d : Montage reconstruction of a longitudinal section through the anterior end of an adult female *P. thornei* showing the relationship between the amphids and the surrounding tissues.



in dorso-sub-lateral, lateral and ventro-sub-lateral positions. The central ridge is situated on a raised oral disc, 2.0-2.3  $\mu\text{m}$  in diameter. Amphidial pores, measuring approximately 0.4 by 0.7  $\mu\text{m}$  are present at the lateral margins of this disc and are angled, dorsally, towards the midline. In *P. crenatus* the surrounding cephalic cuticle forms a smooth dome which does not possess pores or papillae that might indicate the presence and position of other sensilla. Shrinkage in a few specimens reveals « radial ridges » in this region which were found to correspond to the ribs of the underlying cephalic framework.

The cuticular masks of *P. penetrans* and *P. thornei* differ from *P. crenatus* in that the cuticle surrounding the central oral ridge and oral disc is raised dorsally and ventrally to form sub-dorsal and sub-ventral sectors (Figs 1 b, c). In *P. thornei* the lateral boundaries of these sectors are almost radial (Fig. 1 b) whereas in *P. penetrans* they are dorso-ventral giving rise to an « H » configuration (Fig. 1 c). In both males and females of these species the arrangement of the inner labial and amphidial pores resembles that of *P. crenatus*. Again the presence of other cephalic and labial sensilla could not be detected externally.

#### *Internal Structure and Cytology*

With the exception of minor, though possibly significant, details the structural organisation of a given sensillum in each species examined was found to be identical. Anteriorly, each species possesses six inner and six outer labial sensilla, four cephalic sensilla and two amphids. Sexual dimorphism of the sensilla was not observed in adult *P. penetrans* and *P. thornei*.

*The Inner Labial Sensilla.* Each inner labial sensillum comprises two sensory dendrites (Figs 2 b and 3). Perikarya are located in sub-dorsal, lateral and sub-ventral papillary ganglia just anterior to the nerve ring. Their dendrites extend anteriorly for 60 to 65  $\mu\text{m}$  in the papillary nerves to the base of the cephalic framework. Processes of non-neuronal sheath and socket cells accompany the dendrites which they closely resemble. Serial sectioning established the location of the non-neuronal cell bodies of the outer labial and cephalic sensilla to be immediately anterior to the papillary ganglia. Between the stylet protractor muscle processes the sheath cell processes are expanded and contain stacks of parallel membranes (Figs 4 a and 5 b). 1-2  $\mu\text{m}$  posterior to the basal plate of the cephalic framework, the sheath cell processes enclose the inner labial dendrite terminals to which they are sealed by tight junctions (Fig. 3). Within their respective sheath cell processes each dendrite terminal produces lateral processes that interdigitate with the sheath cell cytoplasm and a single, axial dendritic process. In some of the dendrites short striated rootlets were observed in the terminal axoplasm.

A restricted receptor cavity surrounds the bases of the dendritic processes in each sensillum and is continuous with the sensillum canal (see below).

In each inner labial sensillum of each species the two dendritic processes are of unequal length (Figs 3 and 6 c) and have an 8 + 0 microfilament substructure at their bases. In *P. penetrans* and *P. thornei* the shorter of the two processes has a basal diameter of about 0.3  $\mu\text{m}$  and measures 0.85-0.90  $\mu\text{m}$  in length. In *P. crenatus*, the shorter process may measure up to 1.1  $\mu\text{m}$  long and has a basal diameter of between 0.35 and 0.40  $\mu\text{m}$ . In each species, these processes taper distally and bend within the sensillum canal with the larger process (Figs 3, 6 c and 7). In all three species, the larger processes are similar with a basal diameter of approximately 0.4  $\mu\text{m}$  and an expanded, flattened mid-section which contains an electron-dense core (Figs 2 b and 3). Similar material is frequently present in the apical cytoplasm of the shorter processes. The larger process bends within the cuticle of the oral disc and extends to the tip of the sensillum canal which is open to the exterior via one of the six pores that border the prestoma (Figs 3, 6 a, c and 8). The pores are 0.04-0.05  $\mu\text{m}$  in diameter which corresponds to the terminal diameter of the enclosed process. The sensillum canals do not directly interact with the cephalic framework but pass above the densely-staining apical cuticle (Figs 3 and 6 c). The base of the cuticle-lined sensillum canal is totally enclosed by the distal portion of the socket cell process (Figs 3 and 6 c) which forms tight junctions with the surrounding cephalic hypodermis and with the sheath cell process.

*The Outer Labial Sensilla.* The six outer labial sensilla each comprise a single dendrite that gives rise to a single dendritic process. As with the inner labial sensilla, perikarya are located in the six papillary ganglia and their dendrites in the corresponding papillary nerves (Figs 4 a and 5 b). However, unlike the inner labial sensilla, the dendritic processes terminate in the labial cuticle and do not communicate directly with the exterior via pores. The lateral processes are closely associated with the amphidial canals and measure between 1.0 and 1.2  $\mu\text{m}$  in length with a basal diameter of approximately 0.3  $\mu\text{m}$ . These processes are adaxial to the canals (Figs 2 b and 8) and taper distally. Each possesses a sheath and socket cell process that encloses the dendrite terminal and the base of the receptor cavity, respectively.

The sub-medial outer labial processes differ markedly in their structural organisation from those of the lateral sensilla. Each has a similar basal diameter (0.3-0.4  $\mu\text{m}$ ) but they arise from sub-dorsal and sub-ventral terminals and bend laterally, around the latero-dorsal and latero-ventral cephalic dendritic processes. More anteriorly, the outer labial processes migrate back around the cephalic sensilla to their original positions (Figs 2 a, 6 b, 7 and 8). In *P. crenatus* the migration of these processes is approximately 10° from the radial axis

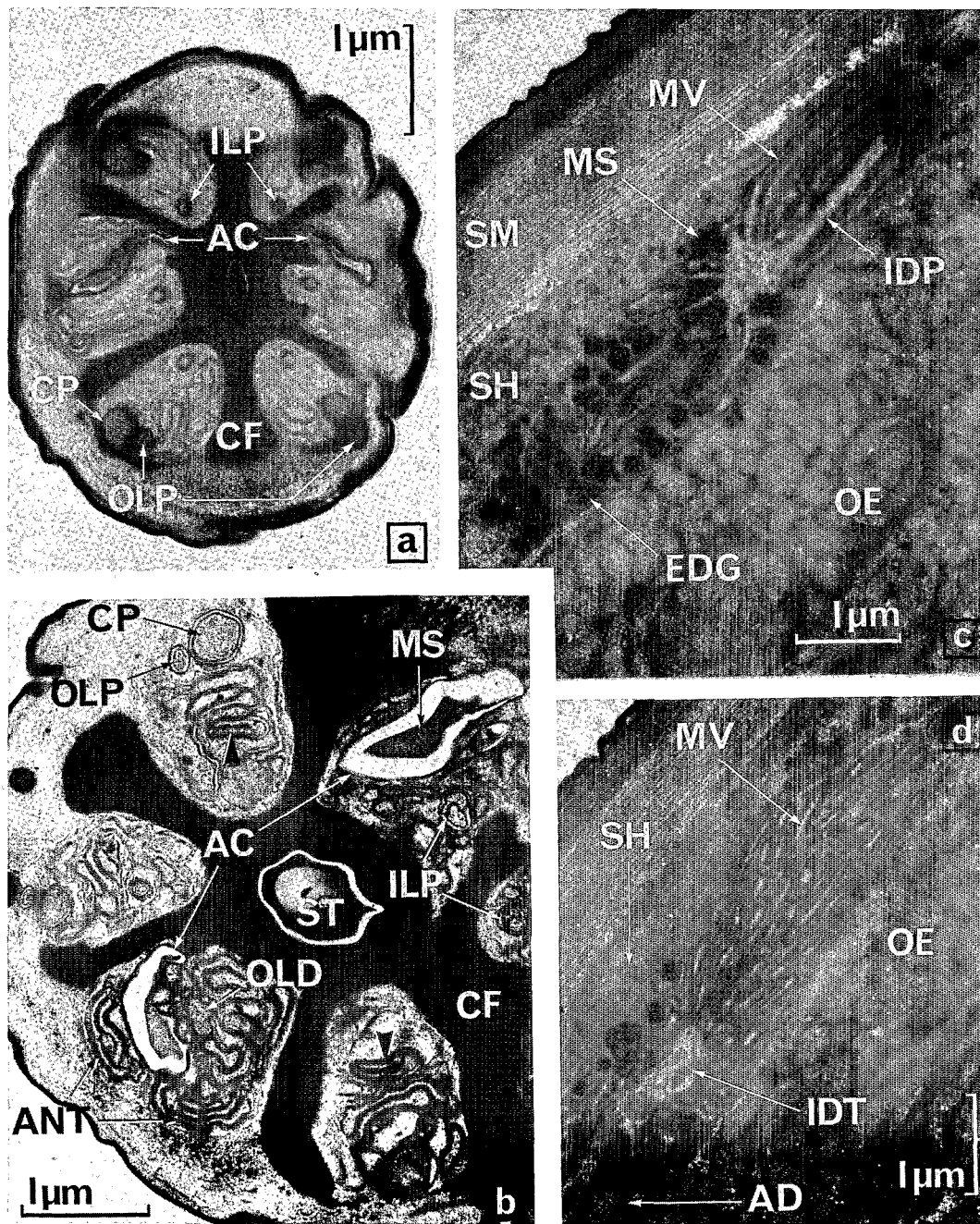


Fig. 2 a and b. Transverse sections through the cephalic tissue of *Pratylenchus* species. a : slightly oblique section from a series through the anterior end of an adult female *P. crenatus*. Note the relative positions of the cephalic and sub-median outer labial dendritic processes. At this level only one process is present in each inner labial sensillum canal. b : Transverse section from the anterior end of an adult female *P. thornei*. Note the twin processes present in the inner labial sensilla and the flattened profile of the processes that contain a densely staining core (arrowheads). Lamellate terminals of accessory neurons (ANT) are visible in a lateral sector. c and d : longitudinal sections of an adult female *P. penetrans* showing the terminal and microvillus-like processes of internal dendrites within amphidial sheath cell processes. Note the densely staining granules in the sheath cell cytoplasm and the matrix substance that fills the receptor cavity. Abbreviations : see page 342.

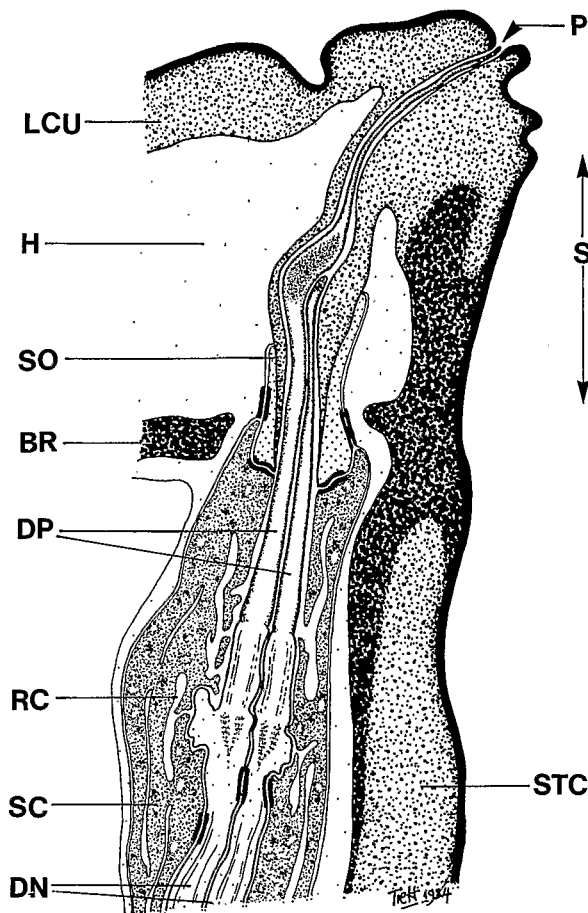


Fig. 3. A diagrammatic reconstruction of a longitudinal section through an inner labial sensillum of the *Pratylenchus* species examined. Abbreviations: see page 342.

whereas in *P. penetrans* and *P. thornei* the degree of enclosure of the cephalic processes is greater involving a migration of  $15^\circ$  or more from the same axis. Each outer labial sensillum retains a discrete receptor cavity (Fig. 2 b) and does not communicate directly with the cephalic process. The combined cephalic and outer labial sensillum units occupy the sub-median tissue between the radial partitions of the cephalic framework. In *P. penetrans* and *P. thornei*, these units end in the cuticle that forms the sub-dorsal and sub-ventral sectors of the cuticular mask (Figs 1 b, c). The outer labial processes of *P. crenatus* terminate at the margins of the oral disc (Figs 6 b and 8). Densely staining cuticle, distinct from that of the cephalic framework, surrounds the tips of all the outer labial sensilla (Figs 2 a, 5 a and 6 b). Cuticle overlying the terminals is thinner than in other cephalic and labial regions and measures only  $0.1\text{--}0.2\ \mu\text{m}$  as opposed to  $0.5\ \mu\text{m}$  or more (Fig. 5 a).

**The Cephalic Sensilla.** All three species examined possess four cephalic sensilla which occupy latero-dorsal and latero-ventral positions in the cephalic tissues (Figs 2 a and 8). Perikarya, in the sub-median papillary ganglia, give rise to two dendrites per sensillum which travel anteriorly in the sub-median papillary nerves. Only one of these dendrites in each sensillum produces a dendritic process (Figs 5 a and 9). The second dendrite ends blindly at the base of the receptor cavity within the sheath cell process. Proximally, the single dendritic processes are  $0.3\text{--}0.4\ \mu\text{m}$  in diameter (Figs 5 a and 9). Distally, the processes expand to about  $0.5\ \mu\text{m}$  in diameter and contain a densely staining, granular cytoplasm interspersed with neurotubules (Figs 5 a and 9). The processes are curved and approximately  $1.5\ \mu\text{m}$  long. Unlike the outer labial processes that enclose them, the cephalic processes remain on the same radial axis. Each ends  $2.5\text{--}3.0\ \mu\text{m}$  from the opening of the prestoma in either the cephalic cuticle (*P. crenatus*) or the sub-dorsal and sub-ventral sectors of the mask (*P. penetrans* and *P. thornei*). As in the outer labial sensilla, densely staining cuticle surrounds the distal portion of the cephalic dendritic processes in each species.

**The Amphids.** Structurally, the amphids are the most complex of the anterior sensilla. Each amphidial nerve contains eleven dendrites which amalgamate with those of the lateral papillary ganglia,  $5\text{--}6\ \mu\text{m}$  anterior to the nerve ring. Cell bodies of the amphidial dendrites are located in the lateral ganglia, immediately posterior to the nerve ring. Approximately  $20\ \mu\text{m}$  posterior from the tip of the head, the amphidial sheath cell process accompanying the dendrites from the nerve ring level, expands and encloses the dendrites (Figs 4 c and 10). Junctional complexes exist between adjacent dendrite membranes and between the dendrites and the sheath cell process. The dendrite terminals are slightly enlarged and contain aggregations of neurofilaments and, in one neuron in each amphid, vesicles  $0.05\text{--}0.20\ \mu\text{m}$  in diameter (see dendrite terminal « 8 »; Fig. 4 c). Seven of the dendrites (designated 1 to 7; Fig. 4 c) produce single dendritic processes that ultimately enter the amphidial canal (Figs 1 d, 4 b and 10). The remaining dendrites give rise to single and multiple processes that either remain in the amphidial sheath cell receptor cavity around the base of the canal (dendrites 8 and 10) or extend anteriorly to innervate accessory neuron terminals (dendrites 9 and 11; see below). In each species, one internal dendrite (number 8) in each amphid produces 25 to 30 microvillus-like processes and a single irregularly-shaped dendritic process (Figs 2 c, d, and 10). Each of these processes is in contact with the receptor cavity. This contains a densely staining matrix substance with similar stain affinities to dense granules,  $0.1\text{--}0.3\ \mu\text{m}$  in diameter, present in the sheath cell cytoplasm (Figs 1 d and 2 c, d). The matrix substance surrounds the amphidial dendritic processes within the canals (Figs 1 d and 11)

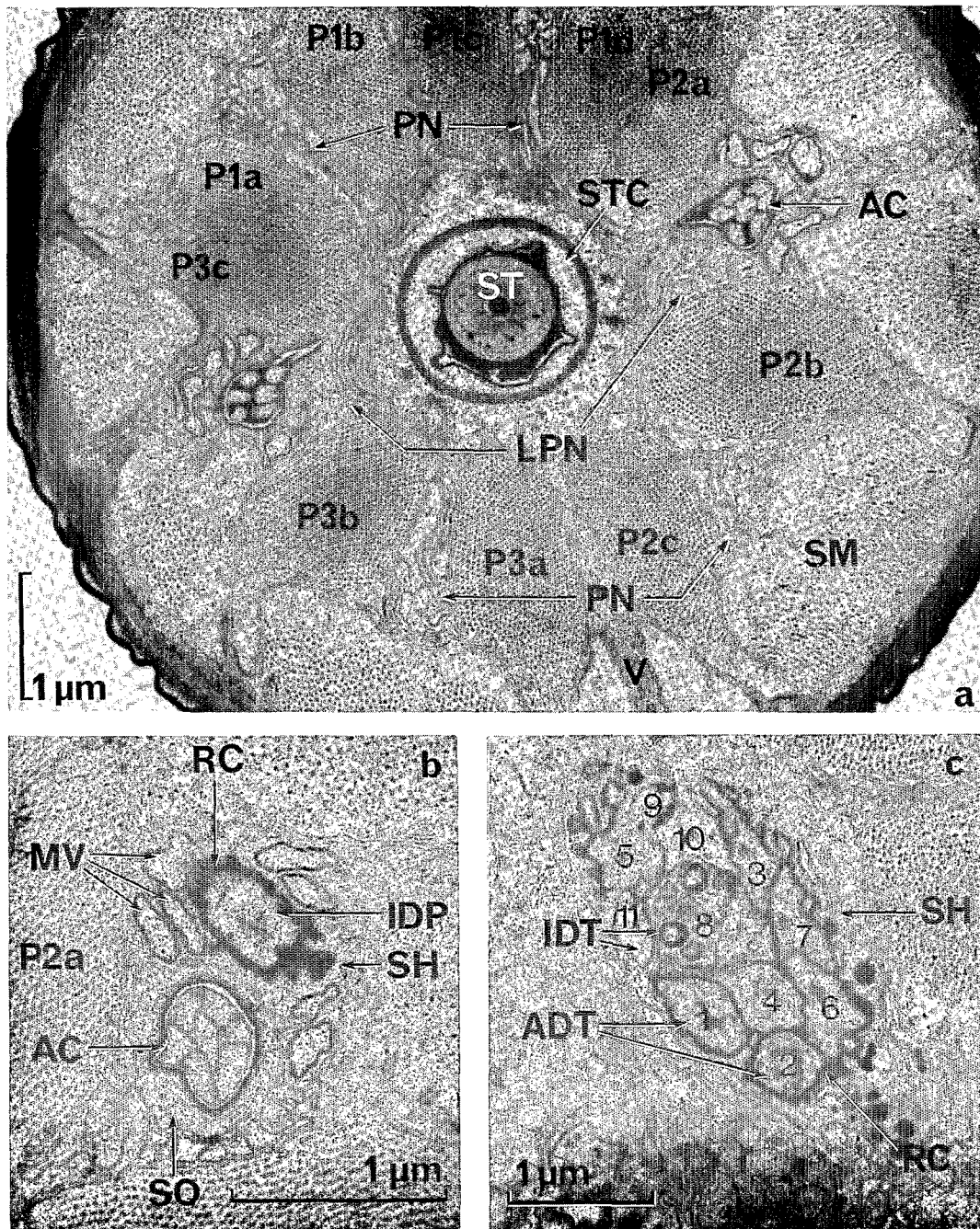


Fig. 4 a. A transverse section through the anterior end of an adult male *P. penetrans* showing the arrangement of the papillary nerves and amphidial canals between the processes of the three stylet protractor muscles. b : transverse section showing a lateral sector of an adult female *P. penetrans*. Note the seven amphidial dendritic processes contained in the canal and the irregular, expanded process of an internal dendrite within the sheath cell receptor cavity surrounded by a densely staining matrix substance. c : transverse section showing amphidial dendrite terminals at the base of the receptor cavity of an adult female *P. crenatus*. Abbreviations : see page 342.

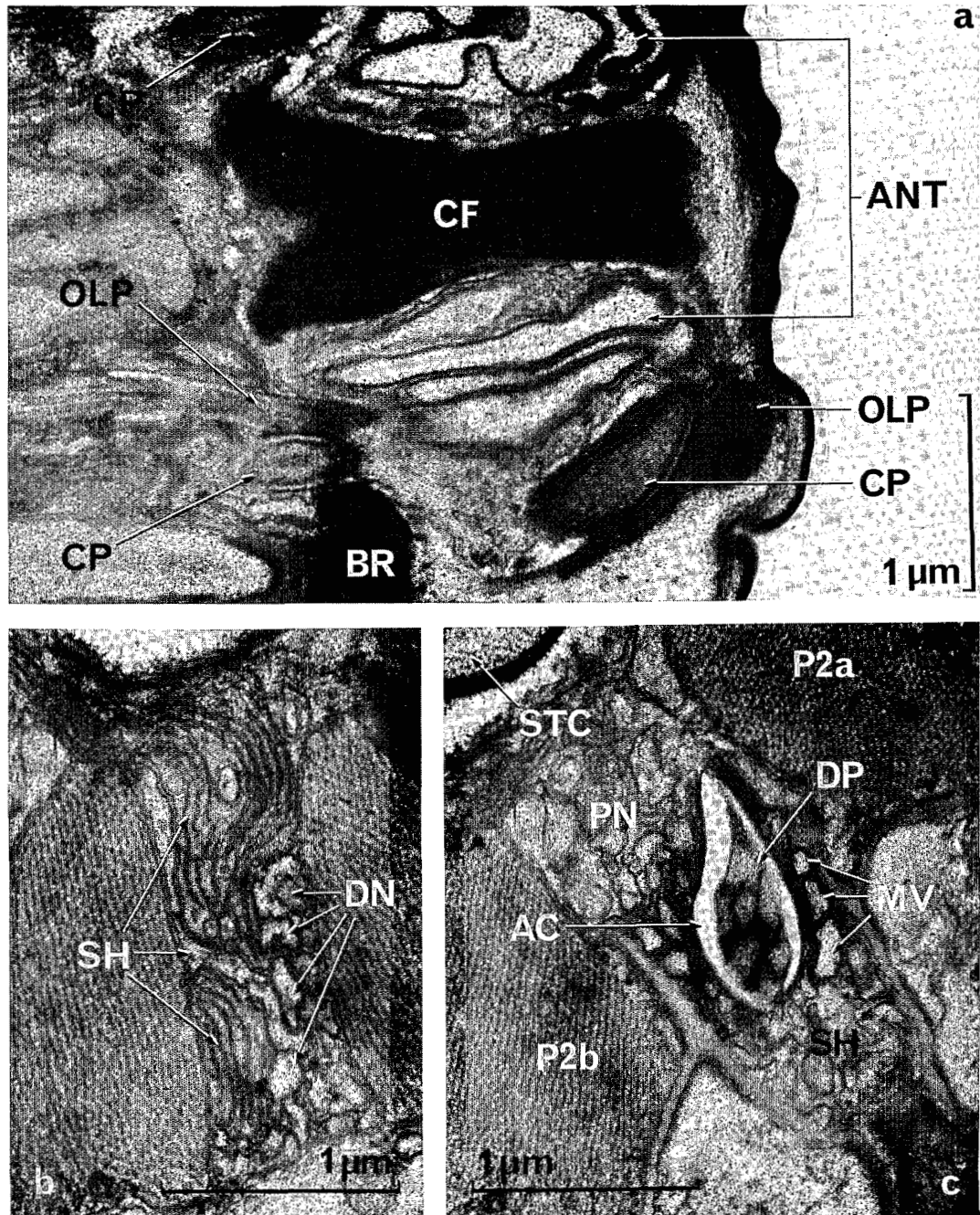


Fig. 5. a. Slightly oblique, longitudinal section through the labial tissue of an adult male *P. penetrans* showing the bases and distal portions of sub-ventral outer labial and cephalic sensilla. Note the expanded process of the cephalic sensillum. b : Transverse section through a sub-ventral papillary nerve of an adult female *P. thornei* showing stacks of membranes present in the sheath cell processes of the outer labial and cephalic sensilla. c : a lateral papillary nerve and amphidial canal recorded from the same section as Figure 5 b. Note the presence of processes of an internal dendrite within the sheath cell that partly encloses the canal. Abbreviations : see page 342.



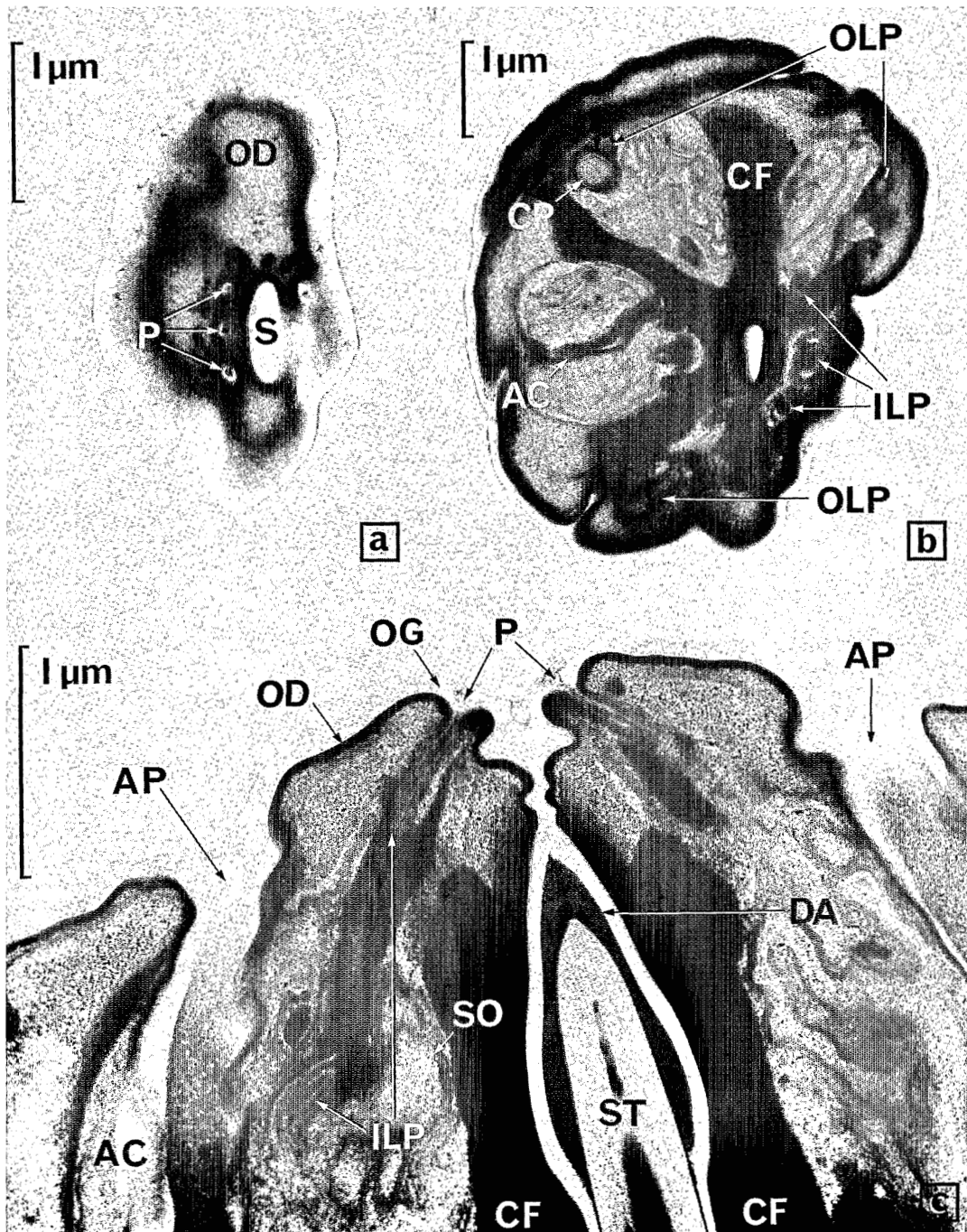


Fig. 6. Transverse and longitudinal sections through labial tissue of *Pratylenchus* species. a : transverse section through the oral disc of an adult female *P. crenatus* showing the arrangement of the inner labial sensillum canals around the prestoma. b : a more posterior section from the same series as Figure 6 a through the apex of the cephalic framework. Note the adaxial migration of the inner and outer labial processes. c : longitudinal section through the anterior end of an adult female *P. penetrans* showing the openings of the lateral inner labial sensilla and amphids. Abbreviations : see page 342.

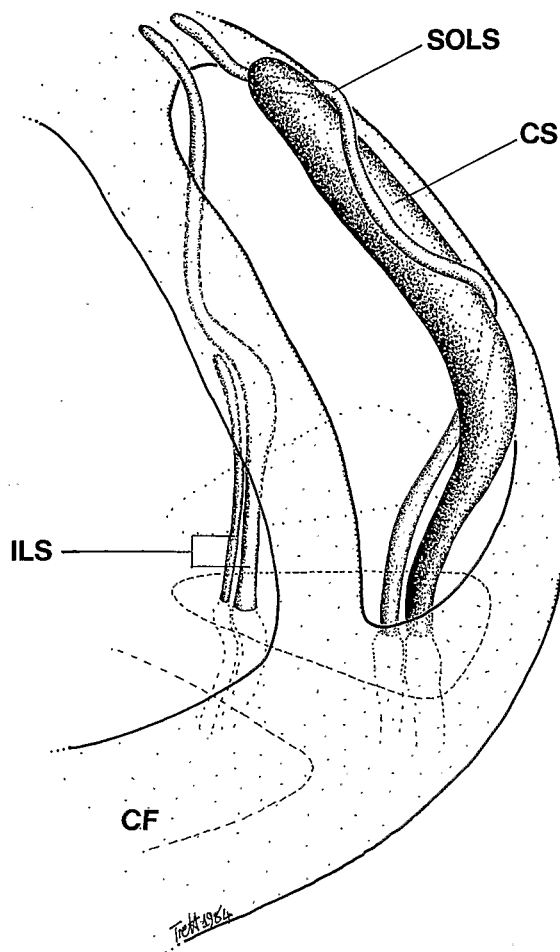


Fig. 7. A generalised diagrammatic reconstruction of the dendritic processes of labial and cephalic sensilla in a sub-median sector of the head of the *Pratylenchus* species examined. Abbreviations : see page 342.

and may exude from the amphidial pores (Figs 1 a and 6 c).

The dendritic processes that enter the canal have a basal diameter of  $0.4 \mu\text{m}$  and measure up to  $16 \mu\text{m}$  long, extending nearly to the apical pore (Figs 1 d and 11). Proximally, the cuticle-lined portion of the amphidial canal is almost filled by the dendritic processes and is enclosed by the socket cell process (Fig. 4 b). At this level, the canal has a near elliptical cross-section which is frequently distended adaxially. Distention is more extreme towards the top of the canal which becomes flattened and obliquely orientated within the lateral cephalic tissue (Figs 2 a, b, 5 c and 11). In some specimens of each species, occlusion of the canal appeared almost complete before it dilated immediately posterior to the pore (Fig. 6 b). With few exceptions, the cuticle-lined portion of the canal measured between 10

and  $12 \mu\text{m}$  in length. The relationship between the amphids and the surrounding somatic and cephalic tissues is summarised in Figure 11.

#### NON-CUTICULAR SENSILLA

Within the sectors delimited by the cephalic framework the terminals of six to eight accessory neurons were identified. These do not interact directly with the cuticle from which they are separated by the cephalic hypodermis. The terminals are folded to form parallel stacks of membranes that enclose a dense intramembranous material (Figs 1 d, 2 b and 5 a). Laterally, two neurons arise from each amphidial nerve and extend anteriorly beside the amphidial canal (Figs 1 d and 10). At the base of the cephalic framework, the neurons diverge and innervate the two lateral accessory neuron terminals (Fig. 2 b). The sub-dorsal and sub-ventral terminals are innervated by single neurons that accompany the papillary dendrites. Non-neuronal cells, resembling sheath and socket cell processes of the cuticular sensilla, have not been identified. Perikarya may be located one in each sub-median papillary ganglion and two in each lateral ganglion.

#### Discussion

The cephalic sense organs of the three *Pratylenchus* species examined correspond in number and relative position to the basic complement and position of nematode sensilla described by De Conninck (1965). Similarly, individual sensilla conform to the fundamental structural pattern observed in other species (McLaren, 1976; Wright, 1980) and possess neuronal, non-neuronal and cuticular elements.

From their structure, the inner labial sensilla appear to have a chemoreceptive capability. The sensillum canals are open to the exterior and one process in each sensillum extends to the tip of the canal. The small size of the apical pores and their location at the edge of the prestoma indicates that they may function as contact chemoreceptors. In association with other stimuli, these may play an important rôle in the initiation and maintenance of feeding behaviour. The significance of the « crooked » structure of the sensillum canals is uncertain but is similar to that described in several other Tylenchoidea (Baldwin & Hirschmann, 1975; Coomans & De Grisse, 1980; Endo, 1980). Endo (1980) considered that in *Heterodera glycines* the bend in the canal might allow for slight flexures of the oral cuticle during probing and feeding. Examination of reconstructions of inner labial sensilla of the three *Pratylenchus* species suggests that forces applied to the oral disc would be concentrated at the bend in each sensillum canal. The shorter of the two inner labial processes terminates at this level and, if mechanoreceptive, may monitor

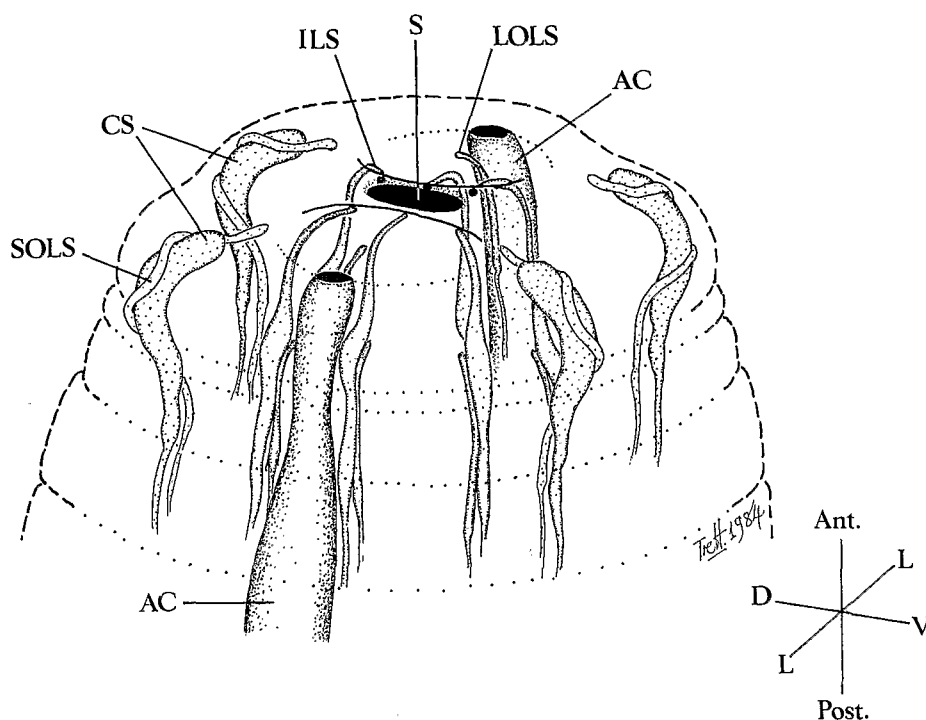


Fig. 8. Diagrammatic reconstruction summarizing the form and arrangement of the anterior sensilla of the *Pratylenchus* species examined. Abbreviations : see page 342.

deformation of the canal cuticle or compression forces within the canal itself. Indirect evidence exists to support this suggestion. Electron-dense material, similar to that present in the tips of the shorter dendritic processes, is frequently associated with putative mechanosensilla in other nematode species (McLaren, 1976; Wright, 1980) and commonly occurs in certain arthropod sensilla for which there is direct electrophysiological evidence for a mechanosensory rôle (Thurm, 1968, 1974). Further, the inner labial sensilla of the free-living, rhabditid nematode, *Caenorhabditis elegans*, also possess two processes, one extending to a pore in the tip of the canal and the other remaining at its base (Ward *et al.*, 1975; Ware *et al.*, 1975); the latter process contains dense, granular material and is absent in certain mechanotactically defective, mutant strains (Ward, 1976, 1977, 1982). If the short processes of the *Pratylenchus* species are mechanoreceptive, the densely-staining core of the longer processes may facilitate the transmission of forces within the canal. Similar dense cores occur in the longer processes of several Tylenchida but are not present in the Criconematoidea in which shorter processes are also absent (Natasasmita, 1979; Coomans & De Grisse, 1980).

The outer labial and cephalic sensilla do not communicate with the exterior via pores and terminate in the

labial and cephalic cuticle. Their structure indicates a mechanosensory rôle. Lateral outer labial processes may transduce mechanical stimuli at the edge of the oral disc and/or monitor lateral forces that lead to compaction of the amphidial canal onto the dendritic process and its receptor cavity. In nearly all Nematoda, somatic muscle activity generates dorso-ventral flexure of the body which may explain the presence of complex, combined cephalic and outer labial sensory units in sub-median positions. The combined processes may provide a greater sensitivity to the broad range of mechanical stimuli encountered during locomotion within the soil microenvironment. Similar combined units are present in other tylenchids (Coomans & De Grisse, 1980) and have also been described in the free-living, soil-dwelling stages of the rat hookworm, *Nippostrongylus brasiliensis* (Trett & Lee, 1982a, b; Trett, 1984). In *P. penetrans* and *P. thornei* the units end in the cuticle of the sub-dorsal and sub-ventral sectors of the cuticular mask. With the support provided by the underlying cephalic framework, this arrangement of cuticle and dendritic processes may be more efficient than the dome-shaped structure of *P. crenatus* in transducing turning moments produced by dorso-ventral displacements of the head. The dome-shaped condition of *P. crenatus* is characteristic of group I species as described by Corbett and Clark (1983) whereas the sectorized condition of *P. penetrans* and

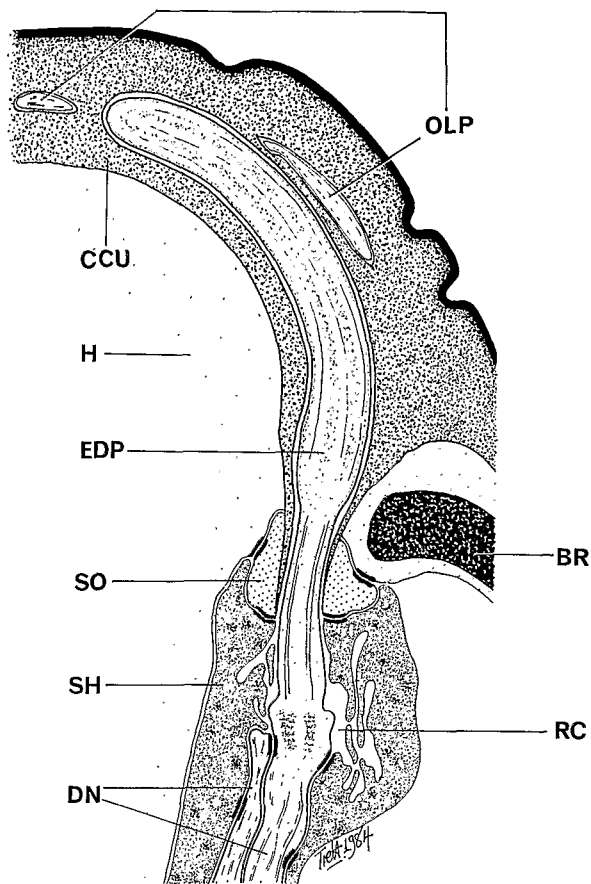


Fig. 9. Diagrammatic reconstruction of a longitudinal section through a cephalic sensillum of the *Pratylenchus* species examined. In each species a secondary neuron, that does not produce a dendritic process, terminates within the sheath cell at the base of the receptor cavity. Abbreviations : see page 342.

*P. thornei* is characteristic of group II and III species, respectively. A further difference between the group I species and those of groups II and III is the degree of enclosure of the cephalic processes by the sub-median outer labial processes. The displacement of the outer labial process is greater in *P. penetrans* and *P. thornei* than in *P. crenatus*. De Grisse (1977) has described a spectrum of interactions between the cephalic and outer labial processes of other tylenchid species ranging from non-interaction in the Aphelenchoidea and Anguinidae to complete enclosure of the cephalic processes in the Hoplolaimidae. Which of these conditions, if any, more closely reflects the plesiomorphic state is uncertain. However, the ultrastructural organisation of the combined receptors described in the present study indicates that *P. thornei* is more closely related to *P. penetrans* than to *P. crenatus*. The differences in the structural organisation of the cuticular mask and combined outer labial and

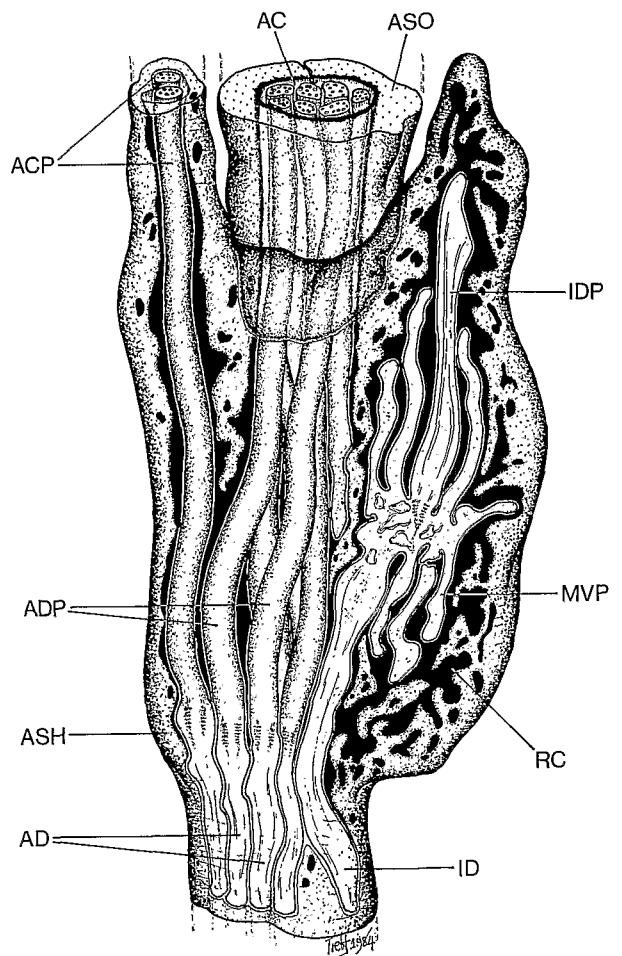


Fig. 10. Diagrammatic reconstruction of the base of the receptor cavity within the distal portion of an amphidial sheath cell process of the *Pratylenchus* species examined. Abbreviations : see page 342.

cephalic sensory units may reflect differences in the preferred habitats of these species; *P. penetrans* and *P. thornei* are more frequently found in heavy soils whereas *P. crenatus* usually occurs in light soils (Webb pers. comm.).

In general the structure of the amphids conforms to that described for other nematode species. They are most probably the primary chemosensory organs (Ward, 1973) and in common with other tylenchids possess seven dendritic processes in each amphidial canal. The effective molecular receptor sites may be restricted to those of the apical membranes of the processes owing to the compaction of the processes within the canals. As suggested for other nematode species, the densely staining matrix substance may be secreted by the amphidial sheath cell process and serve to maintain electrical continuity between the bases of the amphidial canals and

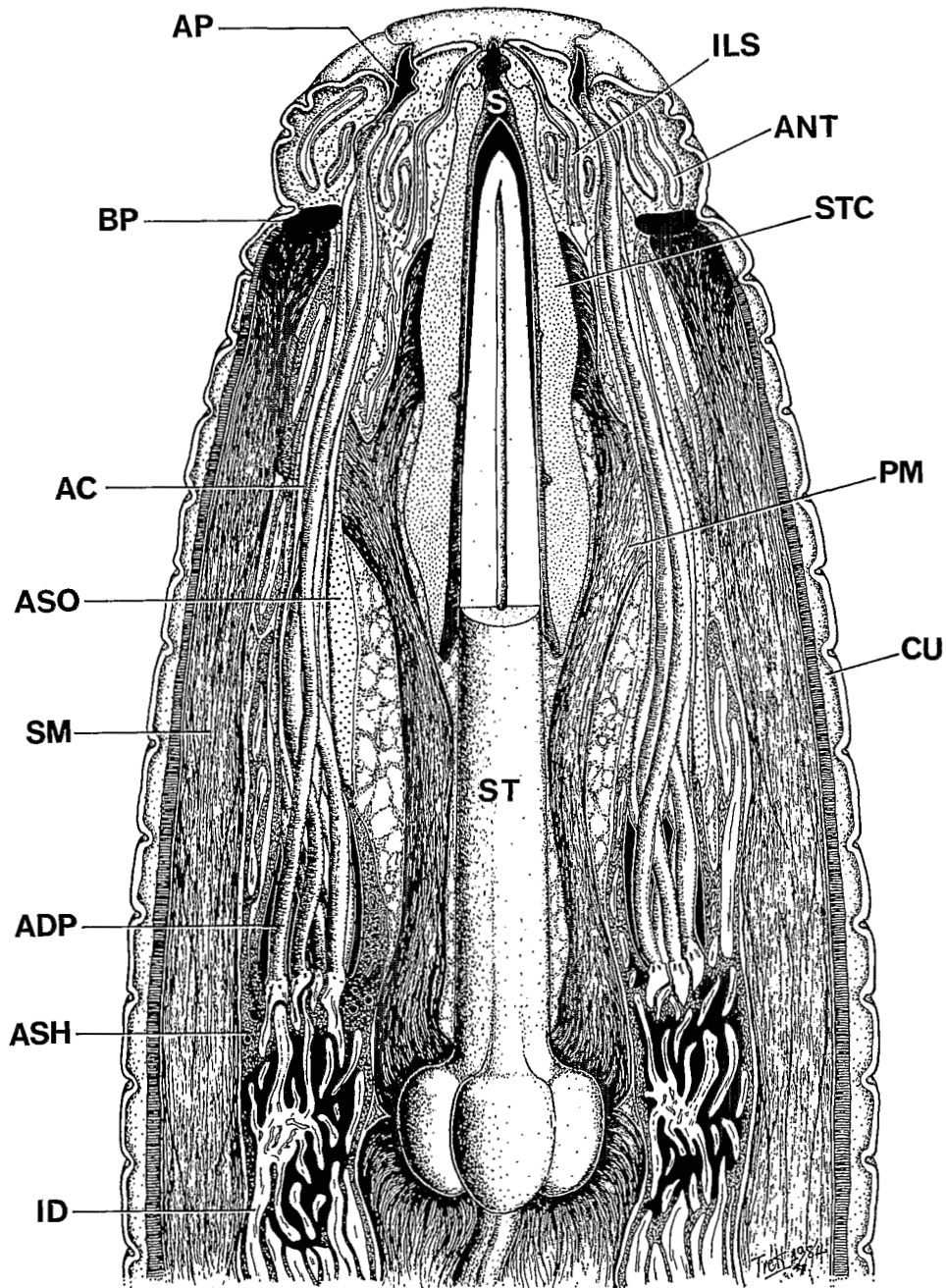


Fig. 11. A generalised diagrammatic reconstruction of the anterior end of the *Pratylenchus* species examined summarizing the relationship of the distal portions of the amphidial sensilla with surrounding cephalic tissue. Abbreviations : see page 342.

the tips of the processes (Trett, 1984). This may be a pre-requisite for the generation of receptor currents and stimulus transduction as in certain arthropod sensilla (Zacharuk, 1980). Several rôles have been postulated for the internal dendrites and their dendritic and multiple, microvillus-like processes. The large surface area presented by these processes may prove to be significant. Whilst it is conceivable that they monitor sheath cell secretions (McLaren, 1976; Wright, 1980) or the net receptor current and ion flux (Trett, 1984), the possibility that they represent exteroceptors cannot be dismissed. Electrical root potentials, for example, might be detected via the dense matrix substance. The rôle of the accessory neuron terminals is also uncertain. These have now been described in secernentean and adenophorean species (Wright, 1980; Wright & Carter, 1980) and may be most highly developed in the plant-parasites. Proposed functions include thermo- and hygroreception (Wright, 1980) and electromagnetic receptors (Coomans, 1979). Further studies using mutant strains of suitable species may provide new evidence for the functions of accessory neuron terminals as well as the internal dendrites of the amphids.

Sexual differences were not observed in the sensilla of adult male and female *P. penetrans* and *P. thornei*. However, owing to the rarity of males, only two were examined in the latter species. In tylenchid males of some species, such as *Heterodera glycines* (Baldwin & Hirschmann, 1975), both processes of each inner labial sensillum extend to the tip of the sensillum canal. In these cases a rôle in pheromone detection has been postulated. If pheromones are produced by *Pratylenchus* females, detection may rely on other sensilla such as the spicular receptors described in several tylenchid males (Clark & Shepherd, 1977).

Several authors have reported that the orientation of plant-parasitic species to known stimuli is impaired by sub-lethal concentrations of carbamate nematicides, although motility is not inhibited. Di Sanso (1973), for example, demonstrated the inability of *P. penetrans* to orientate with respect to tomato roots following exposure to carbofuran. These nematodes also failed to exhibit feeding responses at the root surface. Current studies have shown pathological effects in the amphidial sheath cell processes of adult female *P. penetrans* resulting from treatment with low concentrations of aldicarb (Trett & Perry, in press). It is hoped that these studies will lead to a better understanding of the mode of action of nematicides at levels encountered in the field as well as providing an insight into the functions of the sensilla and their components.

The comparative computer study by Trett (1984) of the sensory neuroanatomy of Nematoda recognised three principal clusters of plant-parasitic species. The Aphelenchoidea and certain members of the Tylenchoidea (Anguininae) and Hoplolaimoidea (*Macrotrophurus* and *Tylenchorhynchus* species) separated from other

tylenchid groups on their possession of two processes in each cephalic sensillum and a complete complement of twelve labial receptors with two processes in each of the inner labial sensilla. The three *Pratylenchus* species exhibit an intermediate condition between that of the two tylenchid groups. Whilst all twelve labial sensilla are present with a full complement of dendritic processes, only one process is present in each cephalic sensillum. However, a second dendrite that does not give rise to a process terminates within the sheath cell of these sensilla. As all stages of *Pratylenchus* species are migratory endoparasites that experience environmental situations inside and outside the host, sensory adaptations reflecting stage specialisations are not required. Thus the reduction in the anterior sensilla and their processes is not as extreme as that of the group which includes the Heteroderidae (Trett, 1984). Cephalic sensory structure and biology of *Pratylenchus* may, therefore, emphasize the separation of their evolutionary pathway from that of the more specialised plant-parasitic species.

#### ACKNOWLEDGEMENTS

We would like to thank Mr. Robin Webb for useful discussion on the biology of *Pratylenchus* species and Mr. John A. Crouch for preparing and checking the manuscript. We also gratefully acknowledge the support of an AFRC post-doctoral research grant for Dr. M. W. Trett held by Prof. J. Green at The School of Biological Sciences, Queen Mary College, University of London.

#### REFERENCES

- BALDWIN, J. G. & HIRSCHMANN, H. (1975). Fine structure of the cephalic sense organs of *Heterodera glycines* males. *J. Nematol.*, 7 : 40-53.
- BANG, B. G. & BANG, F. B. (1957). Graphic reconstruction of the third dimension from serial section micrographs. *J. Ultrastruct. Res.*, 1 : 138-146. 40-53.
- CLARK, S. A. & SHEPHERD, A. M. (1977). Structure of the spicules and caudal sensory equipment in the males of *Aphelenchoides blastophthorus* (Nematoda : Tylenchida, Aphelenchina). *Nematologica*, 23 : 103-111.
- COOMANS, A. (1979). The anterior sensilla of nematodes. *Revue Nématol.*, 2 : 259-283.
- COOMANS, A. & DE GRISSE, A. T. (1981). Sensory structures. In : Zuckerman, B. M. & Rohde, R. A. (Eds) *Plant Parasitic Nematodes*. Vol. III, New York & London, Acad. Press : 127-174.
- CORBETT, D. C. M. & CLARK, S. A. (1983). Surface features in the taxonomy of *Pratylenchus* species. *Revue Nématol.*, 6 : 85-89.
- DE CONINCK, L. A. P. (1965). Classe des Nématodes. In : Grassé, P. (Ed.) *Traité de Zoologie*. Vol. IV, Paris, Masson et Cie : 1-217.

- DE GRISSE, A. T. (1975). The ultrastructure of some ciliary receptors in the cephalic region of tylenchids (Nematoda). *Meded. Fac. Landbouwwet. Rijksuniv., Gent*, 40 : 473-487.
- DE GRISSE, A. T. (1977). *The ultrastructure of the nerves in the cephalic region of 21 species belonging to 19 tylenchid genera*. D. Sc. Thesis, Rijksuniversiteit Gent, 420 p.
- DI SANZO, C. P. (1973). Nematode responses to carbofuran. *J. Nematol.*, 5 : 22-27.
- ENDO, B. Y. (1980). Ultrastructure of the anterior neurosensory organs of the larvae of the soybean cyst nematode, *Heterodera glycines*. *J. Ultrastruct. Res.*, 72 : 349-366.
- FUSCALDO, K. & JONES, H. (1959). A method for the reconstruction of 3-dimensional models from electron micrographs of serial sections. *J. Ultrastruct. Res.*, 3 : 1-10.
- MCLAREN, D. J. (1976). Nematode sense organs. *Adv. Parasitol.*, 14 : 195-265.
- MOUTAIN, W. B. (1955). A method of culturing plant-parasitic nematodes under sterile conditions. *Proc. helminth. Soc., Wash.*, 22 : 49-52.
- NATASASMITA, S. (1979). *Transmission and scanning electron microscope observations on Tylenchulus semipenetrans juveniles (♀ 2), males and females*. Ph. D. Thesis Rijksuniversiteit, Gent, 136 p.
- SPURR, A. R. (1969). A low-viscosity epoxy embedding medium for electron microscopy. *J. Ultrastruct. Res.*, 26 : 31-45.
- THURM, U. (1978). Steps in the transducer process of mechanoreceptors. *Symp. Zool. Soc. Lond.*, 23 : 199-216.
- THURM, U. (1974). Mechanisms of electrical membrane responses in sensory receptors illustrated by mechanoreceptors. In : Jaenicke, L. (Ed.) *Biochemistry of Sensory Functions*. Berlin, Springer-Verlag : 367-390.
- TRETT, M. W. (1981). A handling technique for small specimen critical point drying. *Proc. roy. microscop. Soc.*, 16 : 40-41.
- TRETT, M. W. (1984). *The cephalic sense organs of nematodes*. Ph. D. Thesis, University of Leeds, England, 437 p.
- TRETT, M. W. & CROUCH, J. A. F. (1984). The collection of plastic films on specimen support grids for use in electron microscopy. *Proc. roy. microscop. Soc.*, 19 : 251.
- TRETT, M. W. & LEE, D. L. (1981). The cephalic sense organs of adult female. *Hammerschmidtella diesingi* (Nematoda : Oxyuroidea). *J. Zool., Lond.*, 194 : 41-52.
- TRETT, M. W. & LEE, D. L. (1982a). The cephalic sense organs of the infective, third-stage juvenile of the rat hookworm, *Nippostrongylus brasiliensis*. *Parasitology*, 84 : 26.
- TRETT, M. W. & LEE, D. L. (1982b). A developmental study of the cephalic sense organs of the rat hookworm, *Nippostrongylus brasiliensis*. *Parasitology*, 84 : 69.
- TRETT, M. W. & PERRY, R. N. (1986). Effects of the carbamoylaxime, aldicarb, on the ultrastructure of the root-lesion nematode, *Pratylenchus penetrans* (Tylenchida : Pratylenchidae). *Nematologica*, 31 : in press.
- VENABLE, J. H. & COGGESHAALL, R. (1965). Simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.*, 25 : 407-408.
- WARD, S. (1973). Analysis of chemotactic behaviour of the nematode *Caenorhabditis elegans* using mutants. *Behav. Genetics*, 3 : 417-419.
- WARD, S. (1976). The use of sensory mutants to analyse the sensory nervous system of *Caenorhabditis elegans*. In : Croll, N. A. (Ed.) *Organisation of Nematodes*; New York, Acad. Press : 365-382.
- WARD, S. (1977). Use of nematode behavioural mutants for analysis of neural function and development. *Soc. Neurosci. Res. Symp.*, 2 : 1-26.
- WARD, S. (1982). Genetic analysis of the sensory nervous system of *Caenorhabditis elegans*. In : Mettrick, D. F. & Desser, S. S. (Eds) *Parasites - Their World and Ours*, Amsterdam, Elsevier Biomedical Press : 365-382.
- WARD, S., THOMSON, J. N., WHITE, J. G. & BRENNER, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. comp. Neurol.*, 160 : 313-318.
- WARE, R. W., CLARK, D., CROSSLAND, H. & RUSSELL, R. L. (1975). The nerve ring of the nematode *Caenorhabditis elegans* : sensory input and motor output. *J. comp. Neurol.*, 162 : 71-110.
- WRIGHT, K. A. (1980). Nematode sense organs. In : Zuckerman, B. M. (Ed.) *Nematodes as Biological Models*, New York, Acad. Press : 237-295.
- WRIGHT, K. A. & CARTER, R. (1980). Cephalic sense organs and body pores of *Xiphinema americanum* (Nematoda : Dorylaimoidea). *Can. J. Zool.*, 58 : 1439-1451.
- YAMADA, M. & YOSHIDA, S. (1972). Graphic stereo-reconstruction of serial sections. *J. Microsc.*, 95 : 249-256.
- ZACHARUK, R. Y. (1980). Ultrastructure and function of insect chemosensilla. *Ann. Rev. Entomol.*, 25 : 27-47.

Accepté pour publication le 26 avril 1985.