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# Reconstruction of the pharyngeal corpus of *Aphelenchus avenae* (Nematoda: Tylenchomorpha), with implications for phylogenetic congruence

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The corpus of the pharynx in the nematode *Aphelenchus avenae* (Nematoda: Tylenchomorpha) was three-dimensionally reconstructed to address questions of phylogenetic significance. Reconstructed models are based on serial thin sections imaged by transmission electron microscopy. The corpus comprises six classes of radial cells, two classes of marginal cells, and 13 neurones belonging to eight classes. Between the arcade syncytia and isthmus cells, numbers of cell classes along the pharyngeal lumen and numbers of nuclei per cell class correspond exactly between *A. avenae* and *Caenorhabditis elegans*. The number of radial cell classes between the arcade syncytia and the dorsal gland orifice (DGO) in *A. avenae* is also identical with outgroups. Proposed homologies of the pharynx imply that expression of the anterior two cell classes as epithelial or muscular differs within both Rhabditida and Tylenchomorpha. Numbers of neurone cell bodies within the corpus correspond exactly to *C. elegans*, other free-living outgroups, and other Tylenchomorpha. Neurone polarity and morphology support conserved relative positions of cell bodies of putative neurone homologues. The configuration of cells in the procorpus, including the length of individual cell classes along its lumen, differs across representatives of three deep Tylenchomorpha lineages. Nonhomology of the procorpus challenges the homology of DGO position within the metacorpus, the primary taxonomic character for circumscribing 'Aphelenchoidea'. Comparison of *A. avenae* with *Aphelenchoides blastophthorus* shows that, despite gross pharynx similarity, these nematodes have several differences in corpus construction at a cellular level. The possibility of convergent evolution of an 'aphelenchid' pharynx in two separate lineages would be congruent with molecular-based phylogeny. Putative homologies and conserved arrangement of pharyngeal neurones in Tylenchomorpha expand the experimental model of *C. elegans*.


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## INTRODUCTION

Development of phylogenies based on molecular sequences enables strong tests of evolutionary scenarios, but in some cases has presented a serious challenge to classical hypotheses. One particularly puzzling challenge to traditional classifications, and the transformations they entail, is in the nematodes of Tylenchomorpha. Classical hypotheses have asserted the monophyly of Tylenchomorpha, including the superfamily Aphelenchoidea Fuchs 1937. The synapomorphy fundamental in uniting all Aphelenchoidea is the position of the dorsal gland orifice (DGO) within the metacorpus (wide, posterior region) of the pharynx, in contrast to within the procorpus (thinner, anterior region) as in other Tylenchomorpha

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and free-living outgroups (Andrássy, 1976; Siddiqi, 1980; Hunt, 1993). Several analyses of rRNA sequences (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Smythe, Sanderson & Nadler, 2006; Meldal *et al.*, 2007; Bert *et al.*, 2008) have, however, rejected monophyly of Aphelenchoidea. By contrast, the family Aphelenchidae is supported as the sister group to all non-aphelenchid Tylenchomorpha ('tylenchids *sensu stricto*') with Cephalobomorpha comprising the closest free-living outgroups; the other family of Aphelenchoidea, Aphelenchoididae, is nested within a separate clade of Panagrolaimorpha (Fig. 1). Possible paraphyly of Tylenchomorpha, which are characterized by a highly similar feeding stomatostylet, necessarily has implications for parallel evolution of parasitism (Baldwin, Nadler & Adams, 2004).

Previous studies have shown that rigorous examination of morphology, particularly using transmission electron microscopy (TEM), clarifies discrepancies with conflicting phylogeny. Several elements of feeding morphology are conserved with outgroups in Rhabditida, including the underlying epidermal cells of the stoma and stylet (Bumbarger *et al.*, 2006; Ragsdale *et al.*, 2008) and to some extent the stomatal epithelia (i.e. nonmuscular pharynx cells) and muscles (Baldwin & Eddleman, 1995; De Ley *et al.*, 1995; Baldwin *et al.*, 1997; Dolinski *et al.*, 1998; Dolinski & Baldwin, 2003; Giblin-Davis *et al.*, 2010) and cells of the pharyngeal postcorpus (Zhang & Baldwin, 1999, 2000, 2001; Baldwin, Souza & Dolinski, 2001). Three-dimensional (3D) reconstruction from serial TEM thin sections thus provides a test of homologies of taxonomically important characters. Independent phylogeny predicts differences between Aphelenchidae and Aphelenchoididae in components of DGO position, such as numbers and spatial relationships of surrounding cells.

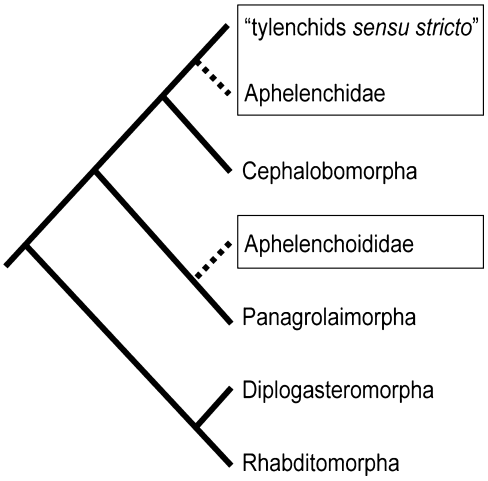



**Figure 1.** Summarized phylogenetic tree of Tylenchomorpha and outgroups, which is a consensus of phylogenies from several phylum-wide analyses (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Meldal *et al.*, 2007; Bert *et al.*, 2008), although placement of the root varies amongst some analyses. Taxa in boxes belong to Tylenchomorpha; taxa on dashed branches belong to 'Aphelenchoidea'. 'Tylenchids *sensu stricto*' designates all non-aphelenchid Tylenchomorpha.

Reconstruction of individual cells in the corpus also enables proposals of homology with more distant outgroups, notably the model organism *Caenorhabditis elegans*. Composition and function of the feeding apparatus and its nervous supply are well characterized for *C. elegans*, but the extent to which the model can be applied to other Rhabditida nematodes, especially Tylenchomorpha, is limited by our understanding of corresponding structures in other taxa. The degree to which cell classes are conserved and readily identified across taxa governs how hypotheses of feeding behaviour might be adapted and tested in those nematodes. Conservation in at least other feeding structures, including the stoma/stylet apparatus, suggests shared components in the pharynx as well. The prospect of disparate feeding types being possible from the same cellular components has significant implications for the evolution of feeding.

Herein we present computer-visualized, complete 3D TEM reconstruction of the pharyngeal corpus and partial reconstruction of the isthmus of *Aphelenchus avenae* (Aphelenchidae). Models are the first TEM-based representation of the entire corpus in a nematode other than *C. elegans*. Hypotheses of pharynx homologies in *A. avenae* are considered with respect to TEM reconstruction in an Aphelenchoididae representative, *Aphelenchoides blastophthorus* (Shepherd, Clark & Hooper, 1980). Furthermore, characters of *A. avenae* are applied to non-aphelenchid Tylenchomorpha, or 'tylenchids *sensu stricto*', insofar as is possible by re-examination of previous TEM studies of the pharynx. Character discovery in various clades of Tylenchomorpha will thereby test pharynx homologies on a cell-to-cell basis. Complete or partial reconstructions of the pharynx in Rhabditida and more distant outgroups (Albertson & Thomson, 1976; Zhang & Baldwin, 1999, 2000, 2001) can polarize characters and inform possible convergence of pharynx structures. In a comparative approach, 3D TEM reconstruction of the pharynx enables re-evaluation of morphology that is the basis of a monophyletic 'Aphelenchoidea' but which is incongruent with molecular evidence.

The generalized pharynx of Tylenchomorpha can be divided into at least two distinct regions: the corpus, which is further divided into the narrower, anterior procorpus and the wider, posterior metacorpus that is often characterized by a medial thickening of the

luminal cuticle; and the postcorpus, which includes the isthmus, gland cell bodies, and in some cases a posterior basal bulb. Pharyngeal tissue comprises radially orientated muscles and epithelia, a semiautonomous nervous system, and typically one dorsal and two subventral glands. The glands extend through the pharynx longitudinally and can have cell bodies that are very large and overlap the intestine; such an arrangement is found in *Aphelenchus*. Distinct in 'Aphelenchoidea' is a large metacorpus that often fills the entire body cavity diameter. The DGO is within the metacorpus in 'Aphelenchoidea', in contrast to its position in the anterior procorpus in tylenchids *sensu stricto*. Nematodes of Aphelenchidae, including *Aphelenchus*, have a long postcorpus. In each part of the pharynx, the cuticle of the central lumen is lined by alternating marginal cells and radial cells or pairs of cells; radial cells are expressed as muscle or epithelium. Spatial connectivity to the cuticle offers strong evidence for positional homologies of cells, which are presumably the source from which adjacent cuticle is secreted (Wright & Thomson, 1981; Endo, 1985). In addition to those cells lining the luminal cuticle in any transverse section are processes of cells that line the cuticle of other regions of the lumen. In particular, cells with luminal attachments at narrower, anterior parts of the nematode often have processes that extend longitudinally to cell bodies in more posterior positions. Determining precise numbers of cells, which is necessary for homological tests of conjunction, requires consecutive serial sections throughout the pharynx. Moreover, the series can elucidate subtle connections between cells and complex positional relationships that are easily missed in isolated sections.

## MATERIAL AND METHODS

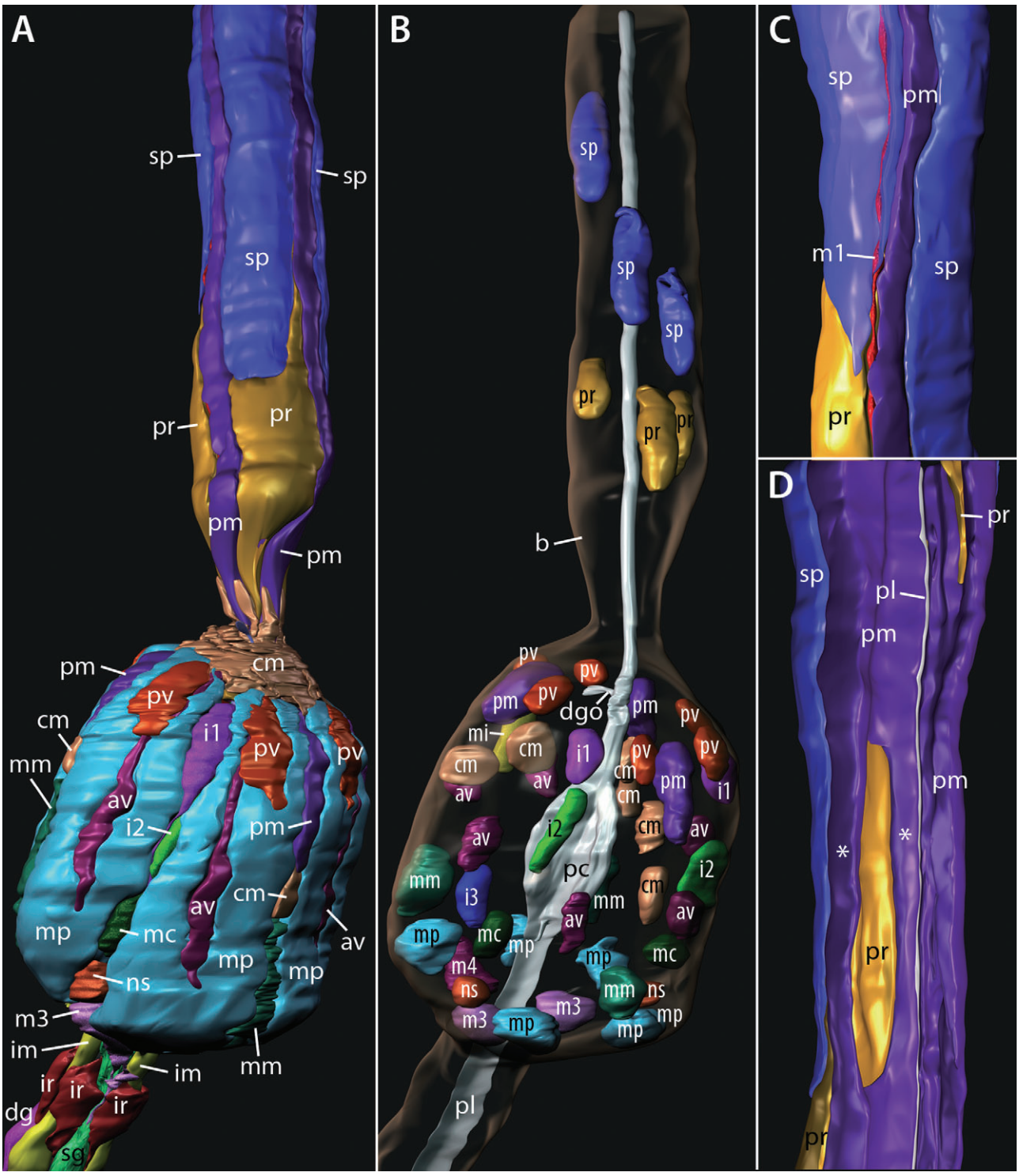
Reconstructions presented herein are based on two complete alignments of serial micrographs, one transverse and one longitudinal, with parts additionally represented by other specimens. Specimens of *A. avenae* (strain RGD103) were from cultures maintained on *Monilinia fructicola* in lactic acid-treated, glycerin-supplemented potato dextrose agar (LGPDA) medium. Nematodes were frozen in a Bal-Tec (Balzers, Liechtenstein) HPM 010 high-pressure freezer in copper specimen carriers filled to capacity, without an auxiliary cryoprotectant. Deep-frozen specimens were then dehydrated, fixed, and stained *en bloc* using a Reichert-Jung (Depew, NY, USA) CS-Auto freeze substitution apparatus in an acetone cocktail of 3% osmium tetroxide and 1% uranyl acetate. Freeze substitution and infiltration regimens, are described in greater detail in Ragsdale *et al.* (2008). Specimens were embedded in Epon 812 in slide-shaped resin moulds (Giammara & Hanker, 1986), and were screened by differential interference contrast (DIC) microscopy. Adult, female nematodes selected for sectioning and TEM were documented in through-focus video vouchers, which were later used to inform measurements for calibrating dimensions of the reconstructed model. Specimens were manually excised from slide moulds and re-embedded in blocks for microtomy. Serial, silver-gold sections (70 nm thick) were taken on a Sorvall MT6000 microtome with a Microstar diamond knife. Sections were post-stained with methanolic uranyl acetate and lead citrate.

Using a Philips Tecnai T12 transmission electron microscope operating at 120 kV, images were acquired as montages with a Gatan US1000 camera and automatically assembled with the Gatan software program Digital Micrograph. Microscopy and imaging were conducted at the Center for Advanced Microscopy and Microanalysis (CFAMM) at the University of California, Riverside. For longitudinal alignments of sections, several acquired images were montaged using Adobe Photoshop (San Jose, CA, USA).

Ordered images of serial sections were converted to the MRC (Medical Research Council Cambridge Image Processing System) stack format and manually aligned, individual cell contours manually traced, and contour-based mesh models of cells constructed using the software package IMOD (Kremer, Mastronarde & McIntosh, 1996). Models were transferred to BLENDER 2.49 (blender3d.org), an open source package for ray tracing, mesh modelling, and animation, for manual correction of meshing errors and production of model figures and animations. All model components visualized herein were reconstructed from a single specimen alignment, except for the transition of the procorpus and metacorpus, which was informed by alternate specimens. To compensate for alignment artefacts caused by nematode curvature over a long series (~1000 sections), model stretching was adjusted in BLENDER as informed by light microscopy video vouchers.

## RESULTS

Three-dimensional reconstruction of the pharyngeal corpus of *A. avenae* identified several classes of muscle/radial and marginal cells (Fig. 2A), distributed serially along its length and with cells within classes positioned radially around the central lumen. All nuclei within the corpus were identified (Fig. 2B). The radial and marginal cells of the pharyngeal isthmus were also partially reconstructed, but not to their definitive end. Limited TEM survey of more posterior parts of the isthmus did not identify any obvious turnover of cells within this region. Cells of the corpus include: six classes of muscle/radial cells,




each with either three single and radial or three pairs of adradial (dorsal, left and right subventral) individuals; two classes of marginal cells, each with three radially distributed (left and right subdorsal, ventral) individuals; the dorsal and two subventral gland cells; cells of the pharyngeal nervous system. Additionally, a class of radial cells and a class of marginal cells have each been identified for the isthmus. Reconstructions of pharyngeal neurones have only been presented for cells in which homologies with *C. elegans* could be confidently predicted, including all with cell bodies in the metacorpus, one anteriorly

**Figure 2.** Three-dimensional reconstruction of pharyngeal corpus of *Aphelenchus avenae* based on serial transmission electron microscopy sections. Top of figure is anterior. Colour key to cells is given in Figure 6G. Neurones are rendered with 'rough' texture; glands, with 'wrinkled' texture. A, subventral view of corpus and anterior isthmus. Cell bodies of anterior and posterior valve muscles are wedged in periphery of metacorpus pump muscles; constraining muscles and marginal cells are in ventral margin. One row of subventral neurone cell bodies is also shown. B, subventral view of corpus showing all corpus nuclei. Basal lamina of pharynx is rendered transparent; pharyngeal lumen is also shown. Labels with narrow face text refer to nuclei. C, right subdorsal view of part of procorpus. Dorsal stylet protractor is rendered transparent to show dorsal procorpus radial cell and M1 neurone behind (medial to) it. Process of M1 neurone lies between right subdorsal marginal cell and dorsal radial cell posteriorly, and between dorsal radial cell and stylet protractor anteriorly. D, left subventral view of procorpus, with left subventral radial cells omitted to show ventral and subdorsal marginal cells. Asterisks indicate strands of loop of ventral marginal cell; part of subdorsal marginal cell loop is also shown. Abbreviations: av, anterior dorsal gland orifice valve muscle; b, basal lamina; cm, metacorpus constraining muscle; dg, dorsal pharyngeal gland; dgo, dorsal gland orifice (DGO); i1, interneurone I1; i2, interneurone I2; i3, interneurone I3; im, isthmus marginal cell; ir, isthmus radial cell; m1, motor neurone M1; m3, motor neurone M3; m4, motor neurone M4; mc, marginal cell neurone MC; mi, motor-interneurone MI; mm, metacorpus marginal cell; mp, metacorpus pump muscle; ns, 'neurosecretory' motor neurone NSM; pc, metacorpus pump chamber; pl, procorpus lumen; pm, procorpus marginal cell; pr, procorpus radial cell; pv, posterior DGO valve muscle; sg, subventral pharyngeal gland; sp, stylet protractor muscle.

terminating pharyngeal neurone described previously (Ragsdale *et al.*, 2008), and processes of two central nervous system interneurones (RIP). All radial cells and marginal cells examined are uninucleate. All radial and isthmus cells form gap junctions and, near the lumen, adherens junctions with laterally adjacent radial or isthmus cells. Nuclei of isthmus muscle cells were not observed in the present study and so are presumably located far posterior within their cells.

## PROCORPUS

The long pharyngeal procorpus is simple in composition, with two sets of three radial cells and one set of marginal cells (Figs 2A, 3A), including the most anterior cells of the pharynx. The most anterior radial cells of the metacorpus, the constraining muscles, taper peripherally within the procorpus at its transition with the metacorpus (Fig. 2A). The procorpus contains only one neurone (Figs 2C, 3B). The pharyngeal lumen is narrow, cylindrical, and its lining is of consistent cuticular composition along the length of the procorpus. Anterior parts of anterior procorporal cells are not shown, having been reconstructed previously by Ragsdale *et al.* (2008).

### *Stylet protractor muscles*

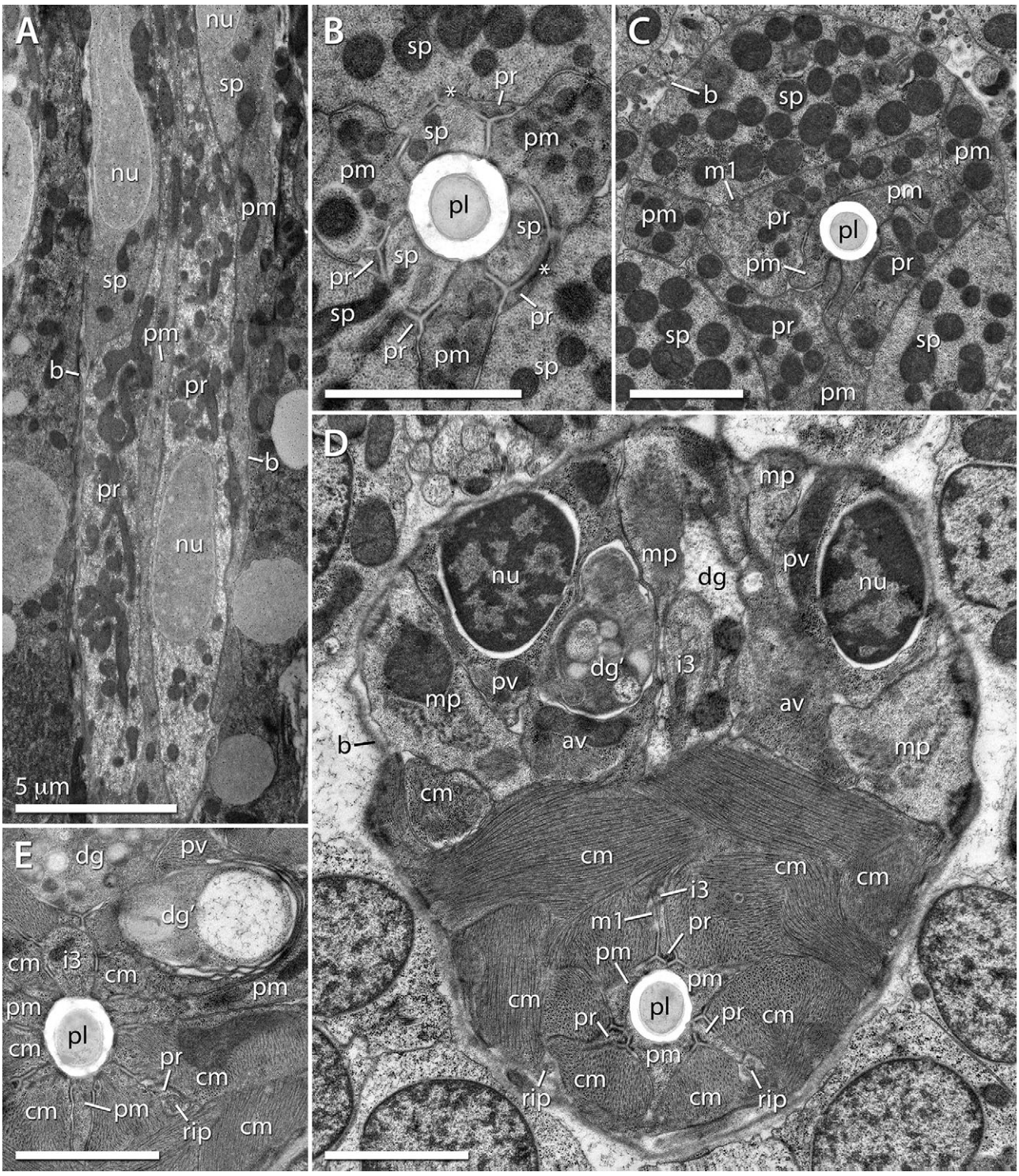
The stylet protractor muscles are a set of three muscle cells that attach to both the base of the stylet and cephalic cuticle (Ragsdale *et al.*, 2008). The cells comprise a contractile region anteriorly and a noncontractile region posteriorly. The stylet protractors attach to the lumen lining at the anterior end of the procorpus (not shown). They extend posteriad in the procorpus along the basal lamina (Fig. 3A, C), persisting for more than half the length of the procorpus (Fig. 2A). The nuclei of the stylet protractors are located in the procorpus (Figs 2B, 3A) at their tapered, posterior tips.

### *Procorpus radial cells*

Along the lumen posterior to the stylet protractor cells is a set of three procorpus radial cells (Fig. 2A). These cells are nonmuscular but have cytoplasm distinct from that of the adjacent marginal cells; the radial cells are less dense with ribosomes and small cellular products (Fig. 3B, C). At their most anterior tips, they enter posterior invaginations in the stylet protractor muscles. Here the radial cells are characterized by transverse filaments, which along with similar filaments in the adjacent marginal cells, form a ring around the lumen (Fig. 3B). Along much of their length in the anterior procorpus, adjacent procorpus radial cells connect to each other through large loops in the marginal cells (Fig. 2D). The posterior ends of the procorpus radial cells extend into the metacorpus, tapering medially along the lumen (Fig. 3D). Their connections to the lumen lining are replaced in position posteriorly by the metacorpus constraining muscles. Nuclei are located in the posterior half of the procorpus radial cells (Figs 2B, 3A). Posterior to their attachment to the lumen lining, the radial cells extend into the metacorpus with swollen processes (Fig. 4A); the process of the dorsal cell is shorter and less distinct (not shown) and is limited in space by the large, adjacent dorsal gland ampulla.

### *Procorpus marginal cells*

The procorpus marginal cells line the luminal cuticle from the base of the stylet to the level of the DGO in the metacorpus (Figs 2A, D, 4B). Over the anterior




half of the procorpus, the marginal cells comprise complete loops (Fig. 2D), with one strand of the loop along the lumen and the other along the basal lamina (Fig. 3C); at the basal lamina they are between the stylet protractors (Fig. 3C). The loop of the ventral marginal cell is longer and more posterior than those of the subdorsal cells. The marginal cells extend into the metacorpus (Fig. 4A, B), within which they separate pairs of the adradial constraining muscles (Fig. 3D, E) and anterior valve muscles around the lumen (Fig. 5A). Posterior to the point of disassociating from the lumen lining, each marginal cell persists

**Figure 3.** Transmission electron microscopy sections of the corpus of *Aphelenchus avenae*. Except for the longitudinal section (A), top of figure is dorsal. In all oblique transverse sections, top of figure is further posterior. A, longitudinal section through procorpus, showing stylet protractor, procorpus radial, and procorpus marginal cells, including the nucleus for a subventral protractor (left) and nuclei for the dorsal protractor and dorsal procorpus radial cell (right). B, anterior procorpus, at the attachment of stylet protractors on the lumen lining posterior to the base of the stylet. Junctions, characterized by bundles of transverse filaments (asterisks) between protractors and anterior tips of procorpus radial cells, are shown. C, mid procorpus, showing stylet protractors along basal lamina and separation of medial and peripheral branches of subdorsal procorpus marginal cells. Anterior process of neurone M1 is between dorsal protractor and procorpus radial cells, near the right, subdorsal marginal cell. D, oblique section through anterior metacorpus. Procorpus radial and marginal cells attach on lumen lining; constraining muscles interdigitate with contractile filaments orientated circumferentially around lumen. Anterior, noncontractile parts of all other metacorpus muscles are shown, including the nuclei for the posterior valve muscles. A portion of the dorsal gland is within an invagination of the right posterior valve muscle cell. The process of neurone I3 crosses the dorsal gland ampulla; processes of neurones I3 and M1 are between adradial constraining muscles. The somatic RIP interneurones are between adradial subventral constraining muscles, shown just posterior to where they enter the metacorpus across the basal lamina. E, oblique section through metacorpus, immediately anterior to dorsal gland orifice (DGO). Constraining muscles attach on lumen lining. A portion of the dorsal gland is within an invagination of the left posterior valve muscle cell. Processes of procorpus radial cell and RIP interneurone are between adradial constraining muscles. Terminus of neurone I3 abuts against lumen lining dorsally. Abbreviations: av, anterior dorsal gland orifice valve muscle; b, basal lamina; cm, metacorpus constraining muscle; dg, dorsal pharyngeal gland; dg', dorsal pharyngeal gland process; i3, interneurone I3; m1, motor neurone M1; mp, metacorpus pump muscle; nu, nucleus; pl, procorpus lumen; pm, procorpus marginal cell; pr, procorpus radial cell; pv, posterior DGO valve muscle; rip, somatic interneurone RIP; sp, stylet protractor muscle. Unless otherwise indicated, scale bars = 2 μm.

in an arc around the periphery of the anterior half of the metacorpus, with its nucleus located at the posterior end of the cell (Figs 4A, B, 5B).

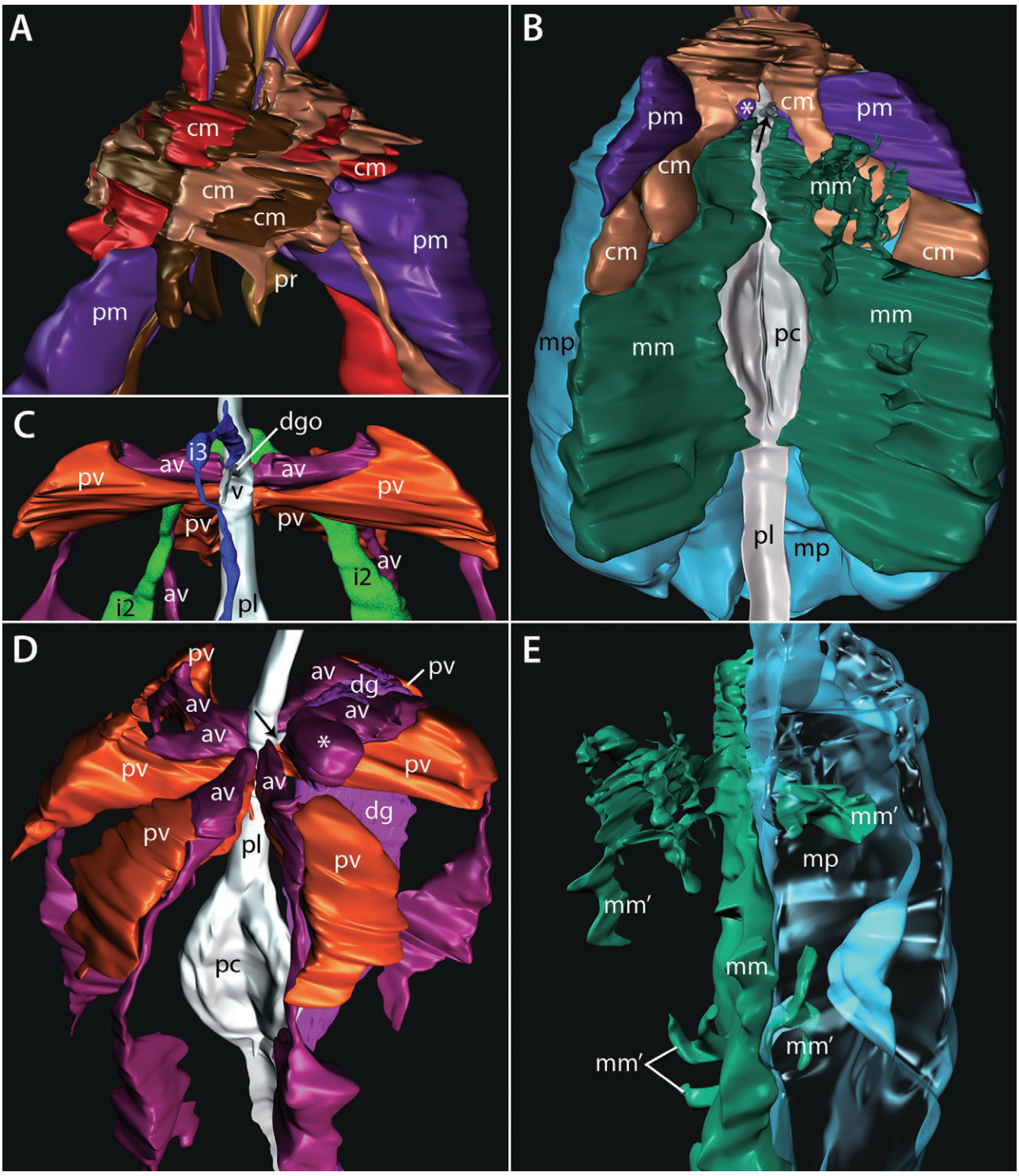
## METACORPUS

The metacorpus comprises four sets of adradial muscle cells and one set of marginal cells (Fig. 2A), in addition to those cells that are primarily associated with the procorpus and the isthmus. The metacorpus pump muscles (Figs 2A, 4B) occupy most of the volume of the metacorpus, whereas all other cells lie either between the pump muscles or embedded within peripheral grooves of these muscles. Posterior to the central 'pump chamber' of the metacorpus, the luminal cuticle is lined by the anterior parts of the isthmus cells (Fig. 6A). Between the central isthmus cells and the more peripheral metacorpus cells is the commissure of the pharyngeal nervous system, from which neurones extend throughout the rest of the pharynx (Fig. 6C).

As in the procorpus, the lumen is narrow and cylindrical anterior to the DGO (Figs 2B, 3D, E). Immediately posterior to the DGO, the lumen lining is a narrow, triradiate 'valve' (Figs 4C, 5B). Posterior to the valve, the lumen remains narrow but is hexagonal in cross section, with shallow grooves between apices of the lining (Fig. 5C). At the centre of the metacorpus is the pump chamber, which comprises three radial plates (Figs 2B, 5D). The apices of the pump chamber plates are thickest peripherally, each having three rounded ridges (Fig. 5D). The cuticle of the pump chamber and lumen posterior to it is slightly more electron-dense than the cuticle of the whole lumen lining of the pharynx anterior to it; an exception where the cuticle is electron-lucent is in the crotches between the apical ridges of the pump chamber plates (Fig. 5D). The lumen is triradiate from the pump chamber posteriad (Fig. 5E) and is wider in total diameter than the lumen anterior to the pump chamber.

### *Constraining muscles*

The six constraining muscles form an anterior 'cap' of the metacorpus at its transition with the procorpus (Figs 2A, 4A, B). The muscles attach to the lumen lining for a short margin posterior to the procorpus radial cells (Figs 3E, 5A), but they extend further anteriad (Figs 2A, 4A, B) with contractile cytoplasm. Each cell has oblique ventral and dorsal branches that interdigitate with those of adjacent constraining muscles (Figs 3D, 4A). Contractile filaments within most of the cell are tangential to the circumference of the lumen (Fig. 3D), but medial filaments are perpendicular to the lumen (Fig. 3D, E), including where the cells insert on the lumen lining. Posterior to their insertion points, the cells extend posteriad and peripherally through the margins between metacorpus pump muscles (Figs 1A, 4B, 5B, C). In each of the one ventral or two subdorsal areas lie the cell bodies (parts of cells containing nuclei) of the two nearest constraining muscles (Fig. 4B). Cell bodies are located just anterior of the middle metacorpus (Fig. 2B).




*DGO valve muscles*

Posterior to the attachments of the constraining muscles are a series of two sets of muscles associated with the region of the DGO and valve, henceforth designated 'valve muscles' (Figs 2A, 4C, D). Both sets of muscles are thin in comparison to other metacorporal muscles, and have small margins of insertion upon the lumen lining (Fig. 4C, D). Contractile filaments in both sets of muscles are perpendicular to the lumen (Fig. 5B, C).

The anterior valve muscles attach to the lumen lining immediately anterior to the DGO, where the

**Figure 4.** Three-dimensional reconstruction of pharyngeal metacorpus of *Aphelenchus avenae* based on serial transmission electron microscopy sections. Except for 'D', top of figure is anterior. Colour key to cells is given in Figure 6G. Neurones are rendered with 'rough' texture; glands, with 'wrinkled' texture. A, subventral view of constraining muscles and procorpus marginal cell bodies. Adjacent constraining muscles cells are different colours, including red and brown (deviating from colour key), to show their interdigitating arms. Posterior tip of procorpus radial cell process also shown. B, dorsal view of metacorpus. Dorsal pump muscle is removed to show metacorpus marginal cells, constraining muscles, and procorpus marginal cell bodies. Procorpus marginal cells line cuticle of lumen (asterisk) posterior to level of dorsal gland orifice (DGO; arrow). Metacorpus marginal cells line apices of pump chamber. Transverse processes of metacorpus marginal cells are in foreground. C, dorsal view of radial cells and neurones around DGO. Dorsal radial cells are omitted for clarity. Posterior valve muscles actuate luminal valve posterior to DGO. Radial cell bodies are peripheral within metacorpus. Neurone I3 terminates immediately anterior to DGO; subventral I2 neurones terminate opposite I3. D, anterior and subventral view of all anterior and posterior valve muscles lining luminal cuticle. Posterior valve muscle cell processes and bodies are arcs following the periphery of the metacorpus. Asterisk indicates prominent process of an anterior valve muscle that is within adjacent pump cell (omitted for clarity). Dorsal gland is between dorsal, adradial valve muscles. E, subdorsal view of subdorsal marginal cell and one adjacent pump cell (transparent); other adjacent pump cell is omitted for clarity. Processes of marginal cell extend transversely into adjacent pump cells. Abbreviations: av, anterior dorsal gland orifice valve muscle; cm, metacorpus constraining muscle; dg, dorsal pharyngeal gland; dgo, DGO; i2, interneurone I2; i3, interneurone I3; mm, metacorpus marginal cell; mm', metacorpus marginal cell process; pc, metacorpus pump chamber; pl, procorpus lumen; pm, procorpus marginal cell; pr, procorpus radial cell; pv, posterior DGO valve muscle.

lumen is circular in cross section (Figs 4C, D, 5A). Of both valve muscle classes, cell bodies of the anterior set are more prominent and in posterior extensions (Fig. 4D). Posterior to their contractile regions, these cells lie in peripheral grooves in the metacorpus pump muscles (Figs 2A, 5C). The cells end posteriorly in cell bodies at the level of the central pump chamber (Fig. 4D) and extend processes into adjacent pump muscles (Figs 4D, 5A). The nuclei are located in the middle metacorpus (Fig. 2B). The marginal cells separating adradial pairs of cells at their insertion points are the procorpus marginal cells (Fig. 5A).

The posterior valve muscles attach to the valve part of the lumen lining immediately posterior to the DGO (Figs 4C, D, 5B) and are thus the operating valve muscles. These cells extend directly perpendicular to the axis of the lumen to include nuclei at their periphery (Figs 2A, 3D, 4C, D, 5B), near the basal lamina. Where the posterior valve muscles insert on the lumen lining, adradial pairs are interspersed with the metacorpus marginal cells (Fig. 5B).

*Metacorpus pump muscles*

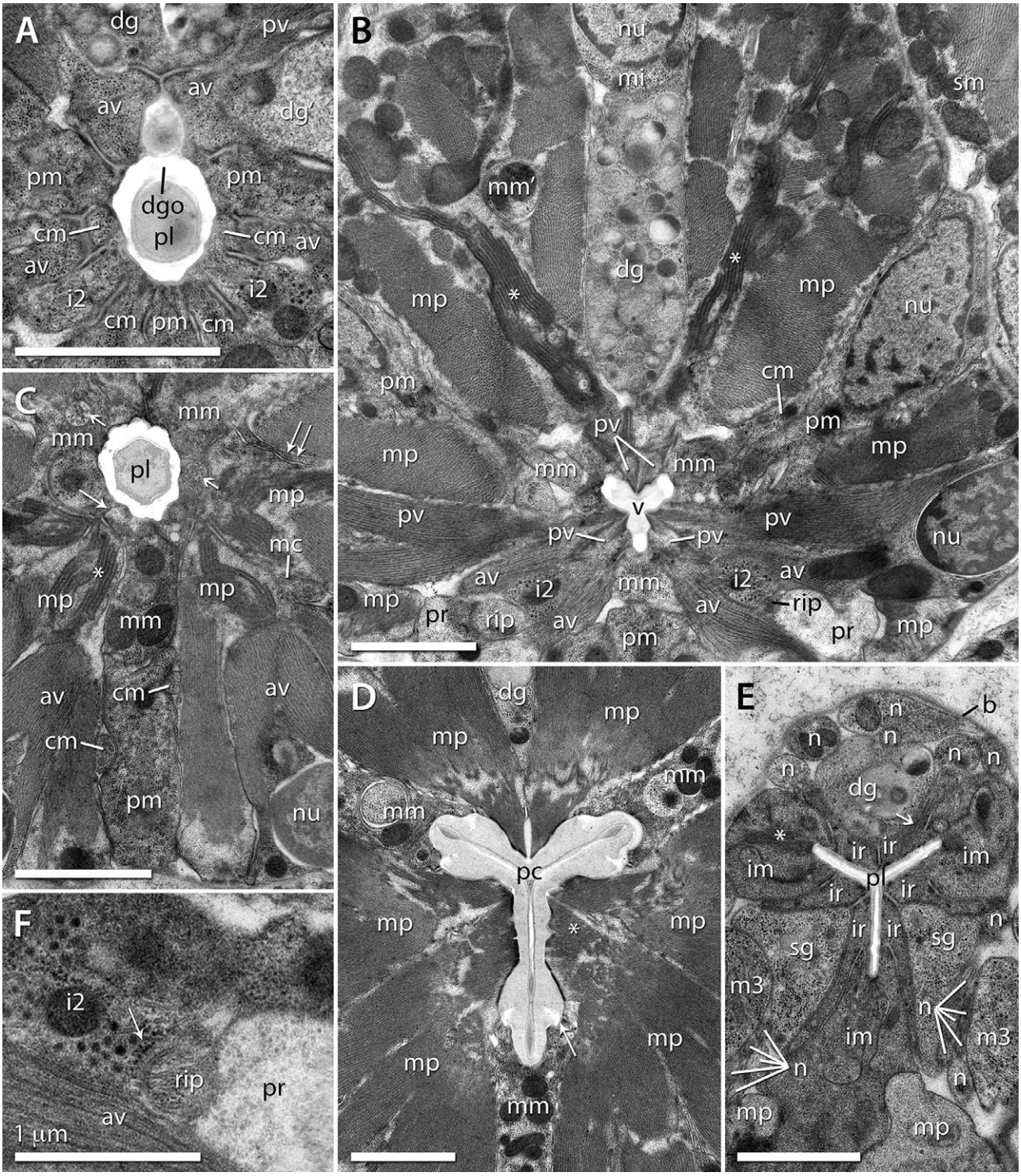
The prominent metacorpus dilator or 'pump' muscles occupy the volume of the metacorpus (Figs 2A, 4B). The relatively thin areas between adradial pairs are where the cell bodies of almost all other pharyngeal cells are located (Fig. 2A). The muscles only insert onto the lumen lining at the pump chamber (Figs 4B, 5D). Large amounts of contractile fibres radiate peripherally from the pump muscle insertions, which are characterized by dense bands of tonofilaments (Fig. 5D). Medially contractile elements are the most densely packed and seem to be mostly thin (actin) filaments. The peripheral parts of the cells include their noncontractile cytoplasm (Fig. 5B). Adjacent marginal cells extend various arms into the pump muscles (Figs 4E, 5B); these marginal cell processes split irregularly and extend between contractile areas throughout the cytoplasm of the muscle cells (Fig. 4E). Anteriorly in the pump muscles, extensive membranous lamellae occupy much of the space not occupied by bundles of filaments (Fig. 5B, C). Nuclei of the pump muscles are found posterior within the cells and thus posterior in the metacorpus (Fig. 2B).

*Metacorpus marginal cells*

From the DGO valve to the posterior end of the pump chamber, the metacorpus marginal cells line the luminal cuticle (Fig. 6E). Between the valve and the pump chamber, where the lumen is hexagonal in cross section, the marginal cells line the luminal cuticle exclusively (Fig. 5C). On the pump chamber, the marginal cells attach to the apices of the lumen lining, between the outer two ridges of each apex (Fig. 5D). Large portions of the cells are in the processes extending in channels throughout adjacent pump muscle cells (Fig. 4E). Anteriorly, the marginal cells share the areas between pump cells with various other metacorpus cells, although in the posterior half of the metacorpus they occupy the margins exclusively. The cytoplasm is rich in vesicles and internal membranes, especially where the marginal cells completely surround the lumen (Fig. 5C, D). The marginal cells contain nuclei in the posterior half of the metacorpus (Fig. 2B).

*Dorsal gland*

In the metacorpus the dorsal gland lies between the successive pairs of dorsal muscles (Figs 3D, E, 4D, 5A,




B, D). Between the metacorpus pump muscles the gland is flattened (Fig. 5B). Anterior to the DGO, between the two sets of valve muscles and the anterior tips of the pump muscles, is the ampulla of the gland (Fig. 4D), which is most swollen anteriorly (Fig. 6B). The ampulla has extensions that enter and swell within both dorsal, adradial anterior valve muscle cells (Figs 3D, E, 5A). A cuticle-lined canal, which originates within the gland cell, extends and opens between the two dorsal anterior valve muscles where they insert onto the lumen lining. Within the gland, the canal is tetraradiate (not shown). The

**Figure 5.** Transverse transmission electron microscopy sections through metacorpus and isthmus of *Aphelenchus avenae*. Top of figure is dorsal. In all oblique transverse sections, top of figure is further posterior. A, oblique section through anterior metacorpus at posterior level of dorsal gland orifice (DGO). Anterior valve muscles are on either side of DGO; junctions between constraining muscles and anterior valve muscles (dorsal members of subventral pairs) are lateral; constraining muscles attaching on the lumen lining are ventral. Termini of I2 neurones abut subventrally on the lumen lining between individuals of constraining muscle pairs. B, oblique section through the metacorpus showing the valve of the lumen lining posterior to the DGO. Posterior valve muscles insert on the valve, with nucleus shown for one subventral cell; subventral anterior valve muscles, with contractile filaments, are shown posterior to their insertions. Metacorpus pump muscles are shown anterior to their insertions; they include extensive internal lamellae (asterisks) and processes of adjacent metacorpus marginal cells. Also shown are the nucleus of procorpus marginal cell and processes of the procorpus radial cells, neurone I2, and somatic interneurone RIP. Dorsal to the dorsal gland is the cell body of neurone MI. C, oblique section through the metacorpus showing lumen (transversely hexagonal) between the valve and pump chamber. Metacorpus marginal cells completely surround lumen lining, forming junctions with each other (single long arrow) and including many large vesicles (short arrows). A process of a marginal cell (double long arrow) is shown entering channel in metacorpus pump muscle. Nuclei and posterior parts of anterior valve muscles are wedged peripherally in metacorpus pump muscles. Asterisk marks internal lamellae of pump muscles. Process of marginal cell (MC) neurone is between pump muscle cells. D, oblique section through metacorpus at pump chamber. Insertions of metacorpus pump muscle cells insert by dense tonofilaments on pump chamber and have a zone of dense thin (actin) filaments (asterisk) around areas of insertion. Metacorpus marginal cells attach to apices of pump chamber and have adherens junctions with pump muscles near electron-lucent areas of luminal cuticle (arrow). E, oblique section through isthmus near transition with metacorpus, showing all three gland cells, isthmus radial and marginal cells, and several pharyngeal neurones including parts of cell bodies of M3 neurones. Tonofilament bands (asterisk) of marginal cell are at luminal apices. Anterior part of dilatory contractile region (arrow) of dorsal radial cell is shown. F, example of synapse in pharyngeal nervous system, between somatic interneurone RIP and pharyngeal interneurone I2. Abbreviations: av, anterior dorsal gland orifice valve muscle; b, basal lamina; cm, metacorpus constraining muscle; dg, dorsal pharyngeal gland; dgo, dorsal gland orifice (DGO); i2, interneurone I2; im, isthmus marginal cell; ir, isthmus radial cell; m3, motor neurone M3; mc, marginal cell neurone MC; mi, motor-interneurone MI; mm, metacorpus marginal cell; mm', metacorpus marginal cell process; mp, metacorpus pump muscle; n, neurone; nu, nucleus; pc, metacorpus pump chamber; pl, procorpus lumen; pm, procorpus marginal cell; pr, procorpus radial cell; pv, posterior DGO valve muscle; rip, somatic interneurone RIP; sg, subventral pharyngeal gland; sm, somatic muscle; v, metacorpus luminal valve. Unless otherwise indicated, scale bars = 2 μm.

nucleus of the dorsal gland is located far posterior within the gland, which has not been reconstructed herein.

*Subventral glands*

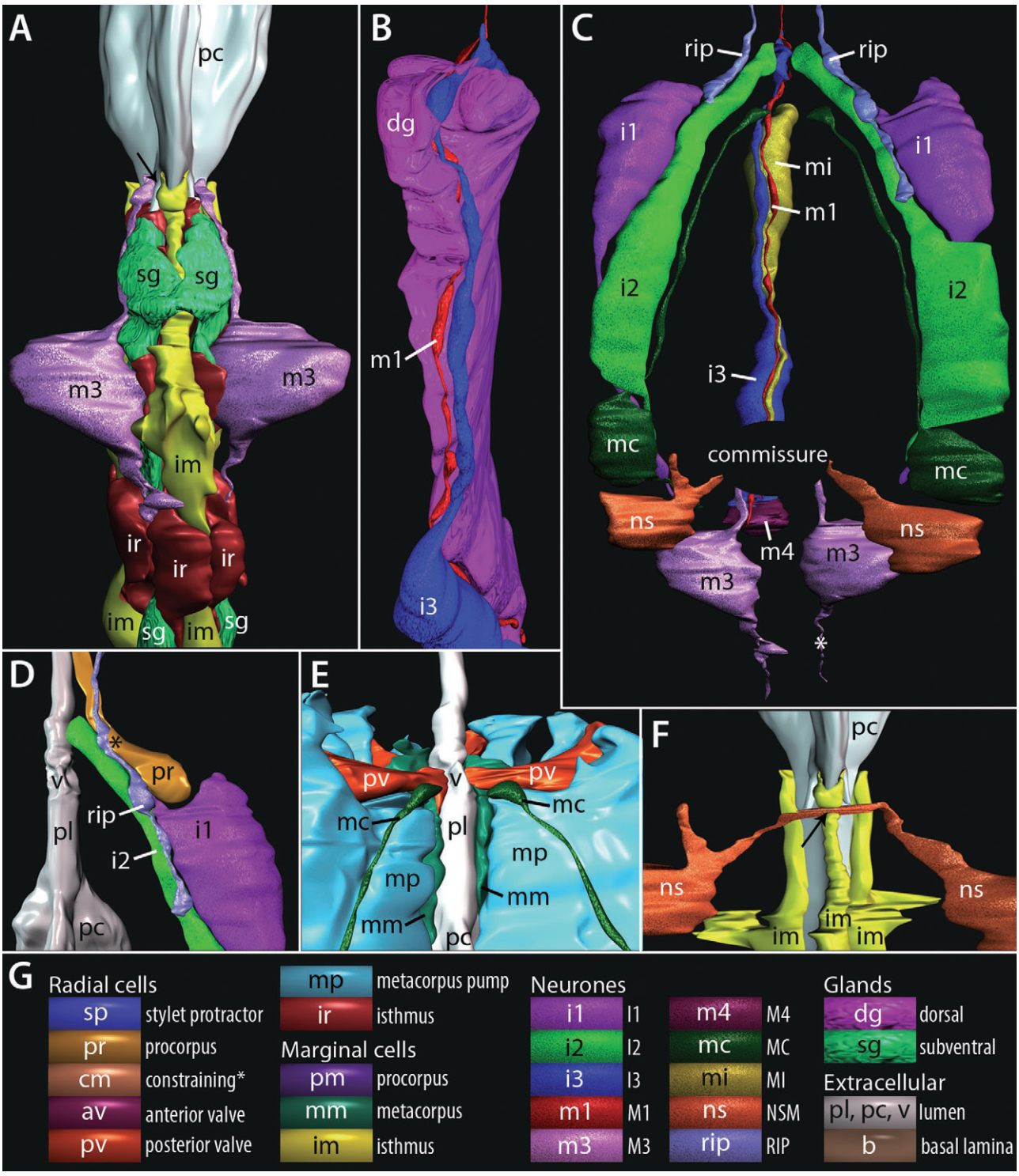
The subventral glands lie between the subventral, adradial isthmus muscles in their corresponding sectors (Figs 5E, 6A). From the anterior ends of the gland cells, the cuticle-lined canals extend medially and anteriorly to open into the lumen; the orifices are in the subventral corners between the luminal apices, immediately posterior to the metacorpus pump chamber (Fig. 6A). Within the gland cells, the canals are tetraradiate (not shown). On either side of each canal are two isthmus muscle cells (Fig. 6A). A subventral neurone terminus also abuts upon the lumen lining immediately dorsal and anterior to the subventral gland orifices (Fig. 6A). Posterior to the orifices are the subventral gland ampullae, which adjoin each other ventrally (Fig. 6A). Posteriorly, where the glands progressively narrow and disassociate from each other (Fig. 6A), they and the other isthmus cells are encircled by the pharyngeal commissure. Like the dorsal gland, the subventral gland cells extend throughout the isthmus and their nuclei are far posterior (not shown).

## Isthmus

The isthmus comprises a set of adradial muscle cells and a set of marginal cells (Figs 2A, 5E, 6A), which extend the length of the isthmus as far as has been examined in the present study. Between the two members of each pair of isthmus muscle cells is one of the pharyngeal glands. Contained within the isthmus are the processes of several pharyngeal neurones (Fig. 5E).

*Isthmus muscles*

The isthmus muscles attach to the lumen lining immediately posterior to the pump chamber. At their anterior tips, the muscles are flattened and noncontractile (Fig. 5E). From the posterior end to the metacorpus to near the most anterior part of the somatic nerve ring, the isthmus muscles are contractile. Medially, the muscles include filaments perpendicular to the walls of the lumen to which they attach, as for dilation (Fig. 5E). Peripherally, the cells are wider in




cross section, especially in the anterior isthmus at the base of the metacorpus (Figs 2A, 6A). Where the cells are wide, they have contractile filaments (not shown) concentric to the basal lamina, suggesting a constrictive function.

*Isthmus marginal cells*

The marginal cells are mostly simple and cylindrical, lining the apices of the luminal wall posterior to the pump chamber (Figs 5E, 6F). At the tapering posterior tip of the metacorpus, posterior to the pump

**Figure 6.** Three-dimensional reconstruction of the metacorpus and anterior isthmus of *Aphelenchus avenae* based on serial transmission electron microscopy sections. Top of figure is anterior in all models. Colour key to cells is given in G. Neurones are rendered with 'rough' texture; glands, with 'wrinkled' texture. A, ventral view of isthmus cells. Within metacorpus (proper metacorpus cells omitted for clarity), isthmus radial and marginal cells line luminal cuticle anterior to base of pump chamber. Ampullae of subventral glands associate ventrally, peripheral to marginal cell. Arrow indicates gland orifice at anterior end of longitudinal canal. Anterior processes of motor neurone M3 neurones terminate at subventral gland orifices, at base of pump chamber. Contractile regions of isthmus radial cells connect peripherally to form a ring of constrictive muscle in anterior isthmus. B, oblique dorsal view of dorsal gland and adjacent neurone processes M1 and interneurone I3. Neurones lie peripheral to gland posteriorly and cross the gland ampulla anteriorly. From metacorpus, M1 extends anteriad into procorpus. C, ventral view of neurones within pharyngeal metacorpus, including all neurones with cell bodies in the corpus. Neurone processes and connections within commissure are not presented herein. Somatic interneurones RIP enter metacorpus anteriorly and subventrally to connect to pharyngeal nervous system. Asterisk indicates isthmus process of M3 neurone. D, ventral view of RIP connections to posterior process of procorpus radial cell and neurones I1 and I2. Connection of I2 and RIP is immediately anterior to cell body of I2. Asterisk indicates radial cell envelopment of RIP. E, ventral view of marginal cell (MC) neurone termini near lumen lining, between pump and posterior valve muscle cells. Ventral marginal cells and most ventral of subventral pump cells are omitted for clarity. Metacorpus marginal cell shown lining luminal cuticle medial to pump cells, between valve and pump chamber. F, ventral view of isthmus marginal cells and neurosecretory motor (NSM) neurones within metacorpus. Transverse expansions of isthmus marginal cells are shown. Ventral, transverse processes of NSM cross each other through peripheral invagination of ventral isthmus marginal cell (arrow). G, key to cells in Figures 2, 4, and 6. *Colour code for constraining muscles applies to all figures except Figure 3A, in which other colours (red and brown) are additionally used. Abbreviations: dg, dorsal pharyngeal gland; i1, interneurone I1; i2, interneurone I2; i3, interneurone I3; im, isthmus marginal cell; ir, isthmus radial cell; m1, motor neurone M1; m3, motor neurone M3; m4, motor neurone M4; mc, marginal cell neurone MC; mi, motor-interneurone MI; mm, metacorpus marginal cell; mp, metacorpus pump muscle; ns, 'neurosecretory' motor neurone NSM; pc, metacorpus pump chamber; pl, procorpus lumen; pr, procorpus radial cell; pv, posterior DGO valve muscle; rip, somatic interneurone RIP; sg, subventral pharyngeal gland; v, metacorpus luminal valve.

muscles and commissure, the cells expand transversely (Fig. 6F). The cytoplasm of the marginal cells contains prominent bands of tonofilaments where the cells attach to the luminal apices (Fig. 5E). The nuclei of the marginal cells are located near the anterior part of the somatic nerve ring.

### Pharyngeal neurones

Several classes of neurones, including all those with cell bodies in the metacorpus, have been identified in the corpus. Names of neurones were assigned based on putative homologies with *C. elegans* (Albertson & Thomson, 1976). Except for in the commissure, neuronal processes and cell bodies in the corpus are located in the dorsal and subventral margins. All neurones with cell bodies anterior to the commissure have posterior processes that enter the commissure, although complete circuits and synapses of cells therein were not mapped in the present study. Information of neural connectivity in general is limited and requires further characterization. In the dorsal margins between the metacorpus pump muscles are cell bodies of three neurones, positioned from anterior to posterior: motor-interneurone MI, interneurone I3, and motor neurone M4 (Figs 2B, 6C). In both subventral margins, there are cell bodies of five neurones: interneurone (I) I1, I2, 'marginal cell' (MC) motor neurone, neurosecretory motor (NSM) neurone, and motor (M) neurone M3 (Figs 2B, 6C).

The most anteriorly terminating neurone is the dorsal motor neurone M1. In the metacorpus, this neurone lies, together with another neurone (I3), along the dorsal margin of the dorsal gland (Fig. 6B). At the anterior end of the gland, the neurone lies in a groove in the right side of the ampulla. Anteriorly the neurone crosses over the ampulla to a position between the dorsal procorpus radial cell and the right dorsal constraining muscle (Fig. 3D). In the procorpus, anterior to the constraining muscles, the neurone lies between the right, subdorsal marginal cell and the dorsal radial cell (Fig. 2C); it terminates in the stylet region (Ragsdale *et al.*, 2008). The cell body for this neurone was not observed in the corpus.

The most anterior neurone cell body in the dorsal margin of the metacorpus, at a level just anterior to the pump chamber, belongs to motor-interneurone MI (Figs 2B, 6C). This neurone is unipolar (Fig. 6C), with a posterior process that enters the commissure. Along this posterior process, between the cell body and the commissure, MI is closely associated with neurones M1 and I3 (Fig. 6C).

The cell body for interneurone I3 is at the level of the posterior half of the pump chamber (Figs 2B, 6C). The neurone is bipolar (Fig. 6C), with an anterior

process that lies along the distal margin of the dorsal gland, and, more anterior, in a groove in the right side of the gland ampulla next to the process of M1 (Fig. 6B); I3 and M1 lie close together (Figs 3D, 6B, C) for much of their length. At the anterior margin of the gland, the neurone crosses over the ampulla (Figs 3D, 6B) to meet the lumen lining. Medial to the dorsal gland, the neurone process is robust and extends shortly posteriad. The neurone forms junctions with the anterior valve muscles (not shown) and constraining muscles (Fig. 3E). It terminates in contact with the lumen lining, between the two dorsal constraining muscles, immediately anterior to and to the (left) side of the DGO (Fig. 4C).

At the level of the commissure, near the posterior end of the metacorpus, is the cell body for motor neurone M4 (Figs 2B, 6C). This neurone contacts I3 at the anterior end of its cell body (not shown). At the posterior end of its cell body, it extends two processes laterally, left and right, into the commissure (not shown).

The most anterior pair of subventral neurone cell bodies, at the level of the DGO, belongs to the I1 interneurones (Figs 2B, 6C). These cells have very short extensions that terminate in connections with somatic interneurones (Fig. 6D), with which they synapse (not shown). The posterior process is much longer, running through most of the metacorpus before entering the commissure.

The I2 interneurones (Fig. 6C) have their cell bodies at the level of the anterior part of the pump chamber (Fig. 2B). These neurones are more distinctly bipolar, with their anterior processes also forming synapses with somatic interneurones (Fig. 5F). The processes of I2 extend further anteriad than those of I1 and abut upon the pharyngeal lumen lining subventrally (Figs 4A, 6D); they terminate between adradial cells within each subventral pair, at the border of the constraining muscles and anterior valve muscles (Fig. 5A). The termini of I2 connect to the constraining muscles with junctions close to the lumen lining (Fig. 5A).

The cell bodies for the MC neurones are at the level of the posterior part of the pump chamber (Fig. 2B). The MC neurones have anterior processes (Figs 5C, 6C) that terminate close to or possibly on the luminal cuticle, between the posterior valve muscles and the metacorpus marginal cells and immediately medial to the subventral pump muscles (Fig. 6E). Each MC neurone also has a posterior process (Fig. 6C) that enters the commissure.

Posterior to the pump chamber, at the level of the commissure, are the cell bodies of the homologues of the NSM neurones (Figs 2B, 6C). These cells each have a process directing medially, toward the commissure. On the ventral side, the processes of this pair of cells cross each other through a groove in the ventral isthmus marginal cell (Fig. 6F). Each connects to both subventral isthmus muscles on its respective side and possibly those on the opposite side (not shown). Other branching of the neurone processes seems apparent at the commissure but is unclear. Presence of neurosecretory elements in cytoplasm is unclear.

At the posterior end of the metacorpus, just posterior to the commissure, are the cell bodies for the M3 motor neurones (Figs 2B, 6C), which are adjacent to the subventral glands. These neurones have anterior processes that extend anteriad along the distal margins of the glands (Fig. 6A). Further anterior, they expand at their termini, surrounding the cuticle of the subventral gland ducts and abutting against the pharyngeal lumen lining at the gland orifices (Fig. 6A). The anterior processes also have dorsal branches that enter the commissure (not shown). Possible synapses with the pump muscles are not clear from present data. In addition, the M3 neurones have posterior, subventral processes that extend along the distal margins of the isthmus (Fig. 6C), where they terminate.

At the anterior cusp of the metacorpus, two somatic neurones, putative RIP, enter the pharynx subventrally (Fig. 6C), crossing the basal lamina. The neurones both enter between two adradial constraining muscles (Fig. 3D, E) and extend posteriad. The neurones articulate with grooves in posterior processes of the subventral procorpus radial cells (Figs 5B, F, 6D), possibly connecting to them by gap junctions (Fig. 5B), posterior to which the radial cells terminate. Posterior to this, each RIP interneurone synapses with anterior processes of interneurones I2 (Fig. 5F) and I1 (not shown) and terminates in its connection with the latter (Fig. 6D).

## DISCUSSION

Components of the pharyngeal corpus, as revealed by 3D TEM reconstruction, show a high degree of conservatism with outgroups. Cell-for-cell correspondence, connectivity between cells, and singular aspects of individual cell morphology are largely consistent with other Tylenchomorpha as well as the more distant taxon *C. elegans*. As previous studies of pharyngeal cells in Rhabditida have supported such conservation of characters to be informative from only a few specimens, and because of the extensive series required for pharynx reconstruction, our hypotheses are based on a limited sample size. We regard our results as hypotheses to be further tested by the addition of more specimens and further character discovery in closely related Tylenchomorpha.

### HOMOLOGIES OF THE PHARYNX WITH RESPECT TO *CAENORHABDITIS ELEGANS*

Complete reconstruction of the pharynx of *C. elegans* (Albertson & Thomson, 1976) offers an extensive point of reference for assessing homologies of individual pharynx cells of *A. avenae* (Fig. 7). Hypotheses of homology can be proposed based on their position within the series lining the luminal cuticle; relative positions of stomatal and pharyngeal cells are highly conserved (Bumbarger *et al.*, 2006; Ragsdale *et al.*, 2008). Numbers of cells or nuclei within each class, connectivity to other cells including pharyngeal neurones, relationships to distinct cuticular features, positions of nuclei, and cellular morphology serve as evidence to test and often support homology hypotheses. Equal numbers of cells, or at least nuclei in the case of cell fusion, allows necessary tests of conjunction for putative homologues between taxa (Patterson, 1982). To limit the set of hypotheses required, especially between putative homologues that show some divergence in expression, the corpus as an entity is defined by boundaries that are most clearly homologous across taxa.

#### *Definition of the corpus*

Crucial for delimiting the corpus as an arena for more specific tests of homology is establishing homology for the isthmus cells. Previous identification of the homologous arcade syncytia surrounding the stylet (Ragsdale *et al.*, 2008) has already distinguished the anterior boundary to the corpus. The isthmus radial cells therefore provide a landmark enabling predictions of homology for all cells lining the luminal cuticle anteriad, to the border of the corpus with stomatal tissue. Evidence for the homologies of isthmus radial cells is supported by their similar morphology across all taxa examined. Details of the junction between metacorpus and isthmus cells are also conserved: immediately posterior to the pump chamber or central metacorporal lumen are the subventral gland orifices, the anterior tips of the isthmus cells, and the pharyngeal commissure. Similarity criteria thus support homology of the isthmus radial cells with pharyngeal muscle (pm) 'pm5' of *C. elegans* as well as other taxa for which this cell has been identified, including the tylenchid *sensu stricto Basiria gracilis* (Baldwin *et al.*, 2001) and other outgroups in the class Chromadorea (Zhang & Baldwin, 1999, 2000, 2001). A posterior set of marginal cells that associates only with the isthmus radial cells anteriorly in *A. avenae* is consistent with marginal cell mc2 in *C. elegans*, further supporting the isthmus as a homologous landmark across taxa.

The corpus of *A. avenae* comprises six sets of radial cells and two sets of marginal cells line the luminal

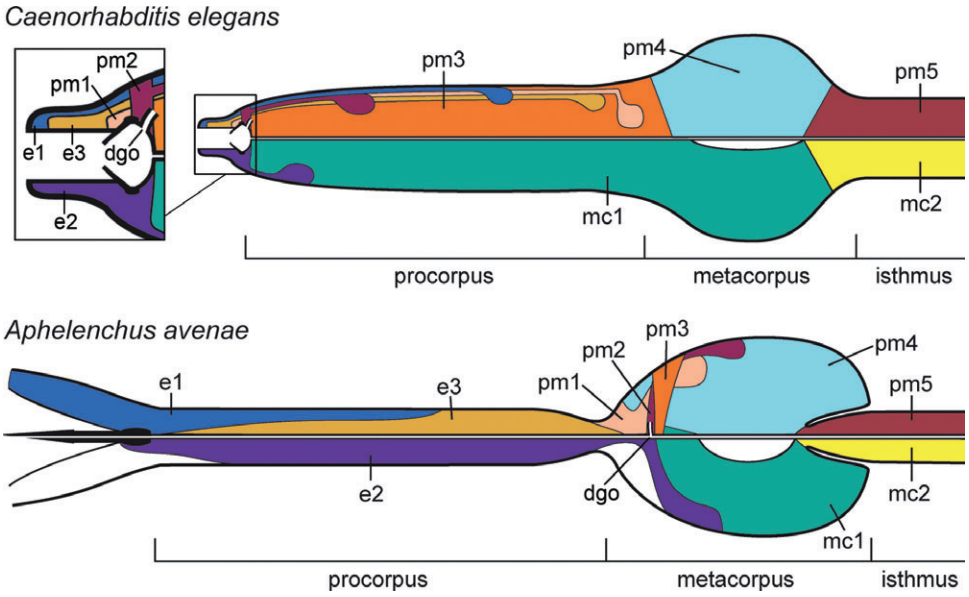



**Figure 7.** Diagrammatic representation of the pharyngeal corpus in *Caenorhabditis elegans* and *Aphelenchus avenae*, showing hypotheses of homology for radial and marginal cells. The top of each diagram shows the cells lining the cuticle of the pharyngeal lumen dorsally; the bottom shows the cells lining the luminal cuticle ventrally. Colours correspond to those used in reconstruction models in Figures 2, 4, and 6 (with colour key in Fig. 6). Drawing of *C. elegans* informed by Albertson & Thomson (1976) and De Ley *et al.* (1995). Abbreviations: dgo, dorsal gland orifice; e1–3, 'epithelial' cells 1–3; mc1–2, marginal cells 1–2; pm1–5, pharyngeal muscle cells 1–5.

cuticle. This is exactly the number of cell classes between the arcade syncytia and pm5 of *C. elegans* (Table 1; Fig. 7), providing a putative cell-for-cell correspondence in the pharyngeal corpus that can then be tested by other criteria.

*Procorpus*

Equating the series of corpus cells across examined taxa suggests that the stylet protractors and procorpus radial cells in *A. avenae* are the homologues of epithelial cells (e) e1 and e3, respectively, in *C. elegans*. Numbers of cells and nuclei, three for each class, are equal in both taxa. Positions of the nuclei of these cells within the procorpus are highly similar between *A. avenae* and *C. elegans*, with those of the anterior set of cells (e1) being located in the middle of the procorpus and those of the posterior set (e3) being at the posterior end of the procorpus. However, the margin of the lining of cells e1 and e3 along the lumen, as well as the volumes of the cells, differs greatly between the two nematodes; in *A. avenae*, these cells comprise the entire procorpus. Another obvious difference is the expression of 'e1' as a muscle cell as opposed to an epithelial cell in *A. avenae*, which challenges similarity based on cell function. This difference in cell expression, in addition to developmental studies of the pharynx, was previously interpreted to reject this homology statement, originally proposed by De Ley *et al.* (1995), in favour of considering the most anterior muscle cells of Rhabditida ('$m_a$' and pm1) to be homologous and excluding e1 and e3 (Dolinski *et al.*, 1998). Although the expression of e1 as epithelial appears to be unique to Rhabditomorpha and Diplogasteromorpha, the present hypothesis for homologies of the anterior two classes of cells in the pharynx is consistent with that of De Ley *et al.* (1995). The expression of e3 as epithelial is present in both *A. avenae* and *C. elegans*, although this differs from examined cephalob taxa where the putative homologue ('$m_b$') is muscular (Van de Velde *et al.*, 1994; Baldwin & Eddleman, 1995; De Ley *et al.*, 1995).

The marginal cells of the procorpus and stomatostylet apparatus in *A. avenae* are hypothesized to be the homologues of the e2 epithelial cells of *C. elegans*. Similarly to cells e1 and e3 in *A. avenae*, e2 lines the pharynx for a much greater length than in *C. elegans*, persisting throughout the procorpus and for the anterior part of the metacorpus. Given the margin of contact of e1 and e3, this finding is consistent with *C. elegans* in that e2 cells are interspersed between individuals of both sets e1 and e3. The position of the nuclei of e2 is posterior in *A. avenae* as compared to *C. elegans*, where they are in the anterior part of the metacorpus. In *A. avenae*, putative e2 cells form gap junctions with two additional classes of radial cells posterior to e3, thus being associated with the most anterior four layers of pharyngeal cells; this is consistent with the putative homologues of e2 in free-living outgroups, including *C. elegans* (De Ley *et al.*, 1995) and several Cephalobomorpha (De Ley *et al.*, 1995; Dolinski *et al.*, 1998). A consequence of this hypothesis is that the stoma or buccal cavity of *C. elegans* and other free-living Rhabditida is equivalent to the basal knobs of the stylet, all of the procorpus, and the anterior part of the metacorpus of *A. avenae*.

*Metacorpus*

Posterior to the e3 cells, the metacorpus comprises the proposed homologues of pharynx muscles (pm) 1–4. Although numbers of individual cells vary between *A. avenae* and *C. elegans* for each class, the total number of nuclei in every class is six for both taxa (Albertson & Thomson, 1976). Putative homologues of at least pm1 and pm2 also have six processes or nuclei per class in representatives of Cephalobomorpha (De Ley *et al.*, 1995; Dolinski *et al.*, 1998) and Panagrolaimorpha (De Ley *et al.*, 1995) and in the possibly more distant outgroup *Myolaimus byersi* (Giblin-Davis *et al.*, 2010).

The homologues of the most anterior set of metacorpus radial cells in *A. avenae*, the constraining muscles, are proposed to be pm1 of *C. elegans*. In *C. elegans*, this class of cells has been described as a single syncytial ring in the stoma (Albertson & Thomson, 1976), although the presence of only a single syncytium has been challenged (De Ley *et al.*, 1995; Dolinski & Baldwin, 2003). The pm1 cells of *C. elegans* have posterior processes extending to cell bodies containing six nuclei. Despite the difference in where these cells line the luminal cuticle between the two taxa, in both cases they have their nuclei in processes posterior to the contractile part of the cell(s).

The anterior valve muscles of *A. avenae* are hypothesized to be homologous with pm2 cells of *C. elegans*. The pm2 cells of *C. elegans* are a set of three radial syncytia that line the posterior region of the stoma and have posterior cell bodies within the procorpus. The positions of putative pm2 nuclei relative to those of other radial cells are highly divergent between the two taxa: they are the most anterior of the pm cells in *C. elegans* but in *A. avenae* they are the second most posterior pm cells. However, as with pm1, putative pm2 in both taxa are characterized by posterior processes extending to cell bodies. Corroborating the hypothesis of the anterior valve muscles as homologous to pm2 is the position of the DGO between these cells in *A. avenae*. The DGO is consistently located in the fourth layer of cells of free-living Rhabditida outgroups, including several Cephalobomorpha and Panagrolaimorpha (Van de Velde *et al.*, 1994; Baldwin & Eddleman, 1995; De Ley *et al.*, 1995), the

**Table 1**. Hypotheses of homology for pharyngeal radial and marginal cells in *Caenorhabditis elegans* and *Aphelenchus avenae*, extended to several representatives of 'tylenchids *sensu stricto*' (non-aphelenchid Tylenchomorpha) and *Aphelenchoides blastophthorus*

| Name of homologue | No. of cells (nuclei) in *C. elegans* | No. of cells/nuclei in *A. avenae* | Cell description in *A. avenae* | Proposed homologue in tylenchids *sensu stricto* | Proposed homologue in *Aphelenchoides blastophthorus* |
|---|---|---|---|---|---|
| e1 | 3 | 3 | Stylet protractor muscles | Stylet protractor muscles | Primary stylet protractor muscles |
| e2 | 3 | 3 | Stylet/procorpus marginal cells | Stylet/procorpus marginal cells | Stylet/procorpus marginal cells |
| e3 | 3 | 3 | Procorpus radial cells | Thin epithelia of 'central core' of procorpus; also 'secondary muscle elements'? | Secondary stylet protractor muscles |
| pm1 | 1 (6) | 6 | Metacorpus constraining muscles | Thin epithelia lining lumen anterior to DGO; part of 'central core' of procorpus | Metacorpus constraining muscles |
| pm2 | 3 (6) | 6 | Anterior DGO valve muscles (metacorpus) | Thin epithelia lining lumen around DGO; part of 'central core' of procorpus | *Muscles anterior to DGO valve muscles? |
| pm3 | 3 (6) | 6 | Posterior DGO valve muscles (metacorpus) | 'Constraining muscles' (posterior procorpus); part of 'central core' of procorpus | DGO valve muscles (metacorpus) |
| pm4 | 3 (6) | 6 | Metacorpus pump muscles | Metacorpus pump muscles | Metacorpus pump muscles |
| pm5 | 3 (6) | 6 | Isthmus radial cells | Isthmus radial cells | *Isthmus radial cells? |
| mc1 | 3 | 3 | Metacorpus marginal cells | Metacorpus marginal cells; part of 'central core' of procorpus | Metacorpus marginal cells |
| mc2 | 3 | 3 | Isthmus marginal cells | Isthmus marginal cells | *Isthmus marginal cells? |

*Not described in text of original study (Shepherd *et al.*, 1980).
e1–3, 'epithelial' cells 1–3; DGO, dorsal gland orifice; mc1–2, marginal cells 1–2; pm1–5, pharyngeal muscle cells 1–5.

diplogasterid *Aduncospiculum halicti* (Baldwin *et al.*, 1997), *C. elegans* (Wright & Thomson, 1981), and the possibly more distant outgroup *Myolaimus byersi* (Giblin-Davis *et al.*, 2010). Thus, whereas the position of the DGO in the corpus is dramatically different between *A. avenae* and *C. elegans*, it is essentially unchanged in its relationship to surrounding cells (Fig. 7).

The hypothesized homologues of the posterior valve muscles in *A. avenae* are the pm3 of *C. elegans*, which in the latter nematode constitute the entire procorpus. Yet despite the difference in cell length along the pharynx, the nuclei of the cells in both taxa are located within the contractile part of the cell and not in posterior cell bodies.

The metacorpus pump muscles of *A. avenae* are most likely to be the homologues of pm4 in *C. elegans*. Aside from this class of cells being a set of three syncytia in *C. elegans*, morphology of these cells is highly similar in both taxa. These muscles constitute most of the swelling characteristic of the metacorpus, insert on thickenings in the luminal cuticle, and have the most posterior nuclei of all corpus radial and marginal cells.

The metacorpus marginal cells (mc) of *A. avenae* are the putative homologues of marginal cell mc1 in *C. elegans*. In both taxa these three morphologically similar cells contain nuclei near their posterior ends, near the level of the metacorpus pump chamber. The homology of these cells also supports the hypotheses of homology for pm3 and pm4, which are radially interspersed with exactly these two sets of cells in both taxa. Unique to *A. avenae* is that the putative mc1 marginal cells surround the luminal cuticle exclusively between the insertion points of putative pm3 and pm4.

A discrepancy in equating the series of metacorpus radial cells to pm1–4 of *C. elegans* is in the relative positions of nuclei within the metacorpus. In particular, the position of the pm2 nuclei, which are the most anterior set of pm nuclei in *C. elegans*, is in the anterior half of the procorpus; in *A. avenae*, these are at a position much further posterior, between the nuclei of putative pm2 and pm4. Aside from the positions of pm2 nuclei, the relative positions of the other radial cell nuclei to one another are relatively conserved with *C. elegans*, with pm3 most anterior and pm4 most posterior, although the positions of these relative to epithelial cell nuclei is more variable between taxa. Furthermore, all nuclei except those of putative e1 and e3 are confined to the metacorpus, whereas in *C. elegans* they are distributed along the entire corpus. This observation is not surprising if the metacorpus and most of the procorpus of *C. elegans* are considered homologous to the metacorpus of *A. avenae*. Apparently more conserved are the positions

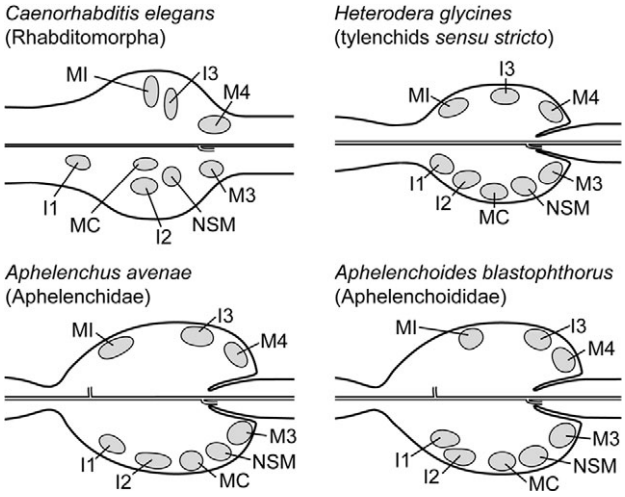



**Figure 8.** Diagrammatic representation of the pharyngeal corpus of *Caenorhabditis elegans* and three different Tylenchomorpha showing positions of pharyngeal neurone nuclei. Neurone homologies in *Aphelenchus avenae* are based on similarity to *C. elegans* in cell body position, dendrite topology, and connectivity. Homologies in *Heterodera glycines* and *Aphelenchoides blastophthorus* are proposed based on positions of nuclei as described by Endo (1984) and Shepherd *et al.* (1984), respectively. The top of each diagram shows dorsal neurone nuclei; the bottom is one of the two subventral rows of nuclei. Diagram of *C. elegans* redrawn from Chiang *et al.* (2006); nucleus positions in *Aphelenchoides blastophthorus* redrawn from Shepherd *et al.* (1984). Abbreviated names of neurones are consistent with Albertson & Thomson (1976). Abbreviations: I1–3, interneurones 1–3; M1, M3, M4, motor neurones 1, 3, 4; MC, marginal cell neurone; NSM, neurosecretory motor neurone.

of nuclei with respect to their own cells; namely, pm1 and pm2 have their nuclei in posterior processes, whereas pm3 and pm4 lack such processes and contain their nuclei within the contractile part of the cell. If homologies of pharyngeal radial and marginal cells can be most reliably based on their position lining the cuticle of the pharyngeal lumen, as results herein suggest, the positions of nuclei raise interesting questions of cell extension during development.

*Pharyngeal neurones*

Relative positions of neuronal nuclei are highly conserved across representatives of Rhabditomorpha, Diplogasteromorpha, Cephalobomorpha, and Panagrolaimorpha (Chiang *et al.*, 2006), and thus provide a strong basis for assigning neurone identities in *A. avenae* (Fig. 8). Although neurones and their interconnections were not fully reconstructed in the present study, many details are apparent that support homology predictions. Findings for *A. avenae* confirm con-

servatism of the relative positions of all nuclei as predicted for the corpus. Polarities of all neurones identified are also consistent between taxa. In the context of the entire pharyngeal corpus, identification of homologous neurones provides additional tests of similarity to other classes of cells to which they connect, including all four classes of pm cells. Descriptions herein of putative neurone homologues are, unless otherwise stated, of features common to both *A. avenae* and *C. elegans*. Comparison with *C. elegans* is based on reconstruction of the pharyngeal nervous system by Albertson & Thomson (1976).

*Motor neurones:* Motor neurone M1 has the most anterior terminus of the dorsal pharyngeal neurones. Its slightly asymmetrical position between the dorsal radial cells and right subdorsal marginal cells is conserved. Although this cell is associated with the dorsal pm1 cells, no ventral circling of the terminus was observed in *A. avenae*. Divergence in the morphology of this neurone in *A. avenae* with respect to *C. elegans* is particularly striking in its anterior branch. The anterior process extends into the procorpus and terminates with prominent swelling, including a process into the dorsal stylet protractor muscle (e1). The exaggerated presence of microtubules in its terminus in *A. avenae* (Ragsdale *et al.*, 2008) suggests a distinct modification of function of this neurone, perhaps to include mechanoreception (e.g. Perkins *et al.*, 1986). The additional association of this neurone with homologues of the e1, as assigned herein, is also novel in *A. avenae*.

The morphology of the M3 motor neurones is complex, with two processes, the anterior of which has multiple branches, patterns of which in at least *C. elegans* vary amongst individuals. Consistent in *A. avenae* and various individuals of *C. elegans* is an anterior, subcuticular terminus at the subventral gland orifices. Also conserved is the dorsal circling of the anterior process as well as a branch extending into the subventral neurone bundles. The posterior processes are simple, extending partially into the isthmus along the distal, subventral margins.

The single M4 motor neurone, the cell body of which is at the level of the commissure, extends two lateral processes ventrally into the latter.

The MC motor neurones have anterior processes that terminate subventrally against or near the cuticle of the lumen, immediately posterior to the pm3 muscle cells. Whereas in *C. elegans* the termini are directly between the insertions of pm3 and pm4, this configuration is precluded in *A. avenae* by the complete enclosure of the luminal cuticle by the metacorpus marginal cells (mc1) posterior to putative pm3. Notwithstanding the peculiarity of the marginal cells in *A. avenae*, the termini are between the same two sets of muscles, pm3 and pm4, given the present assignments of homology.

*Interneurones:* The I1 interneurones are bipolar, with an anterior process extending to connect to the somatic input neurones, the RIP interneurones, at which point they terminate. In *A. avenae* these anterior processes are diminutive, possibly because of the short distance between the I2 cell bodies and the point of entry of the RIP into the pharynx. A difference observed in these putative homologues is the lack of subcuticular anterior termini in *A. avenae*.

Interneurones I2 have the most anterior subventral termini, which prominently abut against the cuticle and attach to the subventral pm1 muscles, supporting the homology of the latter. Unique to *A. avenae* is the connection of the anterior processes of I2 to the RIP interneurones.

The I3 interneurone has an anterior process that terminates subcuticularly, immediately anterior to the DGO. Furthermore, the anterior process inserts on two sets of adjacent, dorsal radial cells, the putative homologues of pm1 and pm2, supporting homology of the latter with the metacorpus constraining muscles and anterior valve muscles, respectively.

*Other neurones:* From the cell body of each NSM neurone, a single process enters the commissure, where a branch crosses ventrally to the other lateral side and connects to subventral isthmus muscle cells on both sides. Association of NSM with pm5 is conserved. The motor-interneurone MI has a single posterior process that enters the commissure. The connections of this neurone in *C. elegans* are known to be variable amongst individuals, but they are apparently consistent in including those to the other dorsal neurones of the corpus, M1 and I3, anterior to entering the commissure; associations with putative homologues of the latter are observed in *A. avenae*. The entry points of the somatic RIP neurones with respect to muscle cells lend support to homology of pm1 with the constraining muscles.

## Comparison with the pharynx in tylenchids *sensu stricto*

Regardless of an extensive body of detailed, insightful ultrastructural studies of the pharynx in tylenchids *sensu stricto* (Yuen, 1968; Byers & Anderson, 1972; Chen & Wen, 1972, 1980; Anderson & Byers, 1975; Baldwin & Hirschmann, 1976; Grootaert *et al.*, 1976a, b; Shepherd & Clark, 1976, 1983; Baldwin, Hirschmann & Triantaphyllou, 1977; Baldwin, 1982; Endo, 1984; Hussey & Mims, 1990; Souza & Baldwin, 1998; Baldwin *et al.*, 2001), no complete reconstruction of the pharynx or pharyngeal corpus is yet available for

any representative. Re-examination of the literature provides some clues as to cell-for-cell correspondence, if one exists, although complete reconstruction of the corpus is required to test more fully hypotheses of homology. Fine structure of the pharynx is discussed for all tylenchids *sensu stricto* under the assumption that numbers and positions of cells are the same across the group. A review of published and unpublished micrographs (J. G. Baldwin, unpubl. data) confirms a lack of obvious discrepancies amongst most ingroups. Based on the sound monophyly of tylenchids *sensu stricto* (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Smythe *et al.*, 2006; Meldal *et al.*, 2007; Bert *et al.*, 2008), and considering the general conservation of pharynx characters shown between *A. avenae* and the distant outgroup *C. elegans*, major differences within the group are not expected. A notable exception is the nematode *Hexatylus viviparus*, which has a completely nonmuscular pharynx (Shepherd & Clark, 1976); a nonmuscular pharynx is consistent with gross observations of other Sphaerularoidea nematodes. The presence of such a pharynx within some Tylenchomorpha provides additional evidence for the plasticity of expression as muscular or nonmuscular, such as in the case of e3 across various Rhabditida representatives (Table 1). Yet because of great divergence in tissue expression, and given the lack of knowledge of individual cells, reliable homology statements cannot be made for the corpus of *H. viviparus*. The interesting case of Sphaerularoidea, including *H. viviparus*, is therefore omitted from the present discussion.

*Procorpus*

The procorpus of representatives of tylenchids *sensu stricto* contains many nuclei. Although some of these nuclei can be attributed to specific cells, a complete count of nuclei for the entire corpus is still relevant for tests of conjunction. The complexity of membrane complexes and posterior processes in the procorpus makes it difficult or impossible to deduce precise numbers of cells from existing data. Nevertheless, micrographs and observations of past studies enable hypotheses subject to more rigorous testing; further character sampling should be driven explicitly to discover numbers and positions of individual cells.

*Primary stylet protractors (e1):* The most anterior cells of the pharynx in tylenchids *sensu stricto*, the primary stylet protractor muscles, are identical in appearance to those in *A. avenae* at their anterior end, including their patterns of branching, their articulation with the arcade syncytia, and attachments to the stylet and cephalic framework cuticle (Ragsdale *et al.*, 2008). Furthermore, their nuclei are located in the procorpus as in *A. avenae*, albeit at a more anterior position (Yuen, 1968; Byers & Anderson, 1972; Anderson & Byers, 1975; Grootaert *et al.*, 1976a; Baldwin *et al.*, 1977; Baldwin, 1982; Shepherd & Clark, 1983; Endo, 1984).

*Procorpus radial cells (e3):* Although homologues of 'e3' in tylenchids *sensu stricto* are ambiguous, they may comprise secondary stylet protractor muscles. A set of 'secondary muscle elements' has been explicitly described for several species of tylenchids *sensu stricto*. The secondary muscle elements are a set of four diminutive strips of muscle attaching posterior to the stylet (Anderson & Byers, 1975; also apparent in Chen & Wen, 1972). These cells seem to have processes extending further posteriad into the procorpus (Endo, 1984), although the numbers and positions of their nuclei are unknown. The suggestion that the two dorsal strips are only one cell (De Grisse, 1977) would be consistent with the prediction of three cell bodies for e3. The expression of e3 cells as muscular, as opposed to epithelial, would contrast with *A. avenae* and *C. elegans*. However, such an expression would be similar to other free-living outgroups such as Cephalobomorpha and Panagrolaimorpha (Van de Velde *et al.*, 1994; Baldwin & Eddleman, 1995; De Ley *et al.*, 1995) as well as *Myolaimus byersi* (Giblin-Davis *et al.*, 2010).

*Stylet/procorpus marginal cells (e2):* The marginal cells lining the stylet knobs between the stylet protractor muscles, putative e2, have nuclei in the anterior procorpus (Anderson & Byers, 1975; Grootaert *et al.*, 1976a; Shepherd & Clark, 1983; Endo, 1984). The presumed posterior extent of the marginal cells along the lumen, implied by the anterior location of their nuclei, is consistent with *A. avenae* and *C. elegans* in being anterior of the DGO.

*Radial cells pm1 and pm2:* Under the hypothesis that DGO position is conserved relative to the lining of radial cells along the lumen, two sets of radial cells (pm1 and pm2) are predicted to line the luminal cuticle anterior to the DGO in addition to the primary and 'secondary' stylet protractors. Lack of complete reconstruction of the procorpus in tylenchids *sensu stricto* precludes the enumeration of corresponding cells necessary for tests of conjunction. However, the evidence for several cells in sequential layers in the anterior procorpus provides some support for the presence of homologues of all cell classes anterior to the DGO.

A set of cells is shown narrowly lining the luminal cuticle anterior to the DGO and an adjacent neurone in *Heterodera glycines* juveniles (Endo, 1984). Conserved spatial relationships would predict this class of cells to be homologous with pm2. Several layers of thin cells separated by junctional

complexes serially lining the cuticle of the procorporal lumen anterior to the DGO can be observed in *Tylenchorhynchus dubius* (Anderson & Byers, 1975), *Meloidogyne incognita* males (Baldwin & Hirschmann, 1976) and females (Hussey & Mims, 1990), and *He. glycines* males (Baldwin *et al.*, 1977). If these cells comprise pm1 and pm2, their expression would differ from their putative homologues in *A. avenae* and *C. elegans* by not being expressed as muscles. The many thin, nonmuscular cells of the anterior procorpus in tylenchids *sensu stricto* have been explicitly described as a 'lumen complex' (Yuen, 1968; Chen & Wen, 1972), although individual cells comprising it have not been traced. Baldwin *et al.* (1977) suggested that several of these cells were nerve endings. Comparison to pharyngeal neurones in *A. avenae* and *C. elegans* predicts that the number of termini so far anterior would be more limited, especially next to the lumen lining (I3 dorsally and the pair I2 subventrally). Fewer neurone termini would open the possibility for the presence of thin epithelial processes with an electron-transparent cytoplasm resembling that of neurones.

Many nuclei have been reported in the procorpus of tylenchids *sensu stricto*, lending additional support to the presence of several cell classes therein. Posterior to the nuclei of the stylet protractors and marginal cells ('stylet shaft tissue'), Endo (1984) counted ten additional nuclei in the procorpus of juvenile *He. glycines*: five in the middle procorpus and five in the posterior procorpus. In a study of *Hoplolaimus* sp., 14 cell bodies were found in the posterior procorpus alone, notwithstanding a line drawing indicating only three sets of radial cells for the entire procorpus (Grootaert *et al.*, 1976a). The posterior procorpus nuclei may correspond to e3, pm1, and pm3, although numbers of corresponding cells as counted are not exact. Nuclei in the anterior metacorpus, in the same plane as the adradial pump muscle nuclei, are also apparent in micrographs of *He. glycines* juveniles (Endo, 1984). These nuclei may belong to homologues of pm2, based on their positional similarity to those in *A. avenae*.

*Radial cells pm3:* The 'constraining muscles' of tylenchids *sensu stricto* are putatively the homologue of pm3, the posterior valve muscles of *A. avenae*. Correspondence amongst the same muscles of a tylenchid *sensu stricto*, *Hoplolaimus* sp., and the DGO valve muscles in *Aphelenchoides blastophthorus* was previously proposed by Geraert (1997) in a review of earlier studies on those taxa (Grootaert *et al.*, 1976a, b; Shepherd *et al.*, 1980). In tylenchids *sensu stricto*, the 'constraining' musculature is not as pronounced as in *A. avenae*. These cells additionally have anteriorly directed contractile filaments extending through a substantial part of the procorpus (Endo, 1984), suggesting these cells are much longer in tylenchids than in *A. avenae* and *Aphelenchoides blastophthorus*. The cell boundary between these cells and the pump muscles can be observed at the anterior of the metacorpus, a separation also shown in Yuen (1968) and Grootaert *et al.* (1976b). The homologue of pm3 in tylenchids *sensu stricto* might include the constraining musculature but also extend through a fair portion of the procorpus. A cell-for-cell correspondence predicts this cell class to line the luminal cuticle for the entire procorpus posterior to the DGO. Narrow cells surrounding the lumen are evident from transverse sections in all TEM studies of tylenchids *sensu stricto*. The case that the cells in the centre of the procorpus are the same cells longitudinally would provide evidence for a conserved number of cell classes posterior to the DGO. Anderson & Byers (1975) suggested this arrangement in their study of *T. dubius*, describing those narrow cells as comprising a 'central core' around the lumen. Peripheral to this core were the posterior cell bodies of the more anterior cells (most notably the stylet protractors). The core cells themselves were larger in the posterior half of the procorpus, presumably as cell bodies. Grootaert *et al.* (1976a) suggested a similar configuration, only proposing a central core comprising two serial sets of cells. Furthermore, longitudinal sections through the metacorpus of *Meloidogyne incognita* (Hussey & Mims, 1990) show no cells between constraining muscles (putative pm3) and pump muscles (putative pm4), confirming the posterior boundary of the constraining muscles. The presumed location of the nuclei within the contractile region supports homology with pm3 in *C. elegans* and *A. avenae*.

Spatial relationships between marginal cells and specific classes of radial cells are apparently conserved in *A. avenae* and *C. elegans*. If similar conservation is found in tylenchids *sensu stricto*, then by the above hypothesis of pm3 homology, mc1 is predicted to line the luminal cuticle from the anterior part of the postcorpus (starting at the DGO) to the posterior end of the pump chamber. Supporting this configuration are sections showing the cell bodies of anterior marginal cells (e2) separated from the lumen lining posterior to the DGO (Anderson & Byers, 1975; Grootaert *et al.*, 1976b).

*Metacorpus*

The homologues of the metacorpus muscles in tylenchids *sensu stricto* are most likely to be the pm4 of *A. avenae*. They comprise six pairs of adradial cells in all cases where they can be discerned (e.g. Yuen, 1968; Chen & Wen, 1972; Grootaert *et al.*, 1976b;

Baldwin *et al.*, 1977; Baldwin, 1982; Endo, 1984). The pump muscles are highly similar in morphology and arrangement to those in *A. avenae*. They also share characteristic cytoplasmic features, notably the presence of extensive membranous organelles in their anterior parts (Endo, 1984).

*Pharyngeal neurones*

Two neurones lie along and are enveloped by the dorsal gland in several representatives of tylenchids *sensu stricto* (Anderson & Byers, 1975; Grootaert *et al.*, 1976b; Baldwin *et al.*, 1977; Baldwin, 1982; Shepherd & Clark, 1983; Endo, 1984). These are most likely to be the homologues of neurones M1 and I3, coinciding in position with the same neurones in *A. avenae* and *C. elegans*. In *H. glycines* juveniles, one of these neurones was observed to terminate at the cuticle immediately anterior to the DGO, similar to interneurone I3 in *A. avenae* and *C. elegans*. Identities of other neurones in the procorpus still await more precise characterization of their termini, connectivity, and cell body position.

Enumeration of neurone cell bodies in the metacorpus implicates remarkable conservation with outgroups. In *H. glycines* juveniles, the metacorpus contains two subventral rows of five and a dorsal row of three nuclei, for a total of 13 (Endo, 1984). Thus the numbers and positions of cell bodies in the corpus correspond exactly to those in *A. avenae* and free-living Rhabditida outgroups (Albertson & Thomson, 1976; Chiang *et al.*, 2006). Based on conservation between outgroups, these neurones in tylenchids *sensu stricto* are very likely to be homologous with those neurones in outgroups with corresponding positions (Fig. 8).

## Comparison with the pharynx in Aphelenchoididae

Reconstruction of the pharynx of *Aphelenchoides blastophthorus* (Shepherd *et al.*, 1980) allows detailed comparison of the corpus with that of *A. avenae.* Expression of radial and marginal cells in *Aphelenchoides blastophthorus* enables robust homology proposals for individual cell classes (Table 1). The corpus also contains the same number of radial and marginal cell nuclei as *A. avenae* and *C. elegans*. Examination of nucleus position (Shepherd *et al.*, 1984), in light of corpus reconstruction of *A. avenae*, also implicates an equal number of radial and marginal cells. Corpus nuclei generally occupy corresponding positions in *Aphelenchoides blastophthorus* and *A. avenae*, with the exception of key differences in the procorpus and anterior metacorpus.

*Procorpus*

*Stylet protractor muscles (e1, e3):* The primary (anterior) stylet protractors of *Aphelenchoides blastophthorus* are similar in their insertion points and hexaradiate branching pattern to those of *A. avenae* (Ragsdale *et al.*, 2008) and tylenchids *sensu stricto* (Baldwin & Hirschmann, 1976). Attaching to the stylet knobs posterior to these are a secondary set of robust protractors, the subventral and two adradial branches of which are also similar to tylenchids *sensu stricto*, in spite of their diminution in the latter group (Geraert, 1997). However, the positions of both sets of protractor nuclei in the corpus differ with respect to *A. avenae* and tylenchids *sensu stricto*. The protractors extend as thin processes far posteriad to cell bodies in the anterior metacorpus, but do not line the luminal cuticle as they do in *A. avenae*. Thus the pharyngeal distance separating the stylet and the DGO in *Aphelenchoides blastophthorus* and *A. avenae* is lined by a different arrangement of cells with differing nucleus positions in each. The disparity of cell body position between *Aphelenchoides blastophthorus* and other tylenchids may reflect a difference in the developmental extension of the procorpus, which in *C. elegans* proceeds by the 'pulling' of pharyngeal cells to connect to the stoma (Portereiko & Mango, 2001).

*Stylet/procorpus marginal cells (e2):* As in *A. avenae*, the anterior marginal cells of *Aphelenchoides blastophthorus* line the luminal cuticle into the anterior metacorpus, near but anterior to the DGO. The marginal cell bodies are also in anterior metacorpus in both taxa. A significant difference in expression of these cells in *Aphelenchoides blastophthorus* is that for most of the procorpus they line the luminal cuticle exclusively, with protractor muscles relegated to the periphery. In *A. avenae* and tylenchids *sensu stricto*, both marginal cells and radial cells line the procorpus for its entire length. This configuration implicates a key difference between *Aphelenchoides blastophthorus* and other Tylenchomorpha in development of the procorpus, namely in the cells responsible for the formation of its lumen and subsequent deposition of cuticle (Leung, Hermann & Priess, 1999).

*Metacorpus*

*Constraining muscles (pm1):* The most anterior muscle cells of the metacorpus, putative pm1, are expressed similarly in *Aphelenchoides blastophthorus* and *A. avenae* as constraining muscles, albeit with notable differences. Most significantly, the constraining muscles apparently do not attach to the lumen lining, a feature aberrant in comparison to all other pharyngeal muscles in all other examined Rhabditida. Such topological discrepancies leave open the

possibility of independent derivation of pm3 cells for a similar purpose, such as the stabilization of the metacorpus during pumping (Shepherd *et al.*, 1980).

*Anterior valve muscles (pm2):* Between the levels of the DGO and the pump chamber in *Aphelenchoides blastophthorus* are a set of muscle nuclei of uncertain designation. Although these nuclei were described as a possible second set belonging to the pump muscles, they correspond well to the position of the anterior valve muscles (pm2) of *A. avenae*. In *A. avenae* the nuclei of putative pm3 are continuous with the contractile part of the muscles by way of thin processes. The diminutive nature of these cells would render them easily missed in the case that they exist in *Aphelenchoides blastophthorus*. Furthermore, in *A. avenae* the cell bodies for pm2 are wedged in the middle of each pair of adradial pump muscle cells, in the same longitudinal plane as the nuclei of pm4; this position confounds their distinction as separate cells. It is possible that they comprise the conjectural 'second set' of metacorpus muscles described by Shepherd *et al.* (1980). Several layers of junctional complexes, which are characteristic of radial cells near their sites of insertion on the cuticle, are apparent near the position of the DGO. The indication of a series of narrow cells in this region supports the possibility of at least another set of muscles attaching to the lumen lining anterior to the posterior valve muscles (pm3).

*Posterior valve muscles (pm3):* The putative pm3 cells are highly similar in their expression in *Aphelenchoides blastophthorus* and *A. avenae*, forming a thin set of radial dilators actuating the valve anterior to the DGO. In both cases the nuclei are wedged within the contractile elements of the cells. The absolute positions of the nuclei in both taxa are at about (in *A. blastophthorus*, slightly anterior to) the level of where the cells attach to the lumen lining.

*Metacorpus pump muscles (pm4):* The cells operating the metacorpus pump in *Aphelenchoides blastophthorus* were described as being either binucleate or two sets of cells. Given the possibility of the 'anterior set' of nuclei belonging to a separate class of cells (i.e. pm2), the more posterior set observed would be congruent with the positions of pm4 nuclei in *A. avenae*. The expression of putative pm4 cells as robust pumping muscles is similar to that observed in *A. avenae*, tylenchids *sensu stricto*, and *C. elegans*. The metacorpus pump cells were also described as being continuous with the isthmus cells in *Aphelenchoides blastophthorus*. This observation presents a challenge to homology proposals for isthmus cells. They are highly similar to the isthmus cells in *A. avenae*, including the orientations of their contractile filaments and their position relative to the pharyngeal commissure and subventral gland openings. Given their similarities, the possibility is considered that the isthmus cells, notwithstanding their original description (Shepherd *et al.*, 1980), are distinct from any in the metacorpus. Such a hypothesis is supported by isthmus cell homologies of *A. avenae*, *C. elegans*, and other Chromadorea for which the isthmus has been TEM reconstructed (Albertson & Thomson, 1976; Zhang & Baldwin, 1999, 2000, 2001; Baldwin *et al.*, 2001).

*Metacorpus marginal cells (mc1):* The second set of marginal cells in the corpus, putative mc1, is a set of cells lining the posterior half of metacorpus in both *Aphelenchoides blastophthorus* and *A. avenae*. A possible difference in expression in *A. avenae* may be their exclusive lining of the luminal cuticle in just anterior to the pump chamber, namely, between the insertions of the valve muscles (pm3) and those of the pump muscles (pm4). A similar configuration was not described for *Aphelenchoides blastophthorus*, although extant observations of the metacorpus cannot rule out such an arrangement.

*Pharyngeal neurones*

Although specific identities were not assigned to neurone nuclei in *Aphelenchoides blastophthorus* (Shepherd *et al.*, 1984), the exact same number of cell bodies in similar positions were observed: five in each subventral sector and three in the dorsal sector. These cell positions correspond closely to those in *A. avenae*, tylenchids *sensu stricto* (Endo, 1984), *C. elegans* (Albertson & Thomson, 1976), and several other free-living Rhabditida (Chiang *et al.*, 2006). It is likely that homologies of neurones are those assigned for *A. avenae* based on their morphological and topological conservation with *C. elegans* (Fig. 8).

Neurone processes were not reconstructed for *Aphelenchoides blastophthorus*, although limited data suggest at least some conservation with *A. avenae* and other outgroups. At least one neurone lies in close association with the dorsal gland and extends anteriorly to the level of the stylet protractors between successive dorsal radial cells and the right subventral marginal cell. The position of this process, in addition to its expanded terminus filled with microtubules, shows great similarity to the anterior process of putative M1 in *A. avenae*. Another neurone or pair of neurones forming a 'gustatory loop' surrounding the stylet shaft was also described for *Aphelenchoides blastophthorus*. The identity for this cell or cells as a pharyngeal neurone homologue is puzzling. The description of its position and morphology likens it to one of the minute arcade syncytia as detailed for *A.*

*avenae* (Ragsdale *et al.*, 2008), which similarly form rings in direct contact with the stylet shaft and include posterior processes in corresponding positions (i.e. dorsolateral and ventral). In the metacorpus, two subventral neurones are explicitly shown as contacting the luminal cuticle near the orifices of the subventral glands, close to thin pairs of radial cells resembling the isthmus radial cells in *A. avenae*. These may be the homologues of the M3 neurones in *A. avenae* and *C. elegans*, which would lend support to general conservation of terminus expression as well as cell body positions of the pharyngeal nervous system in Rhabditida.

*Postcorpus*

Given the general degree of conservation of pharynx components in Rhabditida, the apparent lack of basal bulb cells (pm6–pm8) and possibly the isthmus radial (pm5) and marginal (mc3) cells in *Aphelenchoides blastophthorus* is remarkable. Except for *Aphelenchoides blastophthorus*, a full complement of postcorpus cells has been described for all chromadorean taxa thoroughly examined (Albertson & Thomson, 1976; Zhang & Baldwin, 1999, 2000, 2001; Baldwin *et al.*, 2001), including a representative of tylenchids *sensu stricto*. More complete TEM level information of the postcorpus of *A. avenae* will be required to test this possibility. Given the distinct embryonic lineage of the gut from lineages of the pharynx in Rhabditida nematodes (Sulston *et al.*, 1983; Skiba & Schierenberg, 1992), it is unlikely that homologues of pm6–pm8 are expressed as intestinal epithelia. The possibility remains for homologues to be pharyngeo-intestinal junction cells, which in *Aphelenchoides blastophthorus* persist in the alimentary tract as far posteriad as the mid-intestine. Although some nuclei were described as being in the region of the junction, specific locations of the nuclei for these cells, and of isthmus cells if they are separate from the metacorpus, have not been characterized. Given the presence of their posterior extensions, at least some are presumably far posterior, as are the cell bodies of most postcorpus cells in taxa where they have been reconstructed. The large number of small, closely packed 'accompanying cells' observed along the pharyngeal gland lobes in *Aphelenchoides blastophthorus* may include homologues of otherwise 'missing' postcorpus cells. In either of the cases of alternate expression or of true loss of the postcorpus cells, their absence supports the hypothesis for Aphelenchoididae to comprise a distinct lineage with respect to tylenchids *sensu stricto*.

## Implications for phylogenetic congruence

The similarity of all stomatostylet-bearing Tylenchomorpha has long been reflected in their exclusive membership within a single taxonomic grouping (Thorne, 1949). However, phylogenies based on mostly rRNA sequence alignments have challenged the monophyly of the group, splitting it into clades separated by branches including taxa without stylets, notably the free-living Cephalobomorpha and Panagrolaimorpha (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Smythe *et al.*, 2006; Meldal *et al.*, 2007; Bert *et al.*, 2008). This case of incongruence leads to the question of independent evolution of feeding structures necessary for a plant parasitic or similar lifestyle. Structures directly involved in feeding, namely the stylet and the associated pharynx, need to be re-examined for discrepancies which have been interpreted as homology. Moreover, the same phylogenetic studies predict 'Aphelenchoidea' to be paraphyletic, aligning its type species *A. avenae* with tylenchids *sensu stricto*. New hypotheses of phylogeny therefore contradict the use of characters that have been the hallmark for circumscribing 'Aphelenchoidea': namely (1) the position of the DGO within the metacorpus and (2) the structure of the distinctively large metacorpus itself (Fuchs, 1937; Siddiqi, 1980; Hunt, 1993). Characters revealed herein by 3D TEM reconstruction do not uphold monophyly of Tylenchomorpha and offer some support for the rejection of 'Aphelenchoidea.' Taxonomically important characters as traditionally defined are not homologous.

*Reconstruction suggests plesiomorphy of several corpus characters*

Complete TEM reconstruction of individual components of key characters tests their usefulness for informing natural history within Tylenchomorpha. The 'minuteness of resemblance' (Simpson, 1961) of putative cell homologues tests the possibilities of shared ancestry or propensity for convergence. A comparison of representatives of the three deepest lineages of Tylenchomorpha (Aphelenchidae, Aphelenchoididae, and tylenchids *sensu stricto*) reveals symplesiomorphy for several characters of putative corpus homologues (Table 2; Fig. 9). Across taxa is a general conservatism of some characters, namely in the metacorpus: the structure of the metacorpus pump (pm4) muscles, including arrangement of contractile filaments and presence of extensive membranous lamellae in their anterior parts; the structure of the metacorpus pump chamber, including the distinctive three ridges on each radius, the outer two of which are characterized by a distinct type of electron-lucent cuticle using various preparation methods (Shepherd *et al.*, 1980; Shepherd & Clark, 1983; Endo, 1984). Yet the gross structure of the metacorpus is conserved with free-living outgroups as distant as *C. elegans*, and is indicative of symplesiomorphy rather than exclusive shared ancestry. The anterior

**Table 2**. Summary of characters for putative homologues of pharyngeal radial and marginal cells in *Aphelenchus avenae*, other Tylenchomorpha, and several outgroups (key to taxa below)

| Cell class | * | Characters for putative cell class homologues | *Aa* | T | *Ab* | *Ce* | *Zp* | *Ps* | *Ah* | *Mn* |
|---|---|---|---|---|---|---|---|---|---|---|
| e1 | 1 | Cells epithelial (0) or muscular (1) | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| | | Nuclei in posterior extensions (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 2 | Nuclei in procorpus (0) or metacorpus (1) | 0 | 0 | 1 | 0 | ? | ? | ? | ? |
| e3 | 3 | Cells epithelial (0) or muscular (1) | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 |
| | 4 | Stylet protractors weak (0) or robust (1) | N/A | 0 | 1 | N/A | N/A | N/A | N/A | N/A |
| | 5 | Nuclei in procorpus (0) or metacorpus (1) | 0 | 0 | 1 | 0 | ? | ? | ? | ? |
| e2 | | Line lumen as posterior as level of pm2 (1) | 1 | ? | ? | 1 | 1 | 1 | ? | 1 |
| | 6 | Line part of procorporal lumen exclusively (1) | 0 | 0 | 1 | 0 | ? | ? | ? | ? |
| pm1 | 7 | Cells epithelial (0) or muscular (1) | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 8 | Muscles dilatory (0) or 'constraining' (1) | 1 | N/A | 1 | 0 | 0 | 0 | 0 | 0 |
| | 9 | Cells inserting on (0) or detached from (1) lumen | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | Cell bodies posterior to cell lining of lumen (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| pm2 | 10 | Cells epithelial (0) or muscular (1) | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | Nuclei located in posterior processes (1) | 1 | ? | 1? | 1 | 1 | 1 | 1 | 1 |
| | | Cells at position of DGO (1) | 1 | ? | 1 | 1 | 1 | 1 | 1 | 1 |
| pm3 | | Cells epithelial (0) or muscular (1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 11 | Muscles dilatory (0) or 'constraining' (1) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12 | Muscles associated with a luminal valve (1) | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | Nuclei in posterior part of contractile region (1) | 1 | 1 | 1 | 1 | ? | ? | ? | ? |
| pm4 | | Metacorpus pump muscles (1) | 1 | 1 | 1 | 1 | ? | ? | ? | ? |
| | | Nuclei in posterior part of contractile region (1) | 1 | 1 | 1 | 1 | ? | ? | ? | ? |
| mc1 | | Positioned between at least pm4 cells (1) | 1 | 1 | 1 | 1 | ? | ? | ? | ? |
| | 13 | Line part of lumen exclusively (1) | 1 | 0 | 0 | 0 | ? | ? | ? | ? |

Key to taxa: *Aa*, *Aphelenchus avenae*; *Ab*, *Aphelenchoides blastophthorus* (Shepherd *et al.*, 1980); *Ah*, *Aduncospiculum halicti* (Baldwin *et al.*, 1997); *Ce*, *Caenorhabditis elegans* (Albertson & Thomson, 1976); *Mn*, *Myolaimus byersi* (Giblin-Davis *et al.*, 2010); *Ps*, *Panagrolaimus superbus* (De Ley *et al.*, 1995); T, various representatives of tylenchids *sensu stricto*, i.e. non-aphelenchid Tylenchomorpha (see text); *Zp*, *Zeldia punctata* (Baldwin & Eddleman, 1995; De Ley *et al.*, 1995; Dolinski *et al.*, 1998).

e1–3, 'epithelial' cells 1–3; DGO, dorsal gland orifice; mc1, marginal cell 1; pm1–4, pharyngeal muscle cells 1–4.

*Characters different for two or more taxa (not including missing states), mapped in Figure 9.

expression of the primary stylet protractors is also similar in all three taxa (Ragsdale *et al.*, 2008). However, the positions of their cell bodies differ such that the developmental pathways for arriving at that expression may also differ in separate lineages. The similarity of these muscles and the associated stylet across Tylenchomorpha is still puzzling in the context of independent phylogeny, especially in determining their character polarity relative to the starkly different 'e1' muscles of immediate outgroups (Cephalobomorpha, Panagrolaimorpha).

Other corpus cell characters show more divergence amongst lineages of Tylenchomorpha. The 'secondary' stylet protractor muscles (putative e3) differ in robustness between those groups that have them, Aphelenchoididae and tylenchids *sensu stricto*, and are altogether absent as muscles in *A. avenae* (Aphelenchidae). The shared presence of these muscles in Aphelenchoididae and tylenchids *sensu stricto*, exclusive of Aphelenchoididae, does not support any extant hypothesis of phylogeny. Although the expression of the e3 cells as muscular is probably a primitive condition for all three groups, being thus in immediate outgroups, their apparent insertions on the cephalic framework has more elusive ancestral states. The positions of the cell bodies for e1 and e3 differ in all three taxa, but are at least similar between Aphelenchidae and tylenchids *sensu stricto*. Additionally, the lining by e1 and e3 of the procorporal lumen cuticle is similar for these two clades. Given outgroup character states, this lining is probably plesiomorphic with respect to the procorporal lumen cuticle of Aphelenchoididae, which is lined exclusively by the anterior marginal cells (putative e2) for most of its length.

The pm1 cells differ in all three taxa, but are more similar as 'constraining muscles' between 'Aphelenchoidea' than either is with their nonmuscular counterparts in tylenchids *sensu stricto*. Yet outgroup

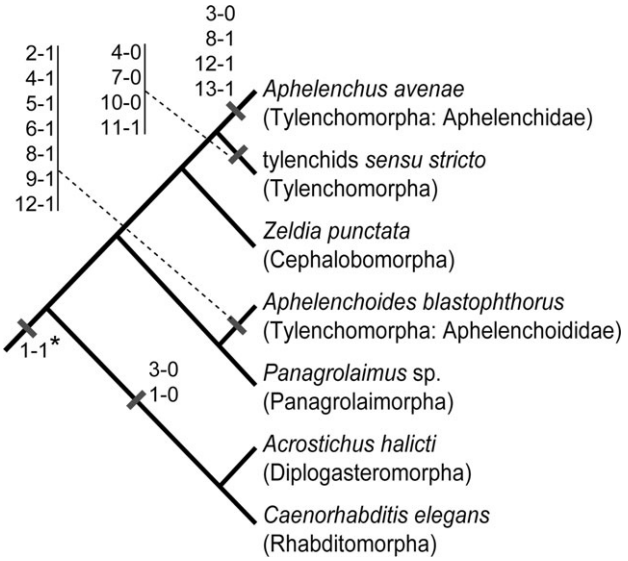



**Figure 9.** Simplified relationships between nematode models with reconstructed sensory anatomies and preliminary mapping of characters based on simple parsimony. Relationships shown are a consensus of published phylogenies inferred from 18S and 28S rRNA sequences (Blaxter *et al.*, 1998; Holterman *et al.*, 2006; Meldal *et al.*, 2007), although placement of the root varies amongst some analyses. Numbers correspond to characters (left of dash) and states (right of dash) drawn from Table 2. *Polarity of character is hypothesized given the state in *Myolaimus byersi* (suborder Myolaimina; Giblin-Davis *et al.*, 2010), which is a possible outgroup to all nematodes in tree (Nadler *et al.*, 2006).

polarity shows these cells to be only primitively muscular, and as muscles they seem to differ between Aphelenchidae and Aphelenchoididae, in that only in the former group do they retain attachment to the luminal cuticle. Similarly, pm2 cells, as hypothesized for Aphelenchoididae, are more similar in expression to those of Aphelenchidae, being muscular as in outgroups. Putative pm3 cells are muscular in all groups, but they are simply dilators in 'Aphelenchoidea' and outgroups; only in tylenchids *sensu stricto* have they taken on an apparent metacorpus 'constraining' function. The presence of a valve in the luminal cuticle just posterior to the DGO seems to be unique to 'Aphelenchoidea'. If convergent as predicted by phylogeny, the presence of a valve need only involve reduction of the lumen diameter, as the preadaptive dilator musculature of outgroups had already been in place.

*Dorsal gland orifice*

The position of the DGO in the metacorpus has traditionally been essential to diagnosing 'Aphelenchoidea'. Reconstruction of the pharynx, however, challenges the definition of the 'metacorpus'. The cells comprising the metacorpus, particularly at its anterior end, vary amongst all three lineages of Tylenchomorpha. The definition of 'procorpus' meets an even greater challenge when comparing all Tylenchomorpha lineages to outgroups. Four layers of radial cells form the stoma or buccal cavity of free-living outgroups (De Ley *et al.*, 1995), underscoring the conservation of DGO position with respect to numbers of cells; these cell layers are putatively homologous with cells extending at least into the procorpus in tylenchids *sensu stricto* and into the anterior metacorpus in Aphelenchidae and Aphelenchoididae (Fig. 7). Thus, any homology implied by the name 'procorpus' is misleading in terms of phylogeny; a more accurate designation of the procorpus of Aphelenchidae and Aphelenchoididae would be as part of the 'stoma'. The distinction of the procorpus is further blurred amongst some tylenchids *sensu stricto*, most notably in the Criconematidae, in which the base of the stylet itself is often near or in the 'metacorpus', resulting in a collapsed 'procorpus' and a more posterior DGO position. The cells within the procorpus also differ amongst the three lineages of Tylenchomorpha, including the cells forming its lumen and the cell bodies it contains. In Tylenchomorpha, only Aphelenchidae and tylenchids *sensu stricto* have a procorpus containing nuclei (particularly e1 and e3) and luminal cuticle totally lined by both radial and marginal cells. The positioning of the DGO so far posterior is the result of different topological arrangements amongst cells, perhaps as a function of different developmental processes; observations of cells during embryogenesis of both Aphelenchidae and Aphelenchoididae could test this possibility.

More detailed characterization of the pharynx in a tylenchid *sensu stricto* is still required to test thoroughly hypotheses of procorpus and anterior metacorpus homology. Identifying the presence and expression of postcorpus cells more completely in Aphelenchidae and Aphelenchoididae may also offer insight into the divergence between these two lineages. The presence of a long isthmus and a well-developed basal bulb in *Paraphelenchus*, the other described genus of Aphelenchidae, suggests that the possible lack of developed muscles in the basal bulb of *A. avenae* may be a secondary reduction or loss relative to outgroups. Selective TEM sections through the postcorpus of *Paraphelenchus* sp. would thus test for the presence of cells pm6–pm8 in a representative of Aphelenchidae. A particularly promising route for testing congruence of 'Aphelenchoidea' would be TEM examination of the pharynx in *Pseudaphelenchus*. This recently described genus is placed basal to all other Aphelenchoididae by rRNA analysis and is an

intermediate between Aphelenchidae and Aphelenchoididae in morphological taxonomic characters (Kanzaki *et al.*, 2009). Because of its phylogenetic position, *Pseudaphelenchus* should offer a stronger test of corroboration or rejection of any possible intermediates in pharyngeal cell homologues. All hypotheses of transformation are dependent upon stronger resolution of independent, molecular-based phylogeny of Tylenchomorpha and outgroup taxa.

### Conserved systems allow expansion of feeding function models

The conservation of the pharyngeal corpus and especially its nervous system in *A. avenae* and other Tylenchomorpha provides a basis for extending the behavioural model of *C. elegans*. An altered configuration of putatively homologous classes of neurones demonstrates the plasticity of function possible from the same set of basic components, in spite of morphological differences on a gross level. With a model of homologous neurones established for *A. avenae*, insight offered into the evolution of feeding can be tested empirically. An understanding of conserved cells in divergent representatives of Rhabditida enables phylogenetic mapping of characters across a broader spectrum of clades and feeding types in Nematoda. From a functional perspective, precise identities of neurones in the corpus, inferred by a preliminary map of cell bodies, termini, and some connections, enable direct experiments of pharyngeal function in Tylenchomorpha. Differences in feeding between Tylenchomorpha and *C. elegans*, such as cues for and control of corpus pumping, may be illuminated by ablations of individual neurone homologues or other tests of expression.

The peculiar anterior process and apparently sensory terminus of M1 in *A. avenae* highlights an interesting functional difference with respect to *C. elegans*. As pharyngeal pumping in *A. avenae* does not occur until after protraction of the stylet (Fisher, 1975), the location of the M1 terminus in the stylet region might transmit a mechanical cue to begin pumping. Control of stylet muscles in *A. avenae* is not clear: M1 was the only direct innervation of stylet protractor muscles observed, but electrical potential created through gap junctions of consecutive radial cells may also be responsible (Avery & Horvitz, 1989).

Although the extent of variability of synapses within *A. avenae* or with respect to other nematodes besides *C. elegans* is unknown, differences as understood from preliminary connectivity observations could provide insight into differences in pharynx function between stylet-feeding and free-living nematodes. For example, the connection of I2 (as well as I1) to the somatic RIP interneurones in *A. avenae* is unique relative to *C. elegans*. In *C. elegans*, I2 connects to all motor neurones; yet because of its lack of connectivity to the somatic nervous system, pumping of the pharynx was predicted to be generated from within itself (Albertson & Thomson, 1976). Experimental ablation of I2 individually has no observable effect on *C. elegans* feeding (Avery & Horvitz, 1989). However, the timing dependent feeding of Tylenchomorpha, in contrast to the more regular pharyngeal pumping of some bacteriovores such as *C. elegans*, might involve links to the somatic nervous system, such as through I2. Connection of pharyngeal neurones to RIP may be engaged in coordinating the response to external feeding cues, including chemical stimuli (Fisher, 1975) or labial contact with the host (Dickinson, 1959; Fisher & Evans, 1967). Extrapharyngeal connectivity is also important for signalling deployment of the stylet. Apparent communication between RIP and the subventral procorpus radial cells may be involved in this process, if neurone potentials are transmitted across from procorpus radial cells to the stylet protractors. Another candidate for identifying feeding cues in Tylenchomorpha is the motor neurone MC, which regulates rate of pumping in *C. elegans* (Avery & Horvitz, 1989), possibly in response to detection of bacteria in the metacorpus by mechanosensory endings on the luminal cuticle (Albertson & Thomson, 1976). Pharyngeal nervous function in *A. avenae* or Tylenchomorpha is necessarily still conjectural, but establishing putative neurone identities provides a starting point for experimental studies in the group.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Movie S1.** Reconstruction of the pharyngeal procorpus of *Aphelenchus avenae*, with cell classes sequentially removed and replaced to show their spatial interrelationships.
**Movie S2.** Reconstruction of the pharyngeal metacorpus of *Aphelenchus avenae*, with cell classes sequentially removed to show their spatial interrelationships.

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