

# Ultrastructure of the post-corpus of *Zeldia punctata* (Cephalobina) for analysis of the evolutionary framework of nematodes related to *Caenorhabditis elegans* (Rhabditina)

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The ultrastructure of the post-corpus of *Zeldia punctata* (Cephalobina) was compared with previous observations of *Caenorhabditis elegans* (Rhabditina) and *Diplenteron* sp. (Diplogastrina) with the goal of interpreting the morphological evolution of the feeding structures in the Secernentea. The post-corpus of *Z. punctata* consists of six marginal, 13 muscle, five gland and seven nerve cells. The most anterior of four layers of muscle cells consists of six mononucleate cells in *Z. punctata*. The homologous layer in *C. elegans* and *Diplenteron* consists of three binucleate cells, suggesting a unique derived character (synapomorphy) shared between the Rhabditina and Diplogastrina. Contrary to *Diplenteron* sp. where we observed three oesophageal glands, *Z. punctata* and *C. elegans* have five oesophageal glands. We question this shared character as reflecting a common evolution between the Cephalobina and Rhabditina, because there are strong arguments for functional (adaptive) convergence of the five glands in these bacterial feeders. Convergence is further suggested by the mosaic distribution of three versus five glands throughout the Nemata; this distribution creates difficulties in establishing character polarity. Although morphological data are often laborious to recover and interpret, we nevertheless view 'reciprocal illumination' between molecular and morphological characters as the most promising and robust process for reconstructing the evolution of the Secernentea and its feeding structures.

**Keywords:** adaptive convergence; basal bulb; *Caenorhabditis elegans*; Cephalobina; *Diplenteron*; Diplogastrina

## 1. INTRODUCTION

Nematodes are proving to be good models for insight into the relationship between morphological and molecular evolution (Baldwin *et al.* 1997*a,b*; Fitch 1997; Nadler & Hudspeth 1998; Dorris *et al.* 1999; Sommer *et al.* 1999). This is particularly true for the class Secernentea, which includes *Caenorhabditis elegans* (Maupas) Dougherty, the only metazoan for which morphology has been fully reconstructed with transmission electron microscopy (TEM), cell lineaging has been mapped and sequencing of the genome has been completed (Sulston *et al.* 1983; Hodgkin *et al.* 1998; White 1988). The phylogenetic position of *C. elegans* within the Rhabditina and in relation to other members of the Secernentea, including the Cephalobina, Diplogastrina and Tylenchida, is becoming increasingly important in interpreting the context and extending the use of the *C. elegans* model. Traditional hypotheses of the relationships between these groups rely heavily on feeding structures, including the buccal capsule (mouth) and oesophagus (pharynx) as interpreted by light microscopy.

The diversity of feeding structures is generally linked to feeding biology. *C. elegans*, like most other Rhabditina and Cephalobina, is a bacterial feeder and has an open buccal capsule. Members of the Diplogastrina may be predators of small invertebrates and often have large teeth, although they also may retain the ability to feed on bacteria. Tylenchida are mostly plant parasites or fungal

feeders and the buccal capsule is always a needle-like stylet. All four groups share the presence of a three-part oesophagus including a corpus (sometimes also including a distinct metacarpus), isthmus and basal bulb, but they vary in their relative proportions of oesophageal musculature and glands. For example, the basal bulb of the Rhabditina and Cephalobina is primarily muscular with a large grinder (a so-called 'valve'), which appears to function in the mechanical digestion of food. Conversely, the Diplogastrina and Tylenchida are described as having a basal bulb that is primarily glandular and that lacks a grinder and associated musculature. In these taxa, mechanical digestion is either limited to the buccal capsule and corpus (i.e. Diplogastrina) or it is lacking (i.e. Tylenchida). In the Tylenchida, where large glands may protrude from the bulb as lobes, feeding may be entirely dependent on the action of gland products on the host (typically plant) cytoplasm (Souza & Baldwin 1999). Classical views of evolution consider buccal capsule morphology as a morphocline culminating in the tylenchid stylet and loss of the basal bulb grinder in the oesophagus in the Tylenchida and Diplogastrina (Andrássy 1976, 1984; Maggenti 1981). Taxonomic systems reflecting these classical views typically include cephalobids and rhabditids combined as separate equivalent sister taxa to tylenchids. However, these classical views differ with respect to including diplogasterids either with the tylenchids (Maggenti 1981) or with the other Secernentea (figure 1).

An alternate view of the phylogenetic relationships of the Secernentea based on molecular characters, including

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- (a) Goodey (1963):  
 order Rhabditida  
   superfamily Rhabditoidea  
     family **Rhabditidae** (Ce)  
     family **Cephalobidae** (Zp)  
   superfamily **Diplogasteroidea** (D)  
 order **Tylenchida**
- (b) Andr assy (1976, 1984):  
 order Rhabditida  
   suborder **Rhabditina** (Ce)  
   suborder **Cephalobina** (Zp)  
   suborder **Diplogastrina** (D)  
 order **Tylenchida**
- (c) Maggenti (1981):  
 subclass Rhabditia  
   order Rhabditida  
     suborder **Rhabditina** (Ce)  
     suborder **Cephalobina** (Zp)  
 subclass Diplogasteria  
   order **Diplogasterida** (D)  
 order **Tylenchida**
- (d) Blaxter *et al.* (1998):
- ```

  graph LR
    Root --- Node1
    Node1 --- Rhabditoidea["Rhabditoidea (Ce)"]
    Node1 --- Node2
    Node2 --- Diplogasterida["Diplogasterida (D)"]
    Node2 --- Node3
    Node3 --- Cephalobidae["Cephalobidae (Zp)"]
    Node3 --- Tylenchida["Tylenchida"]
  
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Figure 1. Evolutionary schemes inferred from classifications based on classical light microscope-based morphology ((a) Goodey 1963; (b) Andr assy 1976, 1984; (c) Maggenti 1981) and 18S rDNA molecular sequences ((d) Blaxter *et al.* 1998). The letters in parentheses indicate the representative taxa: Ce, *C. elegans*; D, *Diploenteron* sp.; Zp, *Z. punctata*. (The taxa in different classifications are in bold for easy comparison.)

18S rDNA and RNA polymerase II, contradicts traditional morphology-based systems in indicating a sister relationship between the Rhabditina and Diplogastrina and that the Cephalobina and Tylenchida share a clade (Baldwin *et al.* 1997a,b; Blaxter *et al.* 1998) (figure 1). Resolution of the apparent incongruence between these morphology-based and molecular-based phylogenetic hypotheses must take into account the following.

- (i) The morphological characters defining the major taxa of the Secernentea are often at the limits of resolution of the light microscope and are subject to misinterpretation and miscoding without more rigorous reconstruction. This problem has been underscored since the application of TEM in redefining previously misinterpreted characters of the buccal capsule (Baldwin & Eddleman 1995; De Ley *et al.* 1995; Baldwin *et al.* 1997b; Dolinski *et al.* 1998).

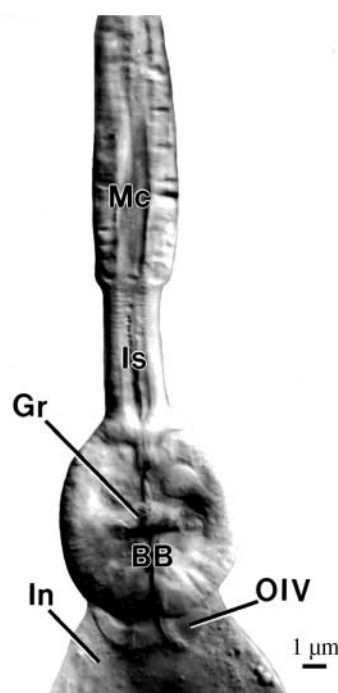


Figure 2. Light micrograph of a dissected part of the oesophagus showing the metacarpus (Mc), isthmus (Is) and basal bulb (BB). A grinder (Gr) occurs in the basal bulb and an oesophageal-intestinal 'valve' (OIV) exists between the basal bulb and the intestine (In).

- (ii) Shared feeding habits and selection pressures in distantly related taxa may result in functionally convergent morphological adaptations, which could confound phylogenetic interpretation.
- (iii) Molecular characters may provide a valuable independent character set for the examination of congruence with carefully defined morphological characters as a tool for understanding the evolution of those morphologies. We have the greatest confidence in this approach where morphological characters are mapped onto branches of molecular-based phylogenetic trees, which are also supported by conservative alignment and by congruence of independent molecular character sets from more than one loci.

As an essential step towards resolution of the morphological and molecular evolution of the Secernentea, we are addressing a redefinition of their feeding characters based on TEM reconstruction of representatives of the Rhabditina, Cephalobina, Diplogastrina and Tylenchida. This approach (as does classical morphology) assumes that these characters are slowly evolving within the major groups and that they can be inferred, subject to further testing, from representatives, as has already been done for the buccal capsule (Baldwin & Eddleman 1995; De Ley *et al.* 1995; Baldwin *et al.* 1997b). Previous TEM reconstruction studies of the oesophagus of *C. elegans* in *Philosophical Transactions of the Royal Society of London* (Albertson & Thomson 1976) have provided the most fundamental basis for a comparison of the Cephalobina, Rhabditina, Diplogastrina, Tylenchida and outgroups (Albertson & Thomson 1976). Recently, Zhang & Baldwin (1999) used TEM to demonstrate a range of similarities between the post-corpus of *Diploenteron* sp. (Diplogastrina) and that of

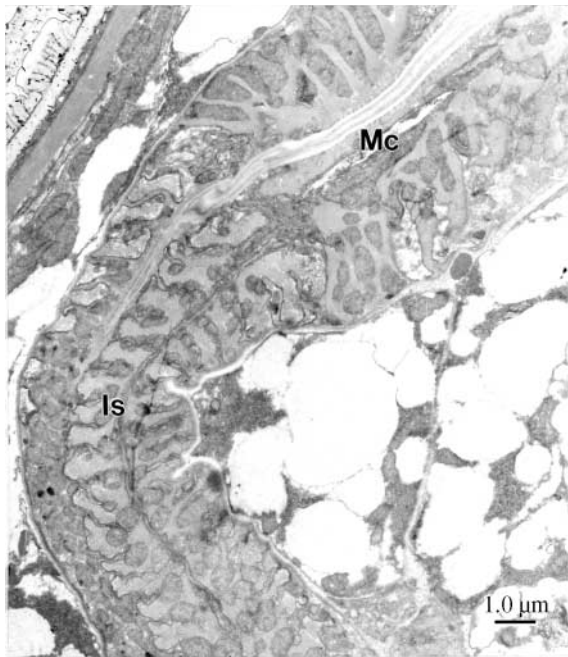


Figure 3. Electron micrograph of a longitudinal section showing the muscles in the metacarpus (Mc) and isthmus (Is).

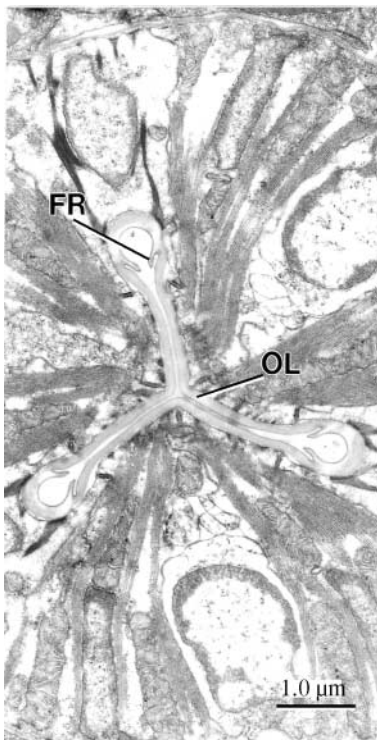


Figure 4. Electron micrograph of a transverse section through the metacarpus showing the flap-like ridge (FR) of the oesophageal lumen (OL).

*C. elegans*. Work is ongoing in our laboratory to reconstruct the corresponding region of the oesophagus in representative species of certain Tylenchida and outgroups (Teratocephalina). In the present study the detailed morphology of the post-corpus of *Zeldia punctata* as a representative of the Cephalobina is examined and compared with that of *C. elegans*. Based on recent molecular-based phylogenetic analyses we do not expect to find oesophageal morphological characters unique

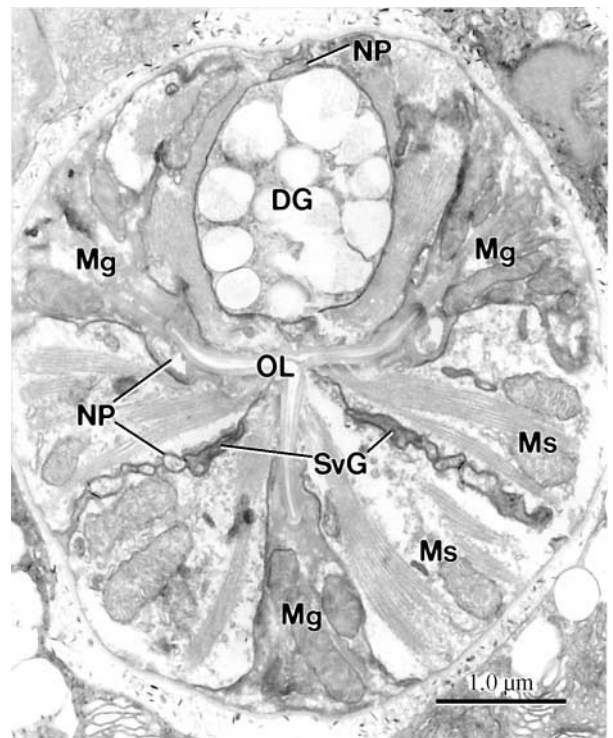


Figure 5. Electron micrograph of a transverse section through the anterior region of the isthmus showing three marginal (apical) cells (Mg) attached to the apices of the triradiate oesophageal lumen (OL). These cells separate the oesophagus into one dorsal and two subventral sectors, which include muscle cells (Ms), dorsal (DG) and subventral (SvG) gland extensions and nerve processes (NP).

(synapomorphic) to the Cephalobina (i.e. *Z. punctata*) and Rhabditina (i.e. *C. elegans*) (figure 1). This is the first time the post-corpus of a Cephalobina has been reconstructed using serial thin sections for TEM.

## 2. MATERIAL AND METHODS

An isolate (JB-121) of *Z. punctata* is maintained in our laboratory on water agar medium seeded with *Escherichia coli* OP50 Miquela, 1895 and living as well as fixed vouchers are curated in the University of California Riverside Nematode Collection. In whole specimens some features of the oesophagus are blocked from view with light microscopy by the nerve ring, associated ganglia and other tissue between the oesophagus and body wall cuticle. However, the oesophagus was completely exposed by contraction of the somatic muscles when adult nematodes were cut at the anterior end of the oesophagus on a slide in 10 mM sodium azide solution. The slide was then sealed with paraffin for observation with a differential interference contrast microscope. For TEM observation, young adult specimens were fixed in 2% osmium tetroxide and stained in 1% aqueous uranyl acetate as described previously (Zhang & Baldwin 1999). The nematodes were blocked in agar and dehydrated in a series to 100% ethanol and then to 100% acetone and infiltrated with epoxy (Spurr 1969). Embedded specimens were cast in moulds and polymerized at 70 °C. Blocks were sectioned with a diamond knife and silver sections (65 nm thick) were picked up on copper grids coated with 0.6% pioloform. Sections were post-stained with lead citrate and uranyl acetate prior to TEM observation on a Hitachi H-600 (Hitachi, Japan) (Reynolds

Table 1. *Comparison of the cells in the post-corpi of Z. punctata, C. elegans and Diplenteron sp.*

(The data for *C. elegans* and *Diplenteron* sp. were obtained from Albertson & Thomson (1976) and Zhang & Baldwin (1999), respectively.)

| cell type      | <i>Z. punctata</i> |                     | <i>C. elegans</i>  |                     | <i>Diplenteron</i> sp. |                     |
|----------------|--------------------|---------------------|--------------------|---------------------|------------------------|---------------------|
|                | cells ( <i>n</i> ) | nuclei ( <i>n</i> ) | cells ( <i>n</i> ) | nuclei ( <i>n</i> ) | cells ( <i>n</i> )     | nuclei ( <i>n</i> ) |
| gland cells    | 5                  | 5                   | 5                  | 5                   | 3                      | 3                   |
| dorsal gland   | 1                  | 1                   | 1                  | 1                   | 1                      | 1                   |
| subventral g1  | 2                  | 2                   | 2                  | 2                   | 2                      | 2                   |
| subventral g2  | 2                  | 2                   | 2                  | 2                   | 0                      | 0                   |
| marginal cells | 6                  | 6                   | 6                  | 6                   | 6                      | 6                   |
| anterior set   | 3                  | 3                   | 3                  | 3                   | 3                      | 3                   |
| posterior set  | 3                  | 3                   | 3                  | 3                   | 3                      | 3                   |
| muscle cells   | 13                 | 13                  | 10                 | 13                  | 6                      | 9                   |
| set 1 (m5)     | 6                  | 6                   | 3                  | 6                   | 3                      | 6                   |
| set 2 (m6)     | 3                  | 3                   | 3                  | 3                   | 3                      | 3                   |
| set 3 (m7)     | 3                  | 3                   | 3                  | 3                   | 0                      | 0                   |
| set 4 (m8)     | 1                  | 1                   | 1                  | 1                   | 0                      | 0                   |
| nerve cells    | 7                  | 7                   | 7                  | 7                   | 11                     | 11                  |
| total          | 31                 | 31                  | 28                 | 31                  | 26                     | 29                  |

1963; Hayat 1993). One young adult nematode was sectioned longitudinally and seven were sectioned transversely. The letter symbols for the oesophageal cells of *C. elegans* are generally adapted from Albertson & Thomson (1976).

### 3. RESULTS

The oesophagus of *Z. punctata* consists of a corpus, isthmus and basal bulb. A metacarpus is not very prominent, but nevertheless the region is distinguishable from the isthmus and we herein use the term 'metacarpus' to refer to the posterior region of the corpus (figure 2). The isthmus and basal bulb together comprise the post-carpus. A grinder occurs in the post-carpus and there is a transition of epithelial cells between the post-carpus and intestine. The isthmus can be differentiated from the metacarpus by its greater optical density and narrower diameter as viewed with a light microscope (figure 2). With TEM the isthmus is more muscular than the metacarpus (figure 3). Unlike the oesophageal lumen in the metacarpus, which has tubular apices with flap-like ridges, the lumen in the post-carpus is basically triradiate (figures 4 and 5). Thirty-one cells have been identified throughout the post-carpus, including six marginal, 13 muscle, five gland and seven nerve cells (table 1). At a given transverse section, three marginal (apical) cells attach to the apices of the triradiate oesophageal lumen and separate the oesophagus into one dorsal and two subventral sectors (figure 5).

One dorsal and four subventral glands occur in the post-carpus of *Z. punctata* (figure 6*a-c*). Each of these glands is a single cell. The dorsal gland has a broad anterior extension that runs through the dorsal radial sector of the isthmus and, at the posterior end of the basal bulb, it expands anteriorly, like the petals of a lotus, into all three radial sectors (figures 5 and 6*a*). The dorsal gland nucleus is located at the right subdorsal area near the posterior end of the post-carpus (figure 7). The anterior two subventral gland cells (g1) have narrow extensions through the isthmus and, at the junction of the

metacarpus and the post-carpus, each of these two g1 cell extensions terminates as a small ampulla through which a gland duct opens into the oesophageal lumen (figure 8). The gland duct terminates in the ampulla as a bladder-like structure. Each of these two subventral glands has one nucleus located laterally (figures 6*b* and 9). The two additional subventral gland cells (g2) are located ventrally and open into the oesophageal lumen slightly anterior to the grinder (figures 9 and 10). Each of these g2 cells has one nucleus posterior to the grinder (figure 6*c*).

The six marginal cells of *Z. punctata* are epithelial. These elongate mononucleate cells are located between the apices of the oesophageal lumen and the basement membrane of the post-carpus and are organized as an anterior and posterior set (sets 1 and 2, respectively) (figures 5, 6*d* and 8). Each of the three anterior marginal cells (set 1) extends from the junction of the metacarpus and the post-carpus through the isthmus and a nucleus occurs in the anterior end of the basal bulb (figure 11). Each of the three posterior marginal cells (set 2) extends into the basal bulb (figure 6*d*). Electron-opaque filaments extend radially in the marginal cells from the cuticular lining of the oesophageal lumen to the basement membrane (figure 8).

There are 13 muscle cells, which are arranged as four sets (layers) in the post-carpus of *Z. punctata*. From anterior to posterior we have designated these as sets 1-4, respectively (table 1 and figure 6*e-h*). Set 1, the most anterior layer, is composed of six muscle cells which primarily occupy the length of the isthmus. The six muscle cells, as viewed transversely, occur in three wedge-shaped pairs and each of the three radial sectors has one pair of these elongate cells. The two muscle cells of a given pair are separated from each other by membrane, gland and nerve extensions and each muscle cell contains one nucleus near its posterior end (figures 6*e* and 11). Posterior to set 1, set 2 includes three mononucleate cells and each cell occurs in one of the three radial sectors (figures 6*f*, 9 and 12). These cells are primarily associated with the anterior half

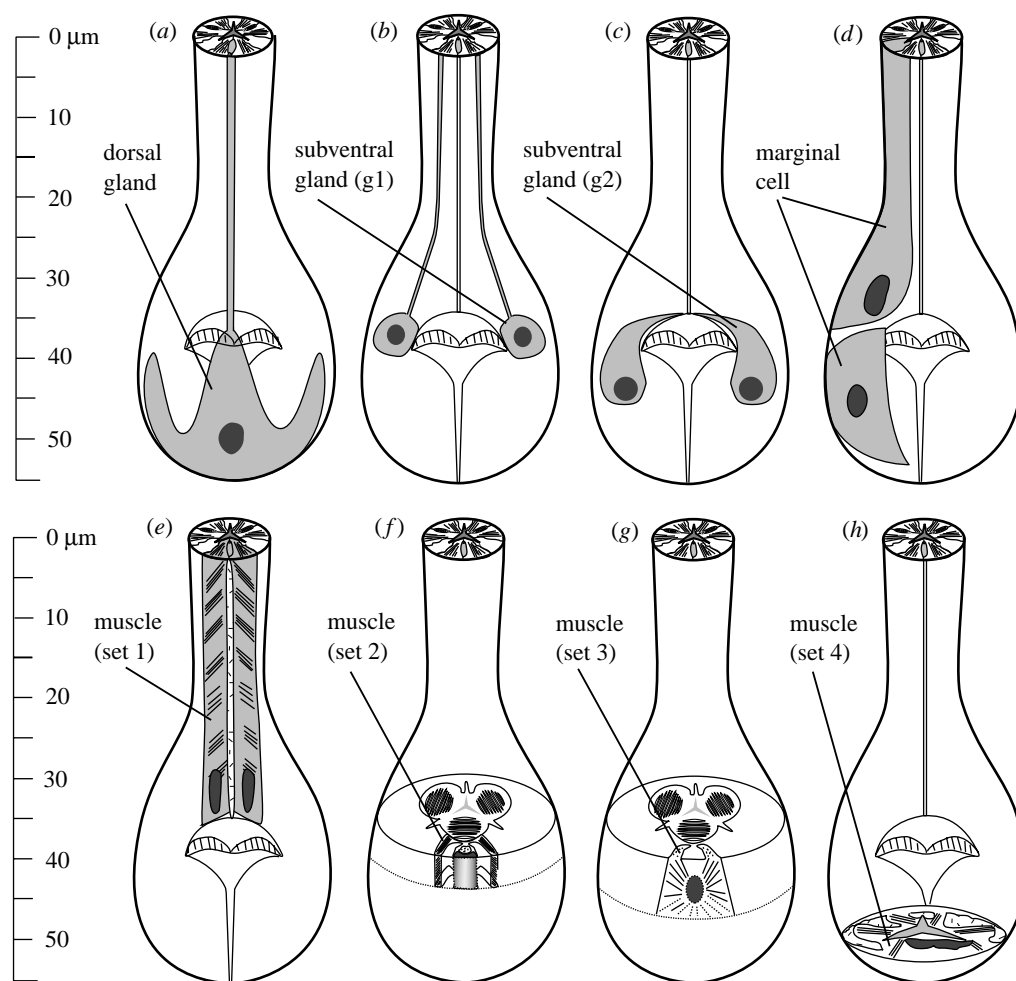


Figure 6. Diagrams of the post-corpus of *Z. punctata* illustrating the configurations of particular cells. The scales indicate the distance from the anterior end of the isthmus. (a) Dorsal view of the dorsal gland, which expands anteriorly like the opening petals of a lotus. (b) Dorsal view of the two anterior subventral glands (g1), which open through the anterior processes into the oesophageal lumen at the junction of the metacarpus and isthmus. (c) Dorsal view of the two posterior subventral glands (g2), which open into the anterior end of the grinder. (d) Dorsal view of one anterior and one posterior marginal cell. (e) Dorsal view of a pair of set 1 muscle cells in the dorsal sector. (f) Dorsal view of one of the set 2 muscle cells in the dorsal sector. (g) Dorsal view of one of the set 3 muscle cells in the dorsal sector. These muscle cells intersect into those of the set 2 muscle cells. (h) Dorsal view of the single set 4 muscle cell at the posterior end of the basal bulb.

of the grinder. Set 3, at the posterior end of the grinder, contains three mononucleate cells with one in each radial sector (figures 6g, 9, 12 and 13). Near each nucleus, the contractile filaments of set 3 muscle cells diverge anteriorly and posteriorly from the lumen lining towards the basal membrane (figure 13). Set 2 muscle cells interlock into set 3 muscle cells and apparently they coordinate with each other in the 'food-grinding' process (figures 9 and 12). Set 4 is a single muscle cell that covers the posterior wall of the basal bulb (figures 6h and 14).

Seven nerve cell bodies, each with one nucleus, occur in the basal bulb of *Z. punctata*. The nerve cells typically have long processes. Five nerve processes are associated with the extension of the anterior subventral glands (g1) and at least one nerve process is associated with dorsal gland extension (figure 5). One nerve nucleus occurs in each of the radial sectors between the two nuclei of the set 1 muscle cells (figure 11). One other nerve nucleus is ventral and two subdorsal near the apices of the oesophageal lumen between the grinder and the basement

membrane (figure 12). An additional nerve nucleus occurs posterior to the grinder.

Five epithelial cells were observed at the posterior end of the basal bulb between the basement membrane of the post-corpus and the intestinal cells. There is no true muscular oesophageal-intestinal valve in this region (figure 14). The grinder is located roughly in the centre of the basal bulb and is a modification of the oesophageal lumen and cuticular lining. Anteriorly the triradiate oesophageal lumen protrudes into the radial sectors at the centre of the lumen and in transverse section appears hexaradiate (figure 15a). Slightly posteriorly the cuticular protrusions enlarge and form one dorsal and two subventral broad leaflets (figure 15b). Further posteriorly the leaflets further enlarge and transverse strips (deep invagination of the lumen between the cuticular ridges) are visible (figure 15c). Each leaflet has a number of ridge-like structures that may be used to grind food (figure 15d). Posteriorly the leaflets are reduced to a triradiate saw-like structure (figure 15e), but still further posteriorly the leaflets are absent and the

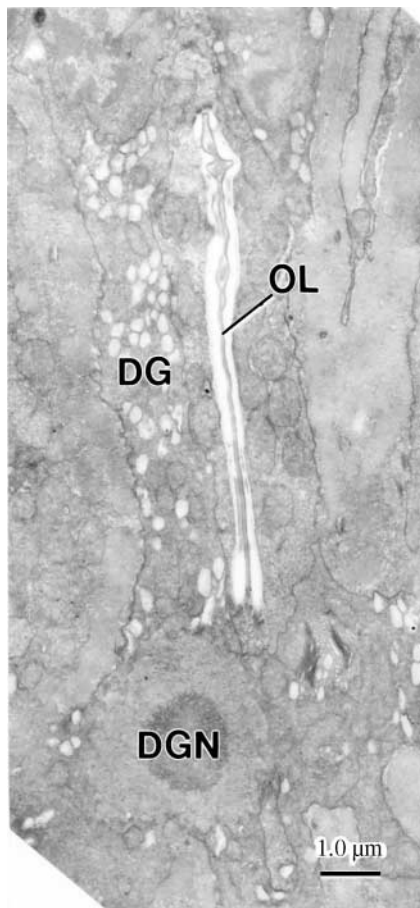


Figure 7. Electron micrograph of a longitudinal section showing the dorsal gland (DG) and its nucleus (DGN) in relation to the oesophageal lumen (OL)

oesophageal lumen becomes triradiate (figure 15*f*). Six muscle cells arranged in two sets are associated with the grinder (sets 2 and 3) (figure 12).

#### 4. DISCUSSION

The post-corpus of *Z. punctata* is surprisingly similar to that of *C. elegans*, with clear correspondence of each cell nucleus (table 1). The major difference is that the most anterior layer of muscle cells (set 1 of the four layers of muscle cells) in *Z. punctata* consists of six mononucleate muscle cells (three pairs) and in *C. elegans* the homologue is three binucleate muscle cells (m5) (Albertson & Thomson 1976). In *C. elegans*, lineages are defined for three separate m5 muscle cells, which apparently become binucleate late in development (Sulston *et al.* 1983). With respect to m5 muscle cells, the homologue in *Diplenteron* sp. (representing the Diplogastrina) is three binucleate cells as in *C. elegans* (representing the Rhabditina) (Zhang & Baldwin 1999). To test the hypothesis that the three binucleate m5 muscle cells are a synapomorphy, we examined homologous muscle cells in the post-corpus of *Teratocephalus*, a genus putatively considered to be an outgroup of the Secernentea by both classical and molecular analysis (Goodey 1963; Blaxter *et al.* 1998). In *Teratocephalus*, as in *Z. punctata*, these cells have six mononucleate muscle cells, suggesting that *Z. punctata* (Cephalobina) conserves the primitive condition and that the

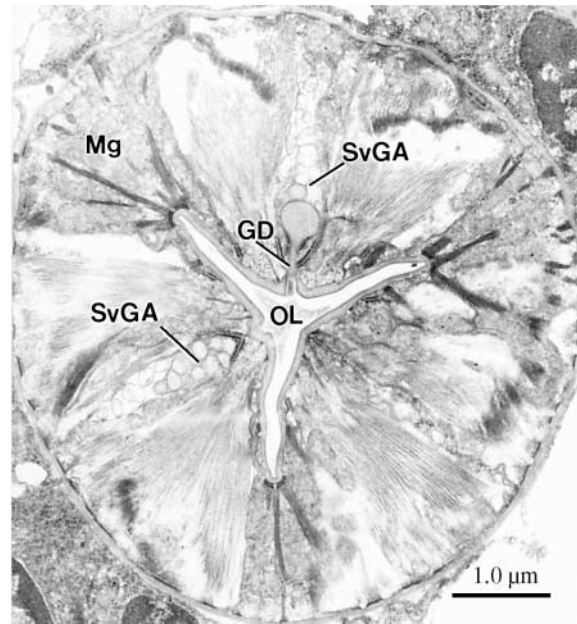


Figure 8. Electron micrograph of a transverse section through the most anterior region of the isthmus showing the two (g1) subventral gland ampulas (SvGA) and the gland duct (GD), which opens into the oesophageal lumen (OL). The marginal cells (Mg) are characterized by electron-opaque filaments.

Rhabditina and Diplogastrina share the derived state indicative of a unique common ancestry; the distribution of this character is thus congruent with phylogenetic interpretations of 18S DNA and RNA polymerase II sequences. This also agrees with the buccal capsule character of a double set of radial epithelial cells revealed by TEM, which are unique to the Rhabditina and Diplogastrina (Baldwin *et al.* 1997*b*).

The Nemata typically have single-celled oesophageal glands embedded in their oesophagus that open through minute ducts into the oesophageal lumen. Based on classical light microscopy, the Rhabditina, Cephalobina and most other Secernentea were previously described with three oesophageal glands, i.e. two subventral glands generally opening at the anterior end of the isthmus and one dorsal gland opening further anteriorly near the buccal capsule (Chitwood & Chitwood 1950). It was not until the application of TEM that an additional pair of small subventral glands was discovered in the basal bulb in *C. elegans* (Albertson & Thomson 1976); herein we demonstrate an identical number and similar arrangement of five oesophageal glands in *Z. punctata* (Cephalobina). This contrasts with only three glands present in other Secernentea examined with TEM, including *Diplenteron* sp., several Tylenchida and the putative outgroup *Teratocephalus* (Baldwin *et al.* 1977; Shepherd & Clark 1983; Endo 1984; Souza & Baldwin 1999; Zhang & Baldwin 1999). However, it must be noted that, outside the Secernentea, while the number of glands may be three, five or more, five glands is widespread and generally considered to be the conserved state at the deepest phylogenetic levels within the Nemata (Maggenti 1981).

Since some shared characters are the outcome of adaptive convergence resulting from shared selection pressures, it is expected that not all characters will be equally transparent in their correlation with evolutionary history

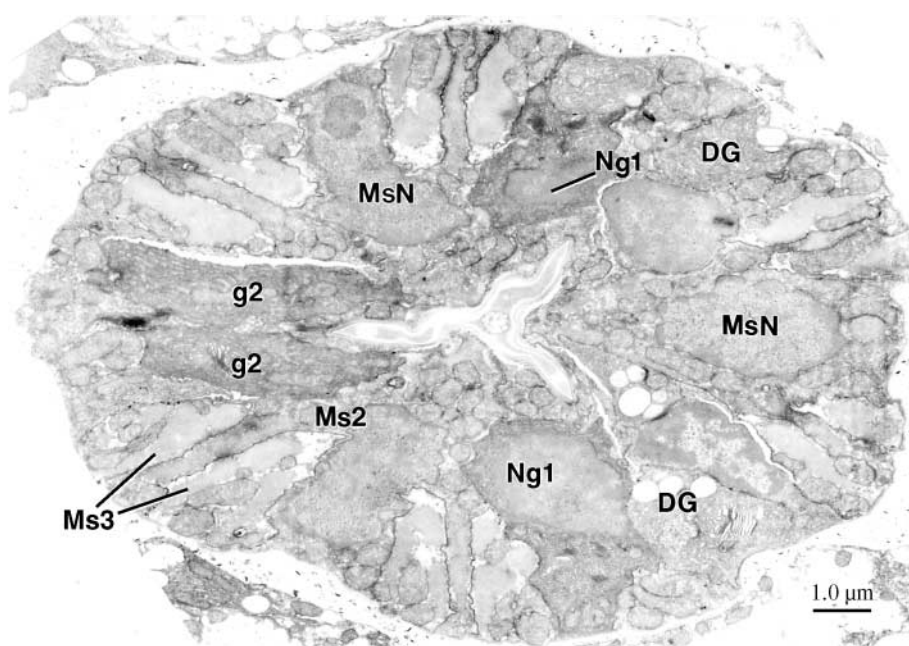


Figure 9. Electron micrograph of a transverse section through the basal bulb showing the four subventral gland cells, g1 and g2 pairs and set 2 and set 3 muscle cells. The set 2 muscle cells (Ms2) and set 3 muscle cells (Ms3) intersect into each other. Ng1, nucleus of a g1 gland cell; MsN, nucleus of one of the set 2 muscle cells; DG, dorsal gland.

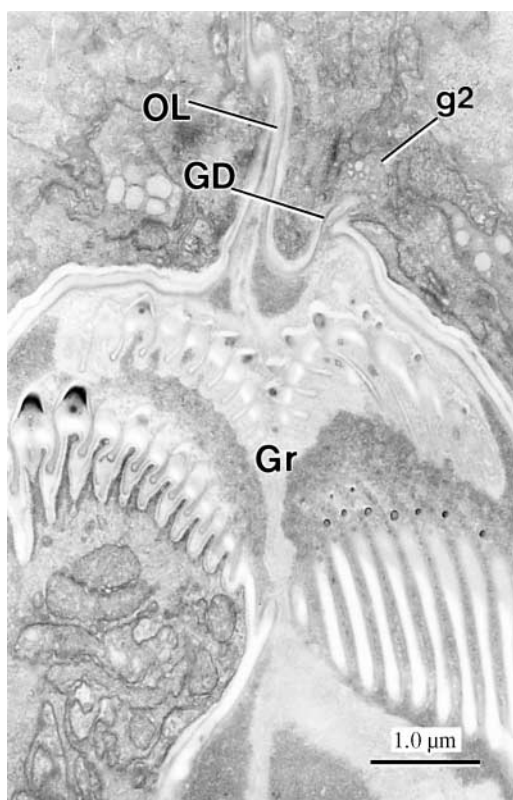


Figure 10. Electron micrograph of a longitudinal section through the grinder (Gr) showing the gland duct (GD) of one of the two g2 gland cells. The grinder is connected to the oesophageal lumen (OL) anteriorly and posteriorly.

(Kluge 1989; Omland 1994). For example, in an analysis of stifftail duck evolution, morphological and molecular data sets appeared to disagree until adaptive morphological convergence was recognized (McCracken *et al.*

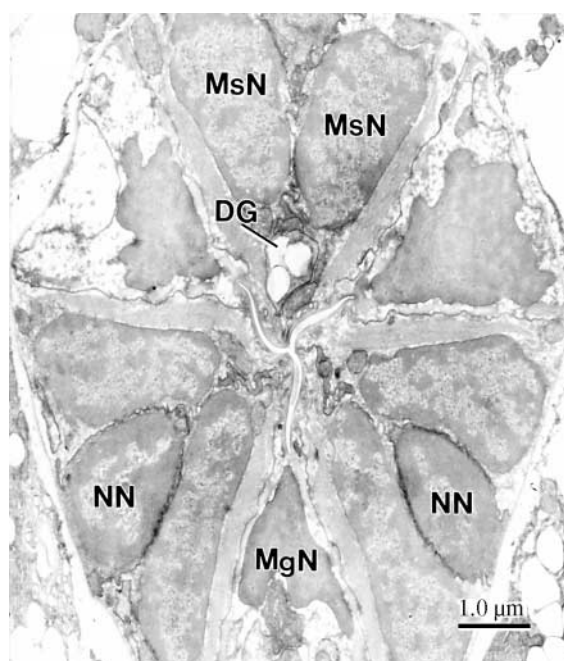


Figure 11. Electron micrograph of a transverse section through the anterior of the basal bulb showing the nuclei of the anterior marginal (MgN), muscle (MsN) and nerve (NN) cells. DG, dorsal gland extension.

1999). For many groups of microscopic nematodes a paucity of reliable morphological characters may confound parsimony approaches in recognizing convergence. The question is how does one choose the most plausible pattern of evolution between equivocal morphological information? Stated more specifically, does the shared character of the binucleate m5 cell in the Rhabditina and Diplogastrina carry greater credibility in defining relationships than the shared character of the five

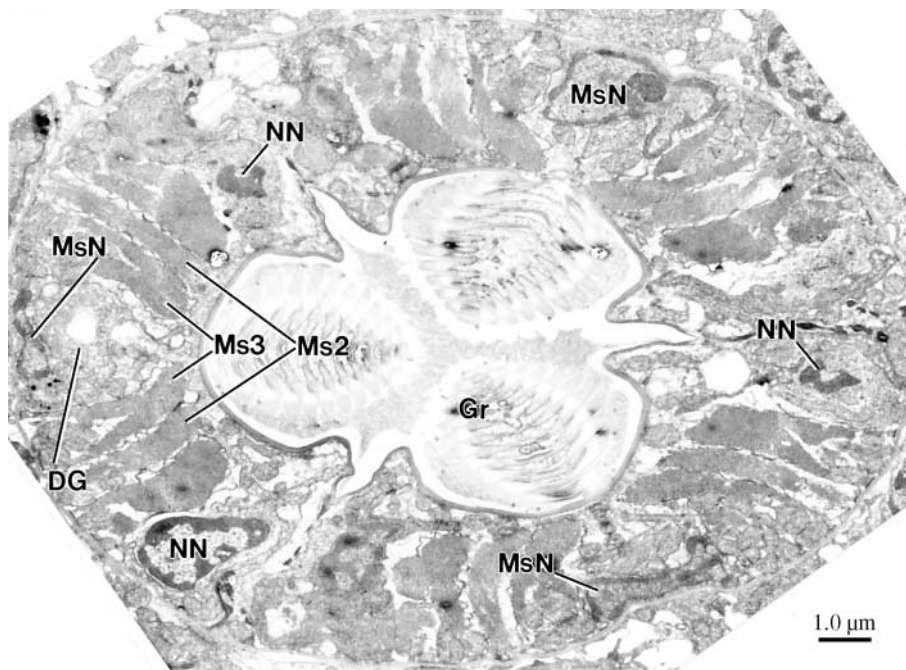


Figure 12. Electron micrograph of a transverse section through the middle of the basal bulb showing the muscle cells associated with the grinder (Gr). Set 2 muscle cells (Ms2) interlock with set 3 muscle cells (Ms3). MsN, nucleus of set 2 muscle cells; NN, nerve nucleus; DG, dorsal gland.

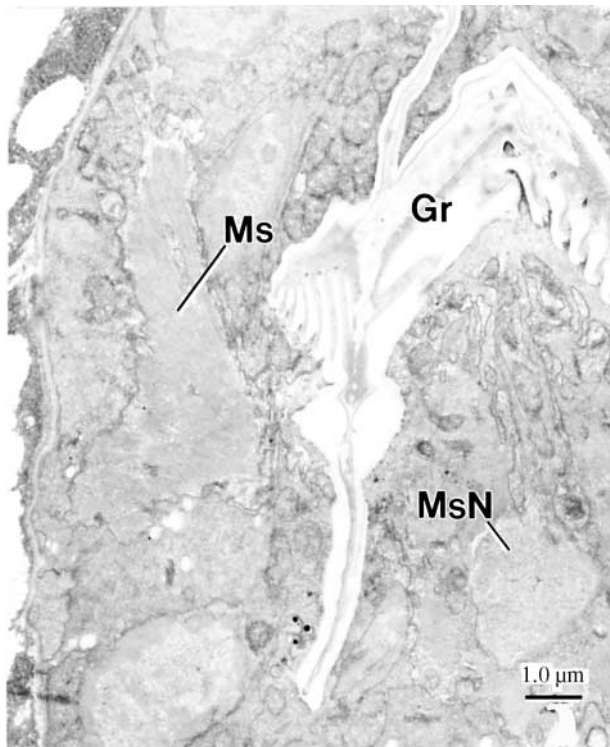


Figure 13. Electron micrograph of a longitudinal section showing one of the set 3 muscle cells (Ms) associated with the posterior end of the grinder (Gr). MsN, nucleus of another set 3 muscle cell.

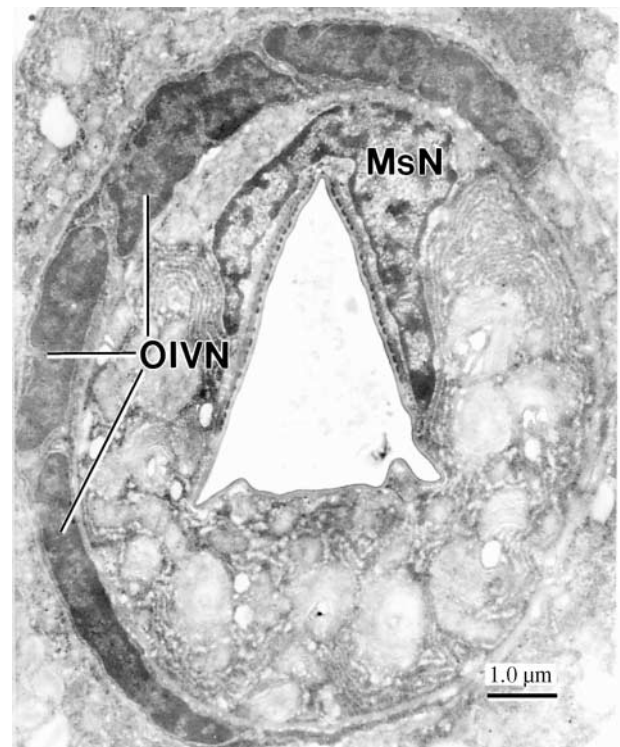


Figure 14. Electron micrograph of a transverse section through the posterior end of the basal bulb showing the oesophageal intestinal valve and the single set 4 muscle cell. MsN, nucleus of the muscle cell; OIVN, nucleus of the valve.

oesophageal glands in the Rhabditina and Cephalobina (*Z. punctata*)? With the limited morphological characters of the present study, parsimony nevertheless supports the argument that the shared character of the binucleate m5 cells is evolutionarily informative because it is congruent

with previous TEM-based morphology (buccal capsule); it is further strongly supported by molecular characters from two independent loci (Baldwin *et al.* 1997*a,b*; Blaxter *et al.* 1998). We do not anticipate that the change from six mononucleate to three binucleate m5 cells will be



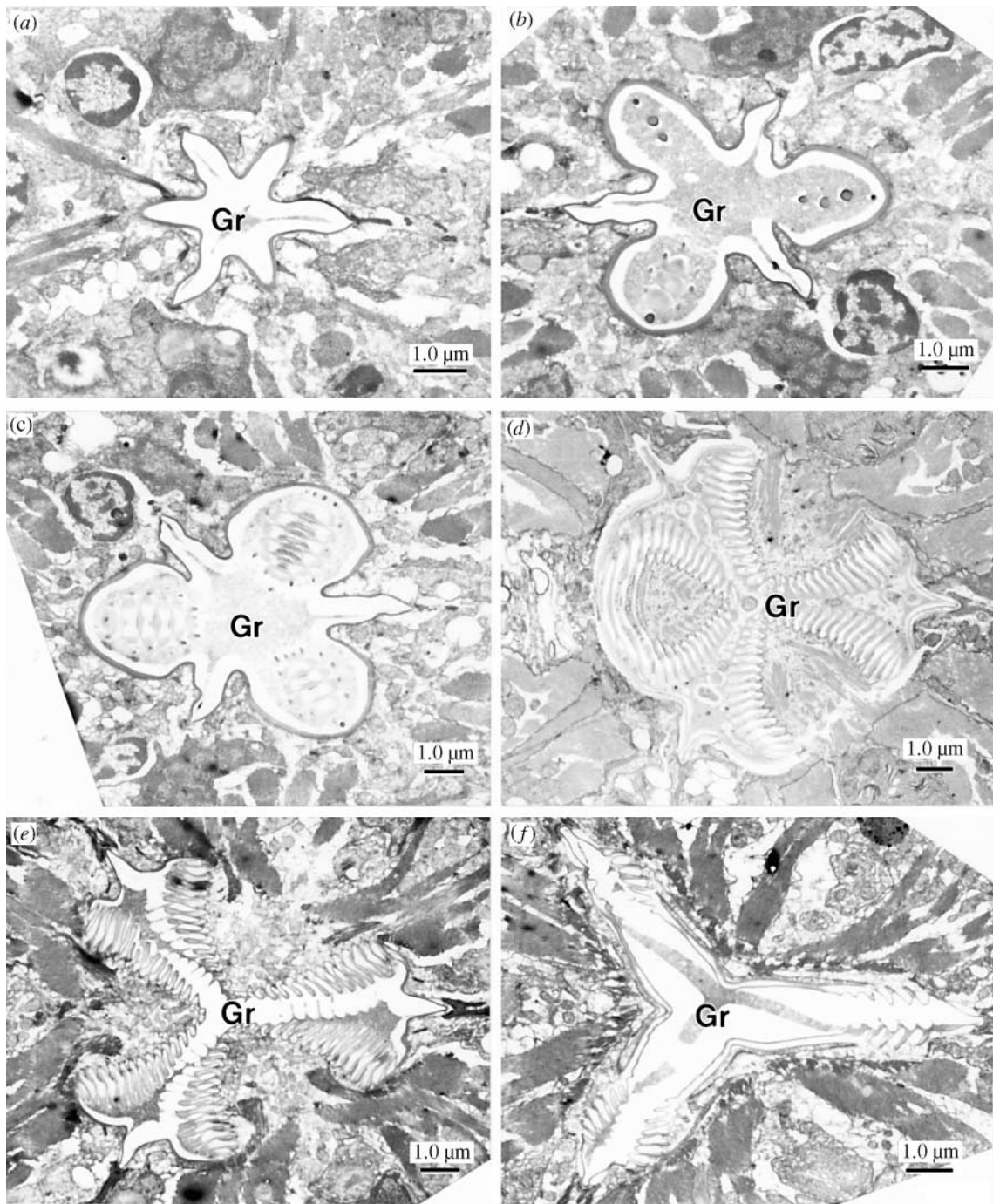


Figure 15. Electron micrographs of serial transverse sections of the grinder. Micrographs (a)–(f) show the gradual change of the grinder (Gr) from anterior to posterior.

functionally linked to any change in feeding and as such this character may be a particularly valuable marker for tracing evolution.

Conversely, there is abundant evidence that, in nematodes, the number of oesophageal glands is indeed highly convergent and less informative in inferring broad patterns of evolution. In many taxonomic orders, either three or five glands occur depending on the genus (Maggenti 1981). Considering the similar feeding habits of most Rhabditina and Cephalobina, including 'grinding' bacteria in the basal bulb, there may be a functional selection pressure (not shared by the Diplogastrina or Tylenchida) for gland products to be secreted within the bulb by the two additional glands that open nearest the site of grinding.

Developmentally this change to five glands might have resulted from a simple second cleavage of the two subventral glands. However, this appears less likely when considering that each of the four subventral glands in *C. elegans* has a lineage somewhat divergent from the others and that, in most cases, the sister cell to the gland undergoes programmed death (Sulston *et al.* 1983). We also consider the plausible explanation that the character of the five glands does not represent a derived state within the Secernentea when considering the occurrence of both three and five oesophageal glands throughout most Adenophorea. Certainly functional convergence and reversals in the outgroup can confound interpretations of character polarity, particularly where the distribution of

the character states are inadequately sampled in the outgroup.

The relationship between molecular evolution and the evolution of morphological characters such as feeding structures in nematodes, where such structures may be at or below the limits of light microscope resolution, requires sophisticated morphological understanding and, among a range of potential tools, this is likely to require TEM. The laborious methodology of some TEM and molecular procedures may promote the hypothesizing of broad meaning from limited numbers of exemplar species. In morphology, we view the process as one of (i) identifying promising characters from TEM in a few representative taxa, (ii) evaluating the potential evolutionary information of the character from cladistic methodology and insight into function as it may explain convergence, as well as by congruence with robust independent character sets (i.e. molecular data), and (iii) extending the resulting hypotheses to additional representative taxa, often by methods less cumbersome than the original TEM reconstruction. This may include directing TEM limited to a few sections in a critical region or it might include the use of light or confocal microscopy in conjunction with specific nuclear or gland stains, which might provide a 'short cut' to mapping the taxonomic distribution of the character states. Although morphological data are often cumbersome to recover and interpret, we nevertheless view reciprocal illumination between molecular and morphological characters as the most promising and robust process for reconstructing the evolution of the Secernentea.

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