JOURNAL OF NEMATOLOGY

VOLUME 31 MARCH 1999 NUMBER 1

Journal of Nematology 31(1):1–19. 1999.

Ultrastructure of the Esophagus of *Diplenteron* sp. (Diplogasterida) to Test Hypotheses of Homology with Rhabditida and Tylenchida[1]

Y. C. Zhang and J. G. Baldwin[2]

Abstract: The ultrastructure of the isthmus and basal bulb (postcorpus) of *Diplenteron* sp. (Diplogasterida) was revealed through transmission electron micrographs of serial sections. The postcorpus is glandular and muscular. There are 26 cells in the postcorpus, including 6 marginal (two sets of three), 6 muscle (two sets of three), 3 gland, and 11 nerve cells. Most of the cell bodies, including the nuclei, are in the basal bulb. Unlike *Caenorhabditis elegans, Diplenteron* sp. has three gland cells. The glands are embedded in a muscular framework in both taxa, but each gland cell is much bigger in *Diplenteron* sp. than in *C. elegans*. Each of the anterior set of three marginal cells is located at the apex of the esophageal lumen and overlaps slightly with one of the posterior sets of three marginal cells. All six marginal cells in *Diplenteron* sp. have homologs in *C. elegans*. The anterior set of radial muscle cells is V-shaped and is homologous to m5 muscle cells in *C. elegans*. The posterior set of muscle cells appears to be homologous to m6 muscle cells in *C. elegans*. *Diplenteron* sp. does not have muscle cells corresponding to the m7 cells associated with the ''grinder'' in *C. elegans,* which is absent in diplogasterids. The single saucer-shaped muscle cell, m8, covering the posterior wall of the basal bulb in *C. elegans* was not observed in *Diplenteron* sp. The structure of the esophageal-intestinal junction in *Diplenteron* sp. is similar to that of *C. elegans* in being composed of five epithelial cells. Neurons appear to be more abundant in *Diplenteron* sp. than in *C. elegans*. Ultrastructure of the esophagus in diplogasterids, rhabditids, cephalobids, and tylenchids will be useful in testing classical and recent competing hypotheses of secernentean phylogeny.

Key words: basal bulb, *Caenorhabditis elegans,* Cephalobina, *Diplenteron,* Diplogasterida, esophagus, homology, nematode, postcorpus, Rhabditida, transmission electron microscopy, ultrastructure.

The phylogenetic relationships of Diplogasterida (Nemata) within the Secernentea including Rhabditina, Cephalobina, and Tylenchida have been addressed by recent morphological (transmission electron microscopy, TEM) and molecular investigations (Baldwin et al., 1997a, 1997b; Blaxter et al., 1998). Of particular significance is the proposed sister relationship of Diplogasterida and Rhabditina because it suggests a context for extending the *Caenorhabditis elegans* (Maupas) Dougherty (Rhabditina) model; this model includes complete TEM morphological reconstruction, cell lineages, and complete DNA sequencing of the genome (Hodgkin et al., 1998; Riddle et al., 1997; Sulston, 1988; Sulston and Horvitz, 1977; Sulston et al., 1983).

Previous classification systems of Secernentea, based on light microscopy (LM), suggest relationships contradicted by recent morphological (TEM) and molecular findings. These systems range from clustering rhabditids, diplogasterids, and cephalobids in one order at equivalent rank to tylenchids (Andrássy, 1976; Chitwood and Chitwood, 1950)[1] to a modification that includes diplogasterids as a separate group from rhabditids and cephalobids combined (Goodey,

Received for publication 26 May 1998.

[1] Portion of the first author's Ph.D. dissertation. This research was supported by National Science Foundation grants (DEB 9318249 and DEB 9712355).

[2] Research Assistant and Professor, Department of Nematology, University of California, Riverside, CA 92521.

E-mail: james.baldwin@ucr.edu

The authors thank Manuel Mundo for his generous contribution of the scanning electron micrograph of the buccal opening and Douglas Eddleman, Paul De Ley, Claudia Dolinski, and Ricardo Souza for discussing the project and for technical assistance.

This paper was edited by E. C. Bernard.

[1] In this case (e.g., Andrássy, 1976) rhabditids = Rhabditina, diplogasterids = Diplogasterina, cephalobids = Cephalobina, and tylenchids = Tylenchida.

1963b). In contrast, Maggenti (1981) introduced even deeper separation when he proposed the separate order Diplogasterida and placed it with Tylenchida (and Aphelenchida) in the new subclass Diplogasteria while keeping rhabditids and cephalobids together in order Rhabditida, subclass Rhabditia.

Key characters in LM morphology-based classifications of Secernentea focused on diverse feeding adaptations of microbivores (e.g., rhabditids, cephalobids), omnivores, predators, and insect associates (e.g., diplogasterids) as well as plant parasites (e.g., tylenchids). Whereas all the taxa share a three-part esophagus, including a postcorpus with a basal bulb, the esophagus varies among and between taxa by the distribution and size of muscles, glands, and valves. Furthermore, the buccal capsule varies from unarmed in microbivores to armed with one or more prominent teeth for predation, to a protrusible stylet essential for plant parasitism. A presumed morphocline in buccal capsule structure (Andrássy, 1976; Goodey, 1963a; Steiner, 1933; Thorne, 1961) combined with the shared character of a reportedly nonmuscular glandular basal bulb has been used to argue for a sister relationship of diplogasterids and tylenchids (Andrássy, 1983; Maggenti, 1981). It has been demonstrated with TEM—and more recently with comparative cell lineages, however, that LM alone is often inadequate for resolving characters and homologies of the feeding apparatus (Baldwin et al., 1997b; De Ley et al., 1995; Dolinski et al., 1998). The difficulty is due not only to the limitations of optical resolution but also to the apparent ubiquity of homoplasy in the morphology of nematode feeding structures, which can be resolved only with sophisticated character analysis and independent character sets (Baldwin, 1992). Further testing of the presumed sister taxon status of Diplogasterida with Rhabditina, as opposed to Diplogasterida and Tylenchida, requires TEM elucidation of additional key morphological characters throughout Secernentea. We are interested in comparing ultrastructure of the basal bulb region of the esophagus in Diplogasterida and Rhabditina as well as the putative outgroups Cephalobina and Tylenchida.

Although the basal bulb region of a diplogasterid or cephalobid never has been examined with TEM, the entire esophagus of the rhabditid *C. elegans* has been reconstructed from serial electron micrographs (Albertson and Thomson, 1976). In contrast to Rhabditina, limited TEM observations suggest that in Tylenchida the postcorpus is entirely glandular (Endo, 1984; Endo et al., 1997; Shepherd and Clark, 1976, 1983; Yuen, 1968), whereas in diplogasterids the glands may be embedded within a distinct muscular region (Chitwood and Chitwood, 1950; unpublished). Thus, there is some basis to question homology of the putative glandular basal bulbs in Diplogasterida and Tylenchida and to further consider the competing hypotheses of sister taxa status between Diplogasterida and Rhabditina. In this first paper in a series we used TEM to reconstruct the basal bulb of a diplogasterid belonging to the genus *Diplenteron* Andrássy, 1964 (Neodiplogasteridae) for detailed comparisons with previous reconstruction of the basal bulb in *C. elegans*.

Materials and Methods

The selected isolate of *Diplenteron* is an undescribed species, collected in 1994 by M. Mundo from soil associated with golf turf at the Tamarask Country Club, Coachella Valley, California. The isolate (JB035) is maintained in our laboratory on nematode growth medium seeded with *Escherichia coli* OP50 Miqula, 1895 (Wood, 1988). Work on the biology and taxonomic description of this species of *Diplenteron* is in progress, and voucher specimens have been deposited at the University of California Riverside Nematode Collection.

Young adult specimens were fixed in 2% osmium tetroxide and stained in 1% aqueous uranyl acetate. They were blocked in agar and dehydrated in a series through 100% ethanol and 100% acetone, and then embedded into epoxy (Spurr, 1969). Infiltrated specimens were cast in molds, which were polymerized at 70 °C. Blocks were sectioned on an ultramicrotome with a dia-

mond knife, and silver-gold sections (70 nm thick) were picked up on copper grids coated with 0.6% pioloform. Sections were poststained with lead citrate and uranyl acetate prior to TEM observation on an Hitachi H-7000 (Hayat, 1993; Reynolds, 1963). Preliminary tests indicated that staining with uranyl acetate both before dehydration and after sectioning greatly improved contrast of the gland region and reduced lead precipitation. Six young adult nematodes were sectioned longitudinally, and 11 were sectioned transversely.

Results

The isthmus and basal bulb in *Diplenteron* sp. are both muscular and glandular. The isthmus is continuous with the basal bulb by broadening gradually into the bulb; hereafter, we refer to the isthmus and basal bulb combined as the postcorpus (Figs. 1,2). The postcorpus is further distinguished as a unit by a deep constriction with the metacorpus, resulting in a narrow (<1.0 μm diam.) connecting passage (Figs. 4,5). Throughout the postcorpus, the triradiate esophageal lumen is surrounded by 26 cells (Table 1). Six marginal cells (three anterior and three posterior) together with the esophageal lumen separate the postcorpus into three radial sectors—dorsal, left subventral, and right subventral (Figs. 1E,F;6). The radial sectors collectively include 3 gland, 6 muscle, and 11 nerve cells (Fig. 6;Table 1). Most of these cells, especially neurons, are polarized; that is, they change in morphology from anterior to posterior end, sometimes having the bulk of the cell separated by a long narrow process from a small region of cytoplasm that includes the nucleus (this region is hereafter referred to as the cell body). The anterior marginal and muscle cells are primarily associated with the isthmus and posteriorly contain one or two nuclei, respectively (Fig. 1C,E). Nuclei of glands and neurons each occur within a cell body. The neuron cells have long processes

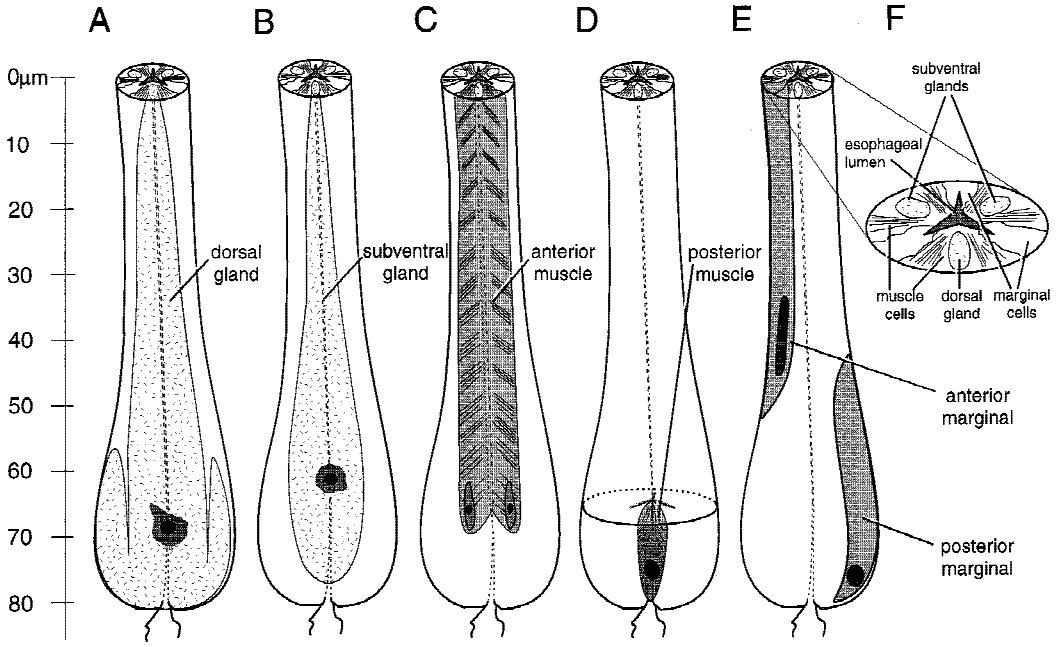
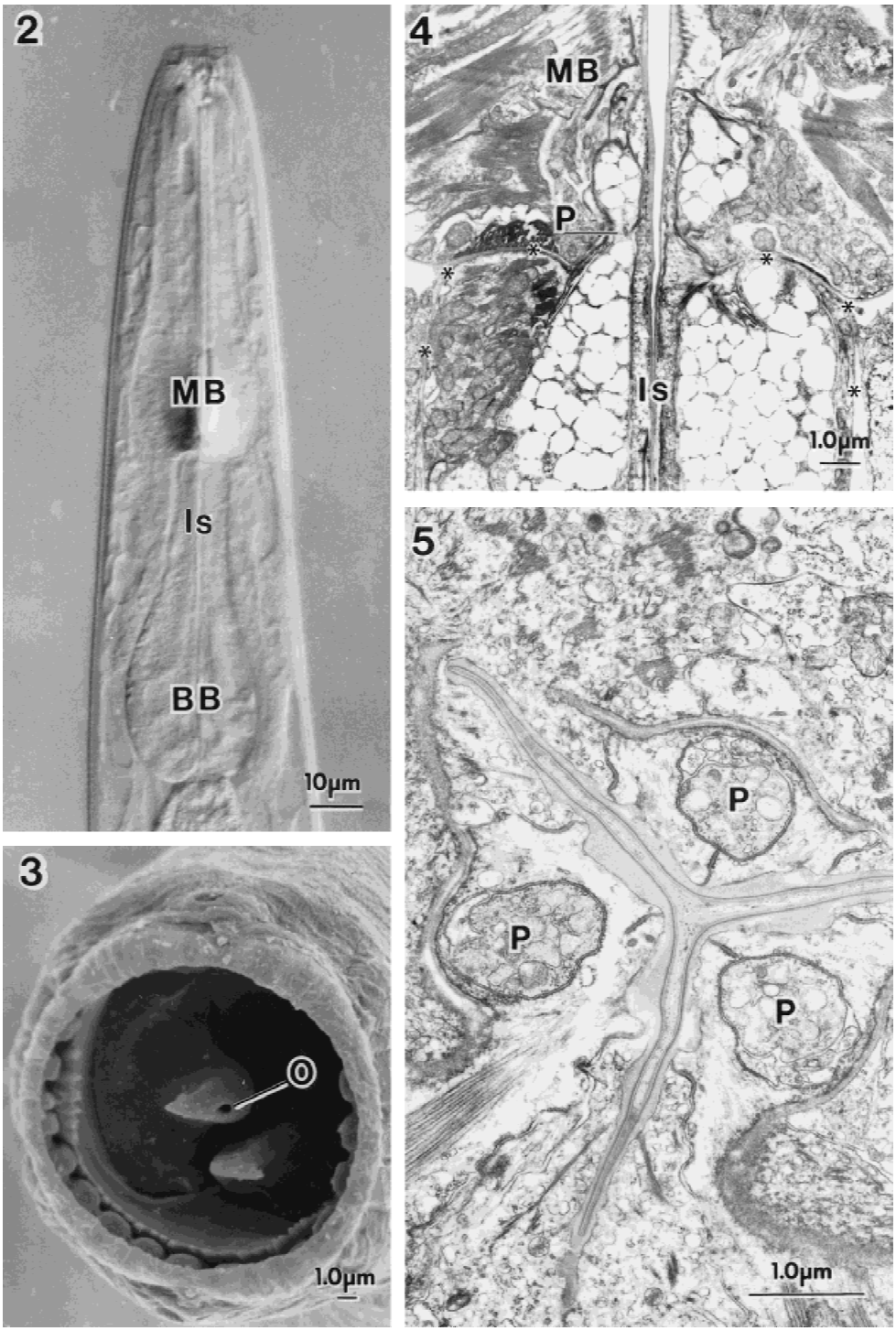


Fig. 1. Diagrams of postcorpus of the *Diplenteron* sp. illustrating the configuration of particular cells. Scale indicates distance from the base of the metacorpus. A) Dorsal view of dorsal gland. B) Subventral view of one of the two subventral glands. C) Dorsal view of one of the three anterior radial muscle cells. Each anterior muscle cell in transverse section is V-shaped and partly wraps around a gland cell. D) Dorsal view of one of three posterior radial muscle cells. The posterior muscle cells are not V-shaped. E) Dorsal view of one of the anterior set and one of the posterior set of marginal epithelial cells located at the apices of the triradiate esophageal lumen. F) Transverse view of a section from the anterior end of isthmus.



FIGS. 2–5. Light, SEM, and TEM micrographs of the buccal capsule and esophagus of *Diplenteron* sp. 2). Light micrograph showing the continuity of isthmus (Is) and basal bulb (BB). MB = metacorpus. 3). SEM of the buccal cavity opening showing the dorsal tooth orifice (O), which is continuous with the dorsal gland duct. 4). Longitudinal section of the narrow connecting passage (P) between the metacorpus (MB) and the isthmus (Is). Asterisks indicate basal lamina marking outer boundary of the isthmus. 5). Transverse section at the junction of the metacorpus and isthmus showing connecting passages (P) of tissue from the metacorpus that extend into the anterior of isthmus.

TABLE 1. Comparison of numbers of each cell type and nuclei in the postcorpi of *Caenorhabditis elegans* and *Diplenteron* sp.

	Caenorhabditis elegans[1]		*Diplenteron* sp.	
Cell Type	Cells	Nuclei	Cells	Nuclei
Gland (total)	5	5	3	3
Dorsal	1	1	1	1
Subventral set 1	2	2	2	2
Subventral set 2	2	2	0	0
Marginal (total)	6	6	6	6
Anterior set	3	3	3	3
Posterior set	3	3	3	3
Muscle (total)	10	13	6	9
Set 1	3	6	3	6
Set 2	3	3	3	3
Set 3	3	3	0	0
Set 4	1	1	0	0
Nerve	7	7	11	11
Total postcorpus nuclei	28	31	26	29

[1] Data for *Caenorhabditis elegans* obtained from Albertson and Thomson (1976).

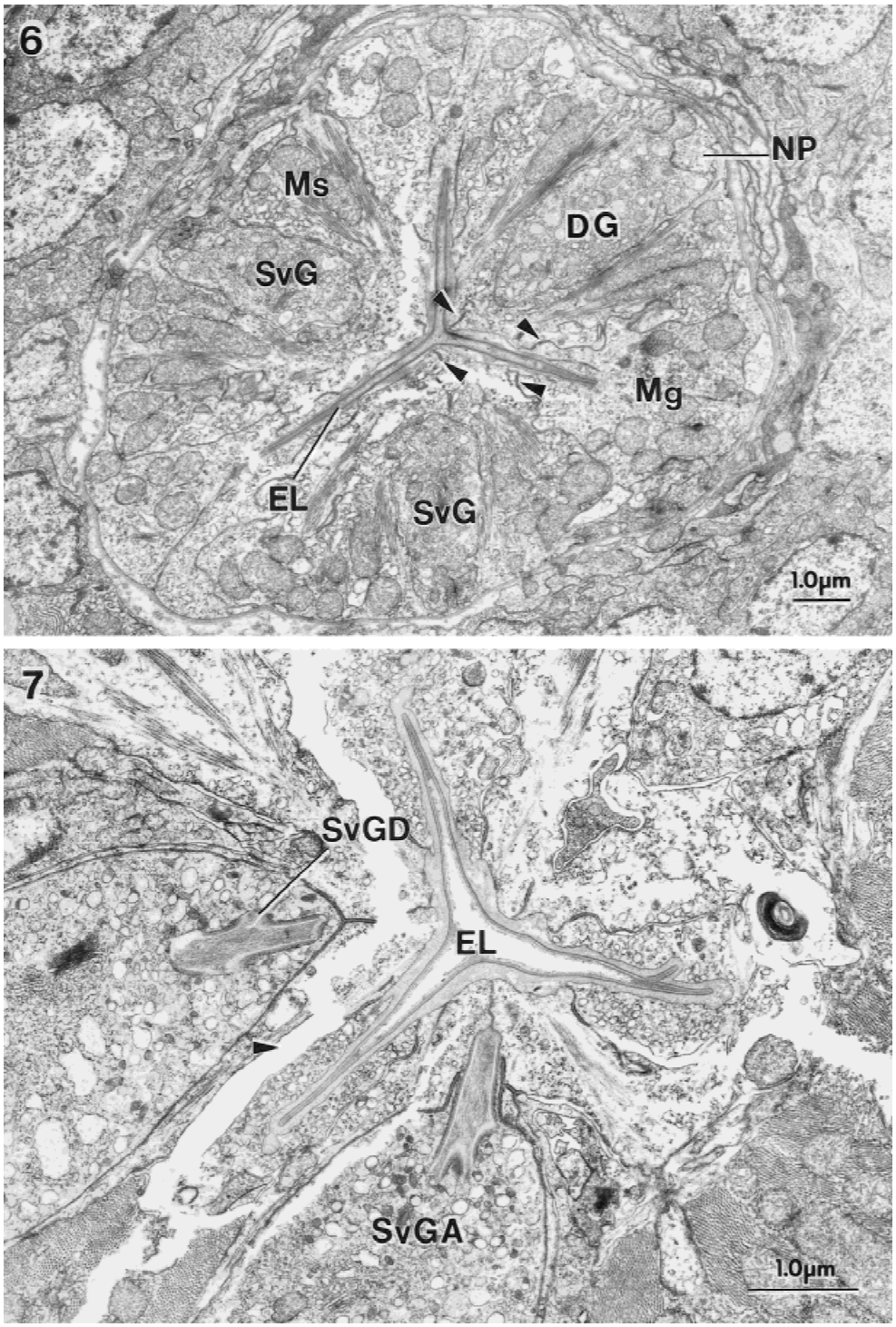
that connect to synapses, and the gland cell bodies connect to cuticle-lined ducts (Figs. 7–9).

Glands: There are one dorsal and two subventral gland cells in the postcorpus, and each has one nucleus (Figs. 1A,B;6,8). The dorsal gland extends through the dorsal radial sector of the isthmus and into the basal bulb, where it expands posteriorly to fill the entire dorsal sector (Figs. 1A;8). Anterior to the postcorpus, the dorsal gland process terminates as an ampulla that opens into the buccal cavity through the duct in the dorsal tooth (Fig. 3). The contents of the dorsal gland are morphologically distinct from those of the subventral gland(s) in having fewer Golgi bodies, a larger and slightly more posteriorly positioned nucleus and nucleolus, and differences in the size and structure of the vesicles (Figs. 1A,B;8).

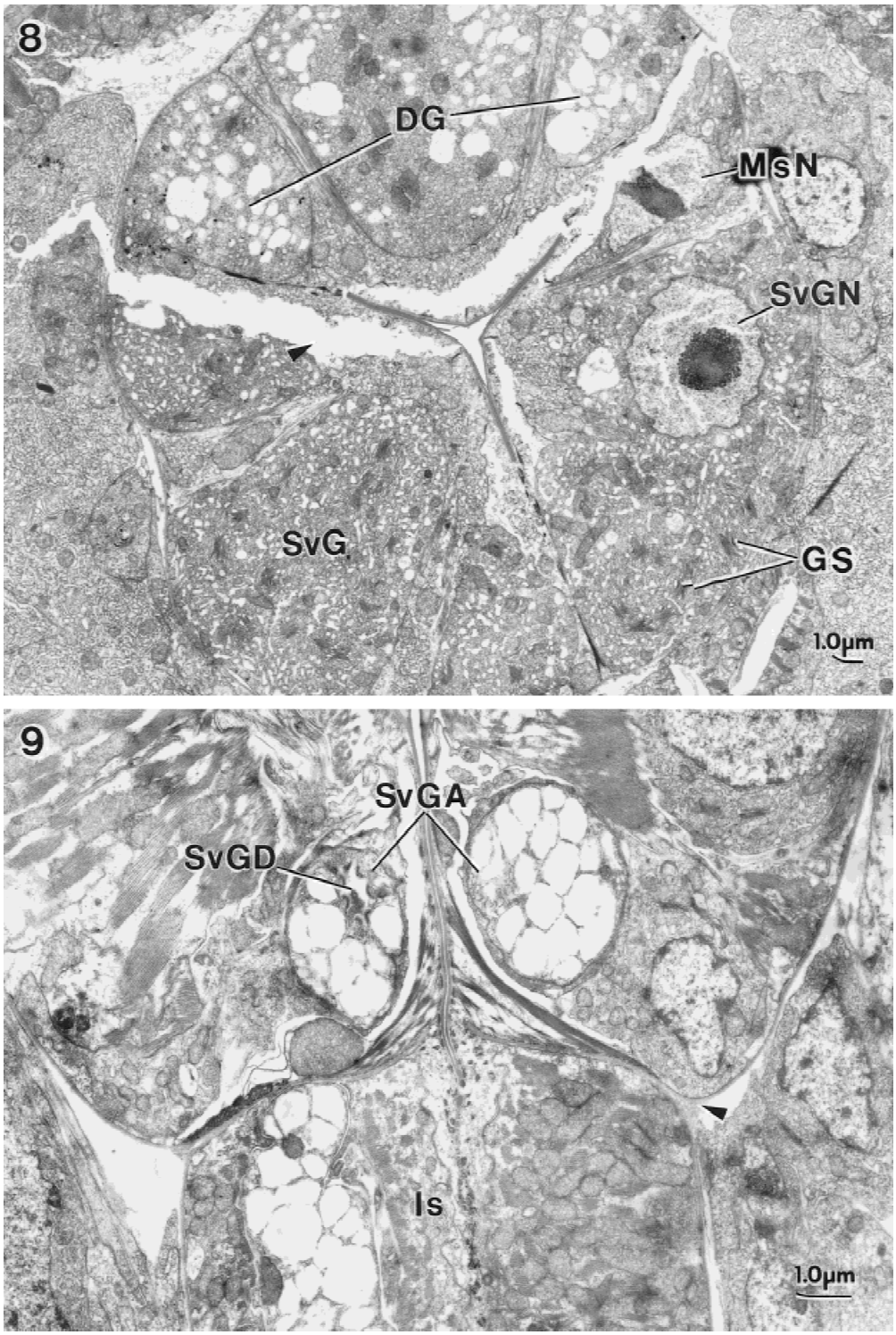
Similarly to the dorsal gland, each of the two subventral gland cells extends through the isthmus (Fig. 1B). Anteriorly, each subventral gland process terminates as an ampulla near the base of the metacorpus, and each is continuous with a duct that opens into the esophageal lumen (Figs. 7,9). The subventral gland cells broaden in the basal bulb region but do not extend beyond the subventral sectors, and near the base of the bulb they narrow and terminate 3 μm anterior to the esophago-intestinal junction (Fig. 1B). The two subventral gland nuclei and nucleoli are each smaller than that of the dorsal gland (Figs. 10,11). Although the subventral gland nuclei are typically near the same transverse level and slightly anterior to the dorsal gland nucleus, the relative positions vary among individuals (Figs. 1B;12). The electron-lucent cytoplasm of each subventral gland has abundant Golgi stacks that apparently may aggregate to form concentric lamellae (Figs. 8,12).

Marginal cells: The six elongate marginal cells of the postcorpus are arranged as an anterior and posterior set (set 1 and set 2) of three epithelial cells each, so that typically at a given transverse section of the postcorpus each marginal cell occupies one of the three apices (two subdorsal and one ventral) of the esophageal lumen (Figs. 1E,F;6). The anterior set of marginal cells extends the length of the isthmus, whereas the posterior set overlaps slightly with the anterior set but primarily occupies the length of the basal bulb (Figs. 1E;13). Throughout their length the marginal cells form junctional complexes with adjacent radial muscle cells (see below), where they meet the cuticle lining of the lumen (Figs. 6,13,21,26).

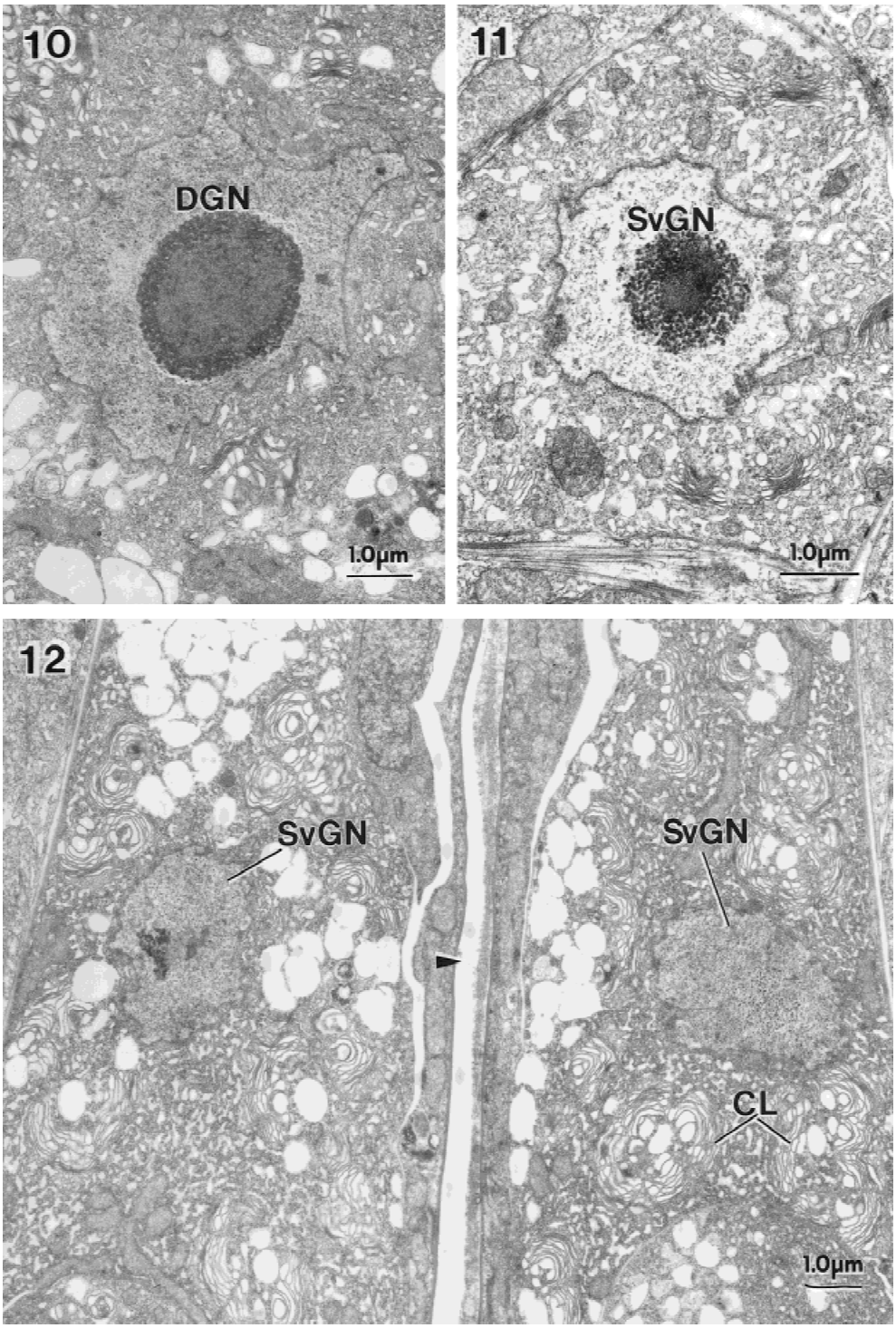
Marginal cells of the anterior set are relatively large and extend along the periphery of the isthmus throughout their length. In a given transverse section they occupy nearly one-third the area of the isthmus and have elongate nuclei (Figs. 1E;6,13,14). The left and right subdorsal marginal cells are each innervated with two neurons, but no neurons were seen associated with the ventral marginal cell (Fig. 15). Marginal cells of the posterior set are thinner than those of the anterior set. This size difference is expected, considering that much of the area of the basal bulb is occupied by large glands and many other cell bodies (Figs. 1E;16). Anteriorly, marginal cells of the posterior set extend to the periphery of the esophagus, but as the cells approach the esophagointestinal junction, they are greatly reduced in size, do not extend to the periphery of the



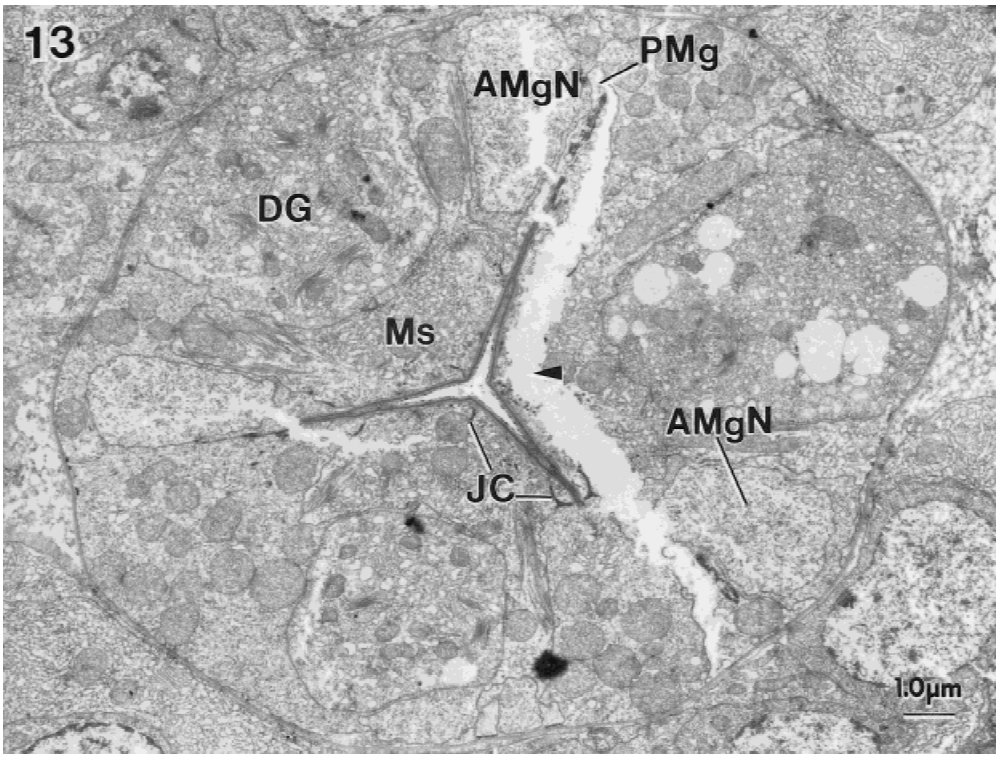
FIGS. 6–7. Transverse TEM sections of the anterior region of the postcorpus and the posterior end of the metacorpus of *Diplenteron* sp. 6). Isthmus (10 µm posterior to the metacorpus) showing three sectors separated by triradiate esophageal lumen (EL) and apical marginal cells (Mg). Arrowheads indicate junctional complexes. DG = dorsal gland; Ms = muscle cell; NP = nerve process; SvG = subventral gland. 7). Posterior end of metacorpus showing the two subventral gland ampullae (SvGA) and part of the subventral gland ducts (SvGD). Arrowhead indicates slight tissue tear in section. EL = esophageal lumen.

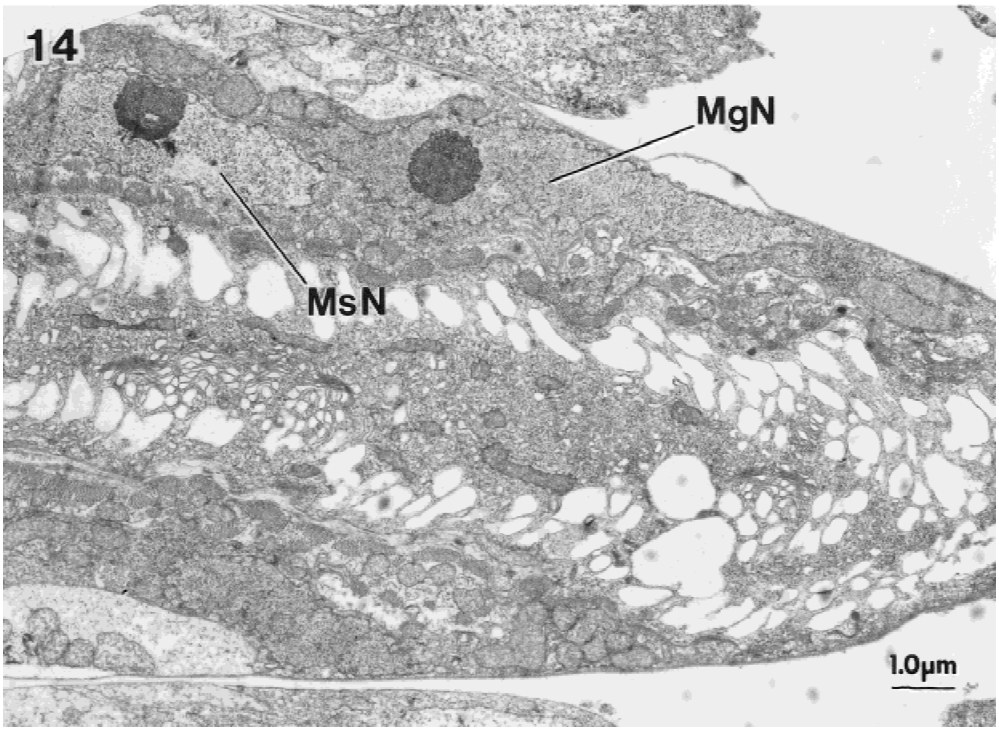


FIGS. 8–9. TEM sections of the postcorpus and transition with the metacorpus including glands of *Diplenteron* sp. 8). Transverse showing expanded region of dorsal gland (DG) and subventral gland (SvG) cells (65–70 µm posterior to metacorpus). Arrowhead indicates tissue tear. GS = Golgi stack; MsN = muscle nucleus; SvGN = subventral gland nucleus. 9). Longitudinal section showing the subventral gland ampulla (SvGA) and part of its gland duct (SvGD). Is = Isthmus.

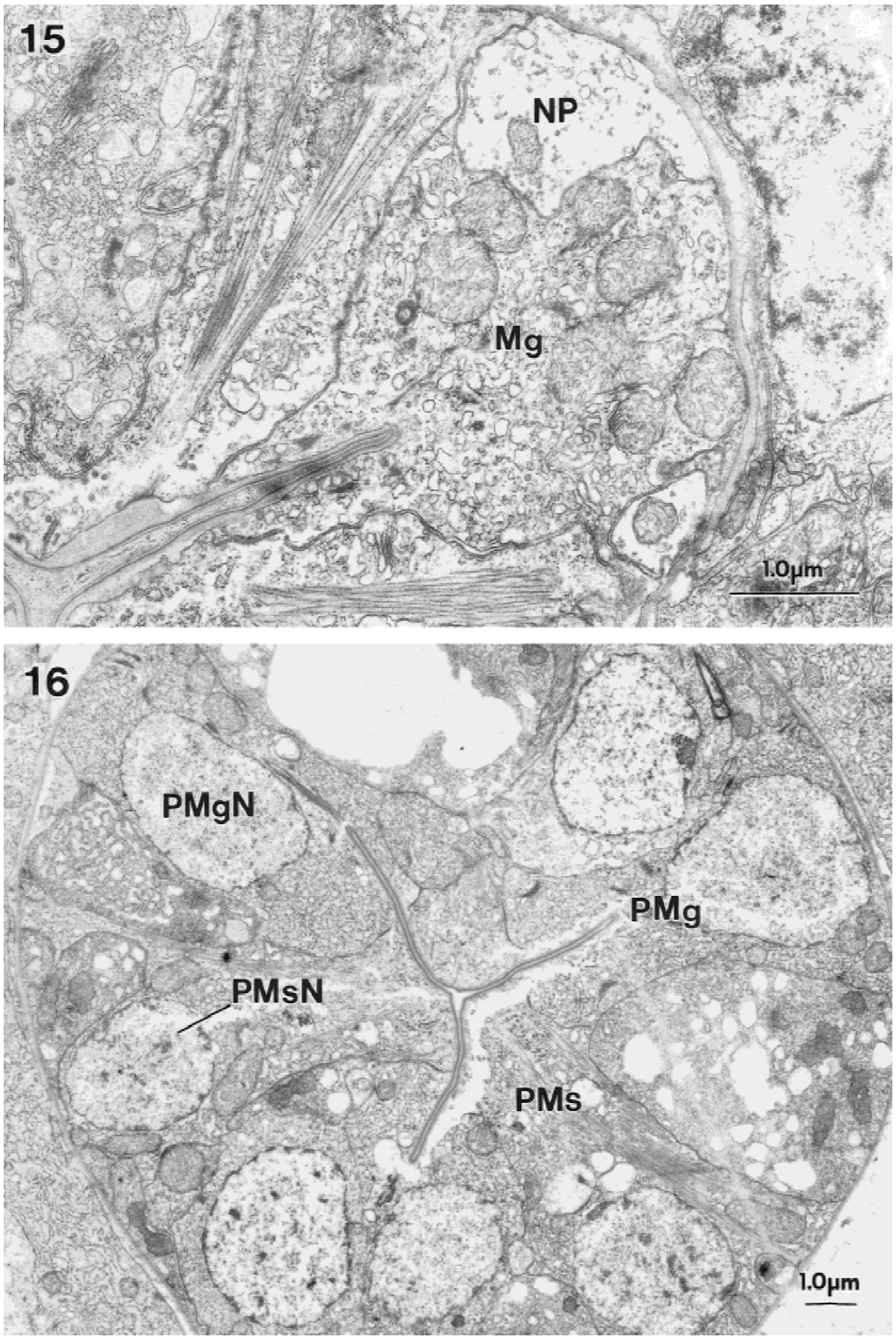


FIGS. 10–12. TEM micrographs of gland cells of *Diplenteron* sp. 10). Longitudinal section of the dorsal gland cell (70 µm posterior to metacorpus) with the dorsal gland nucleus (DGN) and nucleolus. 11). Transverse section of subventral gland (60–65 µm posterior to metacorpus) showing the subventral gland nucleus (SvGN) and nucleolus. 12). Longitudinal section through the subventral glands showing the two subventral gland nuclei (SvGN). Arrowhead indicates slight tissue tear. CL = concentric lamella.

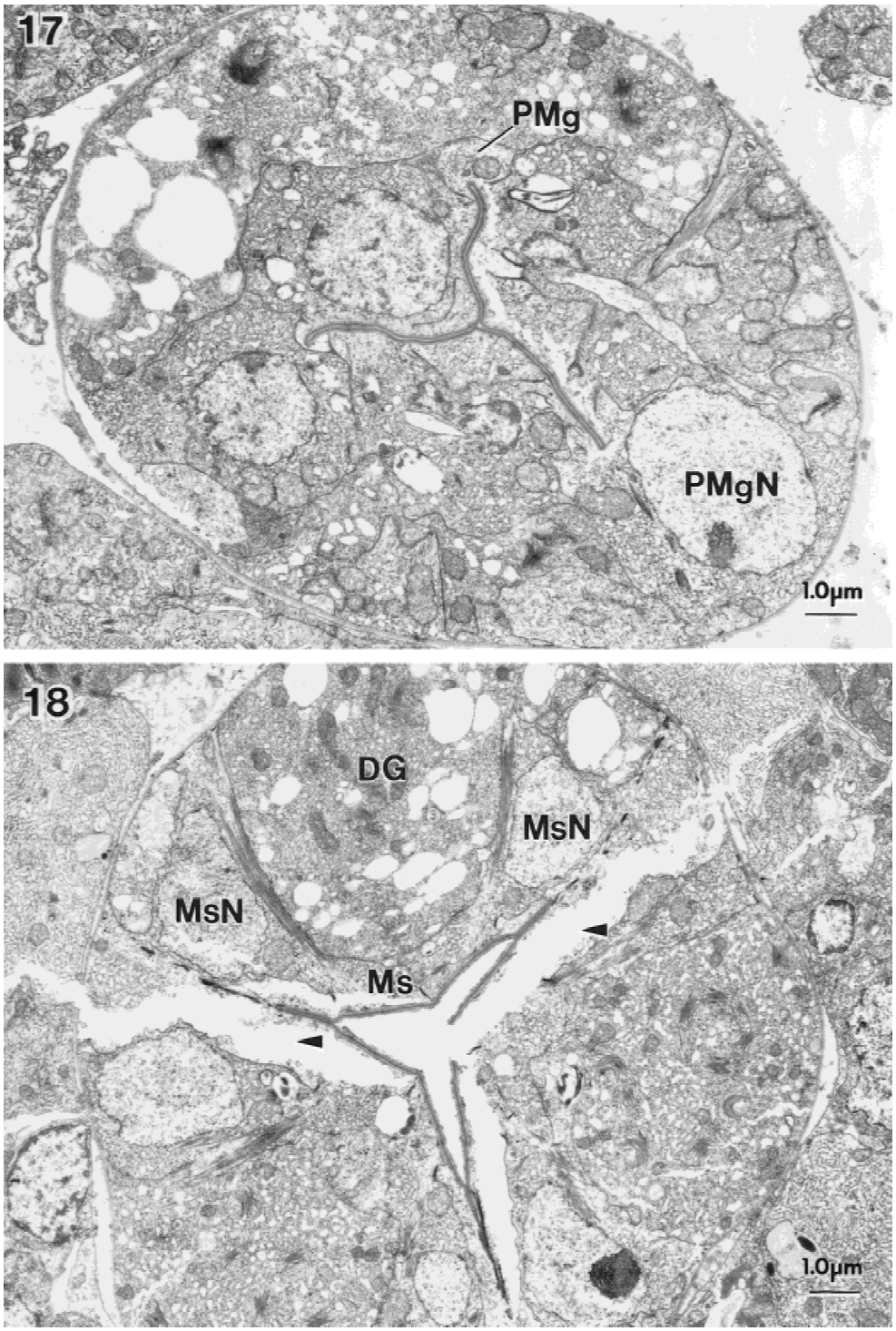




FIGS. 13–14. TEM micrographs of *Diplenteron* sp. showing the marginal and muscle cells. 13). Transverse section through postcorpus (45 μm posterior to metacorpus). Arrowhead indicates slight tissue tear in section. AMgN = anterior marginal cell nucleus; DG = dorsal gland; JC = junctional complex; Ms = muscle cell; PMg = posterior marginal cell. 14). Longitudinal section of the isthumus showing the elongated marginal cell nucleus (MgN) and muscle cell nucleus (MsN).



FIGS. 15–16. Transverse TEM sections of the postcorpus of *Diplenteron* sp. 15). Anterior end of the isthmus (14 µm posterior to metacorpus) showing the neuron innervation to a subventral marginal cell. Mg = marginal cell; NP = neuron process. 16). Basal bulb (75 µm posterior to metacorpus). PMg = posterior marginal cell; PMgN = posterior marginal cell nucleus; PMs = posterior muscle cell; PMsN = posterior muscle cell nucleus.



FIGS. 17–18. Transverse TEM sections through the posterior end and the middle of the basal bulb of *Diplenteron* sp. 17). Posterior end of basal bulb showing marginal cells. PMg = posterior marginal cell; PMgN = posterior marginal cell nucleus. 18). Middle of basal bulb (65 μm posterior to metacorpus) showing two nuclei in one muscle cell. Arrowheads indicate tissue tear. DG = dorsal gland; Ms = muscle cell; MsN = muscle cell nucleus.

bulb, and, while maintaining contact with the lumen, are otherwise nearly surrounded by the glands and other cells (Figs. 1E;13, 16,17). Nuclei, each with a small nucleolus, are not elongated in the posterior set of marginal cells (Figs. 1E;16,17). Neural synapses with these posterior marginal cells were not observed.

Muscles: Six radial muscle cells in the postcorpus occur in two sets of three. Three muscle cells of the anterior set (set 1) extend the entire length of the isthmus and about half the anterior end of the basal bulb; the posterior set (set 2) of radial muscles extends the length of the posterior half of the basal bulb (Fig. 1C,D). The three cells of each set are arranged around the esophageal lumen between the marginal cells in the dorsal and subventral sectors (Figs. 1E,F;6,13). The anterior set, in transverse section, is V-shaped, extends to the periphery of the esophagus, and encloses the cylindrical anterior process of a gland cell between two halves of the muscle, partly separated by a deep fold (Figs. 1C;6,13). Junctional complexes occur not only between muscle and adjacent marginal cells but also between the two halves of a single muscle cell near the center of the esophageal lumen (Figs. 6,13). A separate, elongate nucleus with a single nucleolus is found posteriorly in each half of each cell (Figs. 1C; 14,18). The posterior set of three radial muscle cells, in the basal bulb, is greatly reduced in comparison with the anterior set of muscle cells, and does not enclose the glands (Figs. 1D;16). Each cell, including a few bundles of contractile filaments, extends from the pharyngeal lumen to the periphery of the basal bulb and contains one nucleus (Figs. 1D;16).

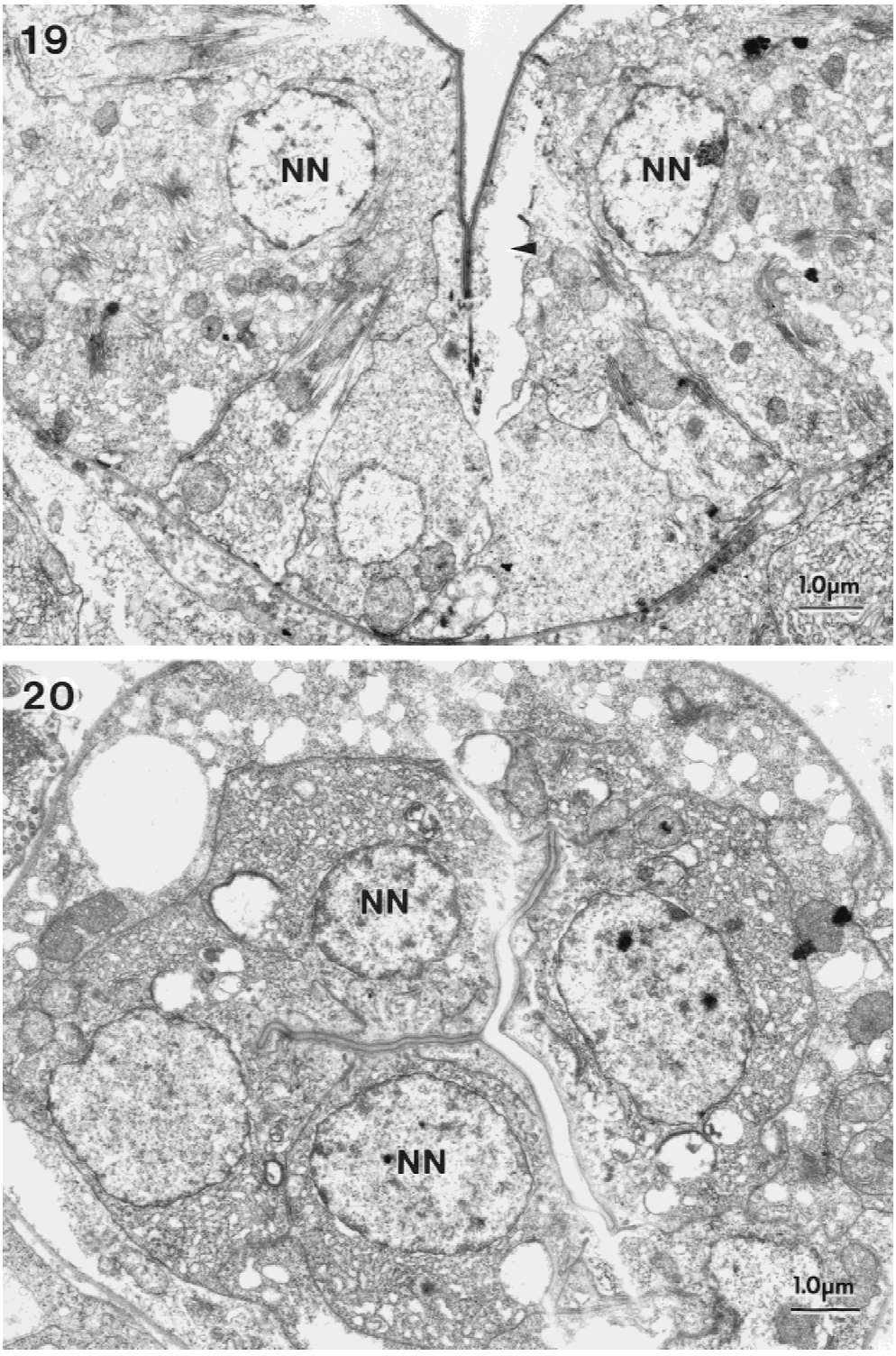
Neurons: The postcorpus includes 11 neurons counted on the basis of the distinctive round nuclei that typically include peripheral chromatin; these nuclei are surrounded by small cell bodies from which neural processes extend. Cell bodies of two neurons are located in the isthmus, slightly posterior to the nuclei of the anterior set of marginal cells (Fig. 19), and cell bodies of nine neurons occur far posterior in the basal bulb, near the esophageal-intestinal junction (Fig. 20). Nerve processes of these cells extend through the postcorpus in four regions as four chords; two nerve cords are associated with the dorsal gland and one nerve cord is associated with each subventral gland (Fig. 21). The neuron in the central dorsal sector apparently terminates in the isthmus about 14 µm from the anterior end of the postcorpus, with a synapse on both the dorsal gland and adjacent radial muscle. The two neurons in the peripheral dorsal position terminate about 24 µm from the posterior end of the metacorpus, where one of them synapses with the dorsal gland (Figs. 22,23).

The neural processes of the left subventral region extend to the anterior of the isthmus where one process lies on the side of the subventral gland adjacent to the lumen, and the additional three processes synapse between the subventral gland and adjacent radial muscle (Figs. 22,24). The right subventrla nerve cord is basically symmetrical with the left subventral nerve cord, but with three processes synapsing on the right subventral gland and the adjacent muscle cells (Figs. 22,25). In addition to these synapses, there are additional synapses between the left marginal cell and a muscle, as well as between the right marginal cell and a muscle cell (Figs. 22,26). The number of neuron bodies is not equal to the number of neural processes, which may indicate that some of the neural processes may originate from cell bodies in the metacorpus or there may be more than one neural process associated with a single cell body.

Esophago-intestinal valve: The postcorpus terminates in a region of five uninucleate, darkly staining, tightly linked epithelial cells that surround the lumen of the esophageal-intestinal junction and form a transition between the esophagus and the intestine (Fig. 27).

Discussion

Recent morphological (buccal capsule) and molecular studies contradict classical hypotheses that Diplogasterida is a sister group of Tylenchida (Andrássy, 1984;



FIGS. 19–20. Transverse TEM micrographs of *Diplenteron* sp. showing neuron nuclei. 19). Postcorpus (55 µm posterior to metacorpus). NN = neuron nucleus. Arrowhead indicates slight tissue tear. 20). Postcorpus just above esophageal-intestinal junction (75–80 µm posterior to metacorpus). NN = neuron nucleus.

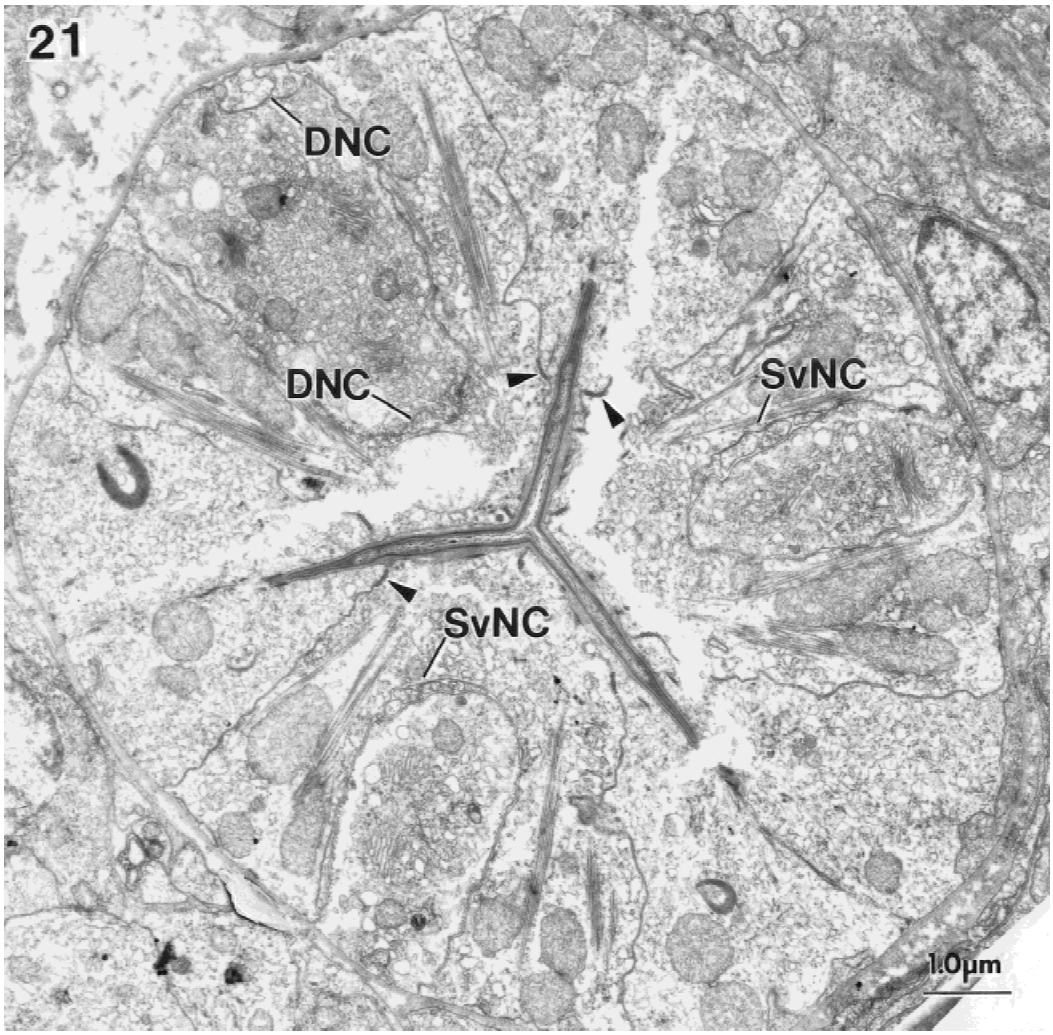
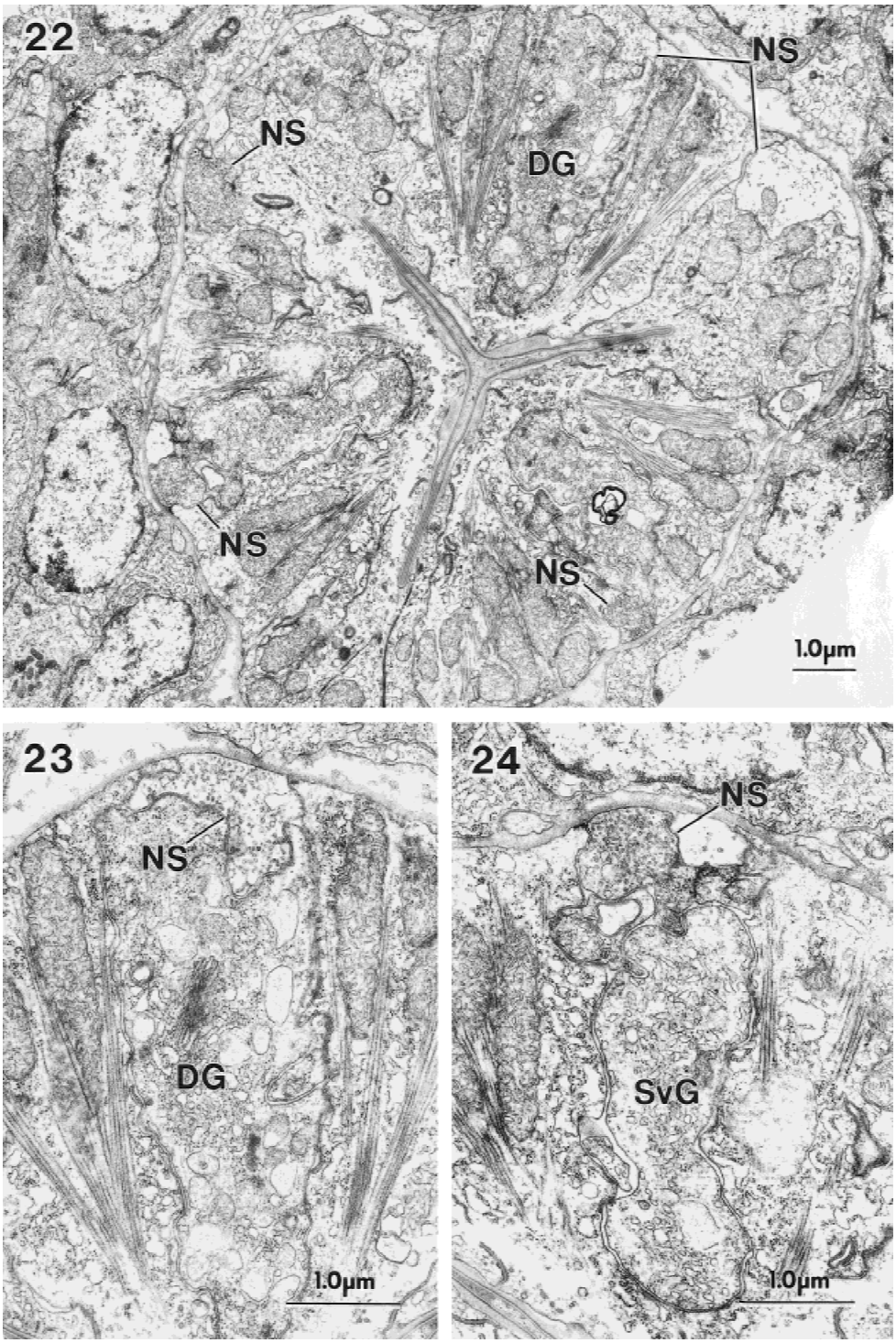
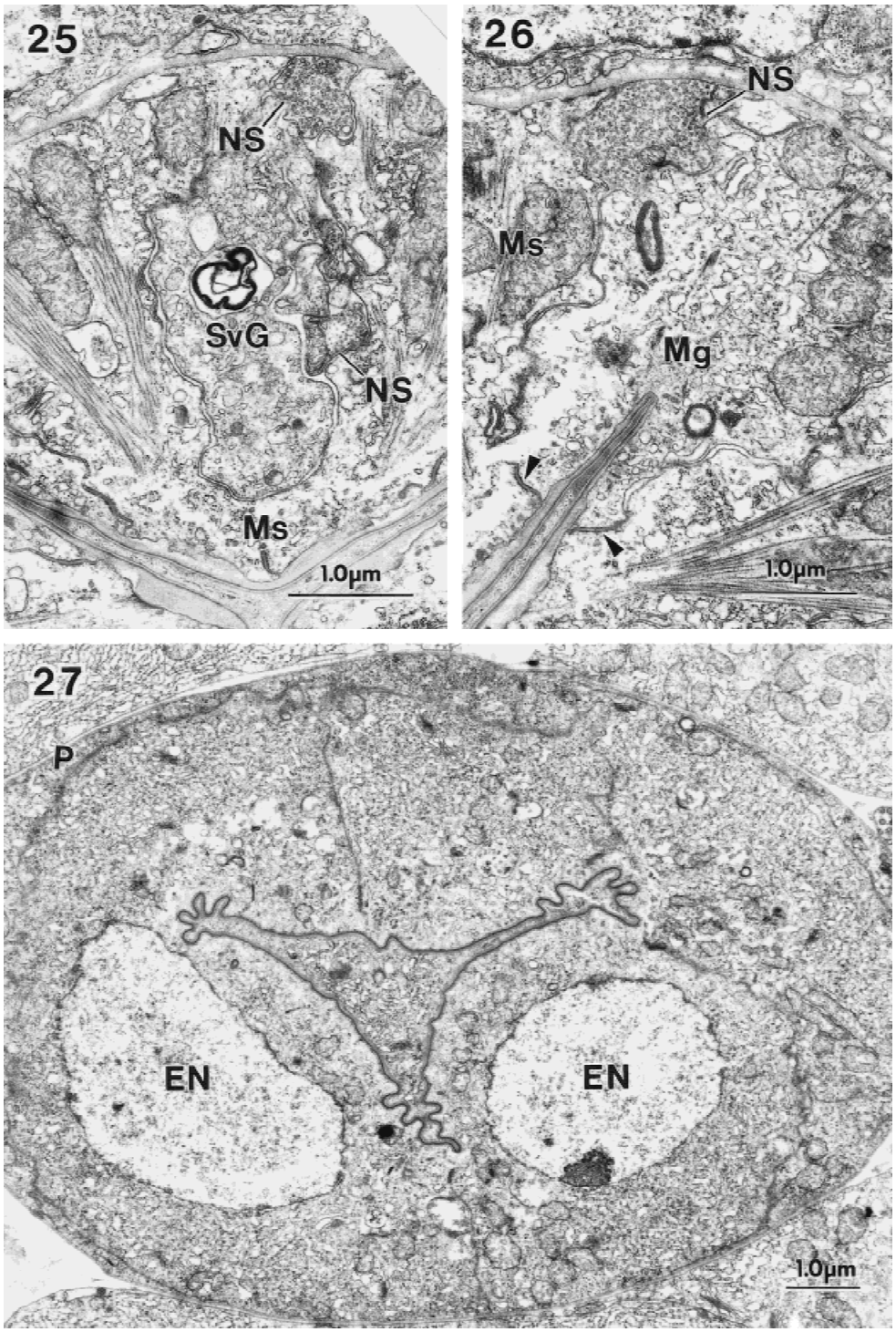


FIG. 21. Transverse TEM section through isthmus of *Diplenteron* sp. (30 µm posterior to metacorpus) showing four nerve cords. Arrowheads indicate junctional complexes. DNC = dorsal nerve cords; SvNC = subventral nerve cords.

Maggenti, 1981) and instead suggest Diplogasterida as a sister taxon of Rhabditina (Baldwin et al., 1997b; Blaxter et al., 1998). The sister relationship of Diplogasterida and Tylenchida has been largely defended on the basis of the shared characters of a ''glandular,'' valveless basal bulb versus a muscular basal bulb in Rhabditida. Our detailed investigation of the esophagus of *Diplenteron* sp. indicates, however, that the basal bulb region of this diplogasterid, while lacking a rhabditid-like grinder, is highly muscular relative to the basal bulb of Tylenchida. The investigation of the esophagus of *Diplenteron* sp. as a representative of Diplogasterida provides a starting point for comparison of esophageal characters of Rhabditida (i.e. *C. elegans*) and a few Tylenchida, previously reconstructed in varying detail with TEM (Albertson and Thomson, 1976; Baldwin et al., 1977; Endo, 1984; Shepherd and Clark, 1983). However, further insight into the relationship between morphological and molecular evolution of Secernentea requires expanding the investigation to include representatives of Cephalobina, and a more complete understanding of the esophagus in basal groups of Tylenchida as well as



FIGS. 22–24. Transverse TEM sections through anterior region of the isthmus of *Diplenteron* sp. showing neuron synapses. 22). Isthmus (5 µm posterior to metacorpus). NS = neuron synapse; DG = dorsal gland. 23). Nerve synapse (NS) (5 µm posterior to metacorpus) on dorsal gland (DG). 24). Nerve synapse (NS) (5 µm posterior to metacorpus) on left subventral gland (SvG).



FIGS. 25–27. Transverse TEM sections of isthmus and esophago-intestinal junction of *Diplenteron* sp. 25). Nerve synapses (NS) (5 µm posterior to metacorpus) on right subventral gland (SvG) and muscle cell (Ms). 26). Nerve synapse (NS) (5 µm posterior to metacorpus) on marginal cell (Mg) and muscle cell (Ms). Arrowheads indicate junctional complexes. 27). Esophago-intestinal junction showing the epithelial cells and their nuclei (EN) (83 µm posterior to metacorpus).

additional representatives of Diplogasterida and Rhabditina. Most importantly, knowledge of esophageal characters in outgroups is needed to distinguish those similarities that are conserved throughout Secernentea versus those that are shared due to a common phylogeny. Detection of characters that are similar due to convergent evolution can be aided by expanding the range of clearly resolved independent morphological characters, as well as by testing for congruence with independent molecular character sets.

Previous TEM reconstruction of the esophagus of *C. elegans* provides the most complete basis for comparison of *Diplenteron* sp. with Rhabditina and establishes homology of many features of the postcorpus of the two representatives. The postcorpus of *Diplenteron* sp., representative of Diplogasterida, is similar to that of Rhabditina in the way that the glands are embedded in a muscular framework. However, in *Diplenteron* sp., the muscles are reduced and the glands are enlarged relative to those of *C. elegans*. In *C. elegans* there are five glands based on the position of gland ducts: (i) one dorsal gland with an opening behind the buccal cavity, (ii) two subventral glands with openings at the posterior region to the metacorpus, and (iii) two additional subventral glands with openings in the basal bulb just anterior to the grinder. Although Albertson and Thomson (1976) referred to this arrangement in *C. elegans* as four gland cells with five nuclei (because the g1 dorsal gland cell fuses with another g1 right subventral gland cell), we consider the five gland nuclei and five gland orifices as indicative of five gland cells (see comments below on cell fusion). On the basis of position of gland ducts and openings, the three gland cells of *Diplenteron* sp. are interpreted as homologs of the dorsal and anterior pair of subventral glands (g1 cells) in *C. elegans*. A homolog of the second pair of subventral gland cells (g2) that opens into the lumen just anterior to the grinder in *C. elegans* is absent in *Diplenteron*. In addition, certain muscles (see below) apparently functionally linked to the grinder in *C. elegans* are absent in *Diplenteron*.

The marginal cells of *Diplenteron* sp. and *C. elegans* are epithelial, organized into two sets of three, and clearly homologous. Based on preliminary observations in a range of Secernentea, we suggest that the number and position of marginal cells is highly conserved and should be considered a symplesiomorphy in Diplogasterida and Rhabditina. Although these cells are similar in position to the marginal cells of the procorpus and buccal capsule (Baldwin and Eddleman, 1995; Baldwin et al., 1997b; Dolinski et al., 1998), in the posterior region of the basal bulb they do not extend to the margin of the esophagus (i.e. basement membrane) and, therefore, more precisely might be called apical cells since they are limited by position to the apices of the lumen lining. The position of marginal cells suggests that their function is structural, anchoring the esophageal lumen; however, synapses of neurons on marginal cells suggest additonal unknown functions.

The two sets of radial muscle cells of *Diplenteron* sp. are clearly homologous with similar sets of cells in *C. elegans,* although in *C. elegans* additional sets of muscles also are present. The anterior set of three muscle cells with six nuclei corresponds in position to the m5 cells in *C. elegans,* and the posterior set of three cells with three nuclei corresponds to the m6 cells in *C. elegans*. In both taxa, the three muscle cells of the anterior set are V-shaped in transverse section, with a nucleus in each of the two arms. Since binuceate cells in *C. elegans* and other nematodes are common, and often the result of two cells fusing late in development (Dolinski et al., 1998; Sulston et al., 1983), we hypothsize that the anterior set of three binucleate radial muscle cells developmentally begins as six cells that subsequently fuse. This hypothesis is further supported by the junctional complex apparently persisting adjacent to the lumen at the point of fusion.

Caenorhabditis elegans has two additional sets of radial muscle cells, designated m7 and m8, for which there are no homologs in *Diplenteron* sp. It is understandable that the three m7 muscle cells, which operate the grinder in the basal bulb of *C. elegans,* are not observed in *Diplenteron* sp., which lacks a

grinder. In contrast, m8 is a saucer-shaped cell that covers the posterior wall of the basal bulb in *C. elegans* but is not observed in *Diplenteron* sp. This m8 muscle cell appears to be closely associated with the esophageal-intestinal valve and might function to contract the bulb and move food to the intestine (Albertson and Thomson, 1976; Doncaster, 1962). The mode of action of ingestion of food and its transportion into the intestine may well be quite different in diplogasterids by comparison to that of Rhabditida (Doncaster, 1962).

Four nerve chords are the prominent features of neurons in the postcorpus, and the function of the many nerve processes in these cords is suggested by specific synapses on the dorsal and subventral glands, muscles, and marginal cells. We recognize 11 neurons in the postcorpus of *Diplenteron* sp., based on 11 nerve nuclei with characteristic cell bodies associated with long processes. However, it is technically difficult to trace irregular paths and branches of neurons with thin sections alone, and to make specific associations between synapses and a particular nerve process and nucleus. Furthermore, it is likely that some nerve processes in the postcorpus originate from cell bodies and nuclei anterior to the postcorpus, a region that was not reconstructed in this study. Recognizing these limitations, we nevertheless suggest that *Diplenteron* sp. has 11 nerve nuclei in the postcorpus, in contrast to seven neuronal bodies in the corresponding region of *C. elegans*. The issue of comparative innervation of the esophagus among Secernentea can be further addressed with technologies complementary to TEM, including computer reconstruction and neuron-specific staining.

It is difficult to interpret the junction of esophagus and the intestine in *Diplenteron* sp. as a true valve with an opening-closing mechanism controlled by muscle. Rather, the esophageal-intestinal junction structure, which consists of five darkly stained, tightly linked epithelial cells, is a transition to the intestine. Any opening-closing mechanism must be related to differential changes in pressure within the digestive tract, or perhaps to rapid osmotic or hydrostatic changes within the epithelial cells. Cells of the esophageal-intestinal junction of *Diplenteron* sp. are homologous to similar cells in *C. elegans*.

TEM reconstruction in representatives of Tylenchida, Cephalobina, and outgroups currently is inadequate to fully extend comparisons of most esophageal characters in *Diplenteron* sp. with these groups. One notable comparison, however, is that unlike *C. elegans* with five gland cells, representatives of Diplogasterida and Tylenchida typically have three gland cells (Baldwin et al., 1977; Endo et al., 1997; Shepherd and Clark, 1983). In the postcorpus of Tylenchida, muscles extend slightly, if at all, posterior to the isthmus (Baldwin et al., 1977; Endo, 1984), whereas in Diplogasterida, as in Rhabditina and Cephalobina (unpublished), muscles extend throughout the postcorpus. In *Diplenteron* sp., most of the postcorpal differences from Rhabditina apparently are interdependent and related to the loss of the grinder. Mapping these characters on extant molecular trees (Baldwin et al., 1997a, 1997b; Blaxter et al., 1998) requires that loss of the grinder occurred independently in Diplogasterida and Tylenchida. In Rhabditina and many other Secernentea, food is primarily concentrated and crushed by mechanical action, whereas in Diplogasterida and Tylenchida, to a large extent, food is digested enzymatically or by other chemical means (cf. massive gland development).

Literature Cited

Albertson, D. G., and J. N. Thomson. 1976. The pharynx of *Caenorhabditis elegans*. Philosophical Transactions of the Royal Society of London, Series B 275:299–325.

Andrássy, I. 1976. Evolution as a basis for the systematization of nematodes. London: Pitman Publishing.

Andrássy, I. 1983. A taxonomic review of the suborder Rhabditina (Nematoda: Secernentea). Paris: ORSTOM.

Andrássy, I. 1984. Klasse Nematoda. Berlin: Akademie-Verlag.

Baldwin, J. G. 1992. Evolution of cyst and noncyst-forming Heteroderinae. Annual Review of Phytopathology 30:271–290.

Baldwin, J. G., and C. D. Eddleman. 1995. Buccal capsule of *Zeldia punctata* (Nematoda: Cephalobidae): An ultrastructural study. Canadian Journal of Zoology 73:648–656.

Baldwin, J. G., L. M. Frisse, J. T. Vida, C. D. Eddleman, and W. K. Thomas. 1997a. An evolutionary framework for the study of developmental evolution in a set of nematodes related to *Caenorhabditis elegans* (Nemata: Rhabditina). Molecular Phylogenetics and Evolution 8: 249–259.

Baldwin, J. G., R. M. Giblin-Davis, C. D. Eddleman, D. S. Williams, J. T. Vida, and W. K. Thomas. 1997b. Buccal capsule of *Aduncospiculum halicti* (Nematoda: Diplogasterina): An ultrastructural and molecular phylogenetic study. Canadian Journal of Zoology 75:407–423.

Baldwin, J. G., H. Hirschmann, and A. C. Triantaphyllou. 1977. Comparative fine structure of the esophagus of males of *Heterodera glycines* and *Meloidogyne incognita*. Nematologica 23:239–252.

Blaxter, M. L., P. De Ley, J. R. Garey, L. X. Liu, P. Scheldman, A. Vierstraete, J. R. Vanfleteren, L. Y. Mackey, M. Dorris, L. M. Frisse, J. T. Vida, and W. K. Thomas. 1998. A molecular evolution framework for the phylum Nematoda. Nature 392:71–75.

Chitwood, B. G., and M. B. Chitwood. 1950. An introduction to nematology. Baltimore, MD: Monumental Printing.

De Ley, P., M. C. Van de Velde, D. Mounport, P. Baujard, and A. Coomans. 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae, and Rhabditidae with a proposal for a revised stoma terminology in Rhabditida (Nematoda). Nematologica 41:153–182.

Dolinski, C., G. Borgonie, R. Schnabel, and J. G. Baldwin. 1998. Buccal capsule development as a consideration for phylogenetic analysis of Rhabditida (Nemata). Developmental Genes and Evolution 208: 495–503.

Doncaster, C. C. 1962. Nematode feeding mechanisms. 1. Observation on *Rhabditis* and *Pelodera*. Nematologica 8:313–320.

Endo, B. Y. 1984. Ultrastructure of the esophagus of larvae of the soybean cyst nematode, *Heterodera glycines*. Proceedings of the Helminthological Society of Washington 51:1–24.

Endo, B. Y., U. Zunke, and W. P. Wergin. 1997. Ultrastructure of the lesion nematode, *Pratylenchus penetrans* (Nematoda: Pratylenchidae). Journal of the Helminthological Society of Washington 64:59–95.

Goodey, J. B. 1963a. Speculations on the identity of the parts of the tylenchid spear. Nematologica 9:468–470.

Goodey, J. B. 1963b. Soil and freshwater nematodes. New York: John Wiley and Sons.

Hayat, M. A. 1993. Stains and cytochemical methods. New York: Plenum Press.

Hodgkin, J., H. R. Horvitz, B. R. Jasny, and J. Kimble. 1998. *C. elegans*: Sequence to biology. Science 282:2011.

Maggenti, A. 1981. General nematology. New York: Springer-Verlag.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal of Cell Biology 17:208–212.

Riddle, D. L., T. Blumenthal, B. J. Meyer, and J. R. Priess, eds. 1997. *C. elegans* II. Plainview, NY: Cold Spring Harbor Laboratory Press.

Shepherd, A. M., and S. A. Clark. 1976. Structure of the anterior alimentary tract of the passively feeding nematode *Hexatylus viviparus* (Neotylenchidae: Tylenchida). Nematologica 22:332–342.

Shepherd, A. M., and S. A. Clark. 1983. A reexamination of oesophageal ultrastructure in *Ditylenchus dipsaci* (Nematoda, Tylenchida) with some observations on intestinal structure. Nematologica 29: 151–170.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31–43.

Steiner, G. 1933. The nematode *Cylindrogaster longistoma* (Stefanski) Goodey, and its relationship. Journal of Parasitology 20:66–68.

Sulston, J. 1988. Cell lineage. Pp. 123–156 *in* W. B. Wood, ed. The nematode *Caenorhabditis elegans*. Plainview, NY: Cold Spring Harbor Laboratory Press.

Sulston, J. E., and H. R. Horvitz. 1977. Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Developmental Biology 56:110–156.

Sulston, J. E., E. Schierenberg, J. G. White, and J. N. Thomson. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Developmental Biology 100:64–119.

Thorne, G. 1961. Principles of nematology. New York: McGraw-Hill.

Wood, W. B. 1988. The nematode *Caenorhabditis elegans*. Plainview, NY: Cold Spring Harbor Laboratory Press.

Yuen, P. H. 1968. Electron microscopical studies on *Ditylenchus dipsaci*. II. Esophagus. Nematologica 14:554–564.