Journal of Nematology 17(2):175–186. 1985.

Ultrastructure of the Phasmids of *Scutellonema brachyurum*[1]

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Abstract: Electron microscopical studies reveal that the phasmids of *Scutellonema brachyurum* include socket and sheath cells which appear to be secretory. The phasmid includes an external cup-shaped ampulla filled with electron-dense material which may be lipopolysaccharide. The ampulla is continuous, with a receptor cavity surrounded by the socket and sheath cells. The dendrite receptor originates from a neuron and directs posteriorly through a receptor socket into the receptor cavity ending near the electron-dense plug. Serial sections through the dendrite receptor indicate that it does not conform to the typical 9+2 arrangement of microtubules characteristic of cilia in higher animals.

Key words: receptor socket, morphology, phasmid, receptor cavity, sensory organ, transmission electron microscopy.

Cobb (1) proposed the term phasmid for each of the pair of small lateral organs in the caudal region of nematodes, and it has been used as an important characteristic for nematode classification. Electron microscopy of phasmids includes animal parasites *Necator americanus* (10), *Baylisascaris tasmaniensis* (18), *Ophidascaris papuanus* (17), *Dracunculus medinensis* (11,12), the microfilariae of *Dirofilaria immitis, Dipetalonema viteae, D. setariosum, Loa loa, Litomosoides carinii* (5–9), and the free-living nematode *Caenorhabditis elegans* (21).

Phasmids are considered sensory organs because they are innervated by dendrite receptors (16). Cytochemical techniques using light and electron microscopy have detected esterases (10,12,15) and cholinesterase in phasmids (9).

Coomans and DeGrisse (2) recently published some electron micrographs of phasmid openings of *Rotylenchus robustus, Hoplolaimus* sp., and *Scutellonema* sp., but no detailed ultrastructural studies of the phasmids of plant-parasitic nematodes have been reported. Therefore, our objective was to elucidate the fine structure of the phasmid of a plant-parasitic nematode.

Scutellonema brachyurum was chosen because it is an important plant-parasitic nematode and its phasmids are large. Males of *S. brachyurum* are rare, one occurring for about every 6,000 females in our greenhouse culture; thus, only three males were collected. Data presented here are from a single male specimen. Therefore, they do not represent the average data for male nematodes of this species.

The terms dendrite, socket cell, sheath cell, and receptor cavity used here are adopted from Coomans and DeGrisse (2) and Wright (25,26).

MATERIALS AND METHODS

Adult male and female *Scutellonema brachyurum* (Steiner, 1938) Andrassy, 1958 were propagated on jade plants, *Crassula argentea,* in a greenhouse and prepared for transmission electron microscopic examination.

To avoid distension of the phasmids or receptor cavities by hypertonic solutions (12,14,20), the nematodes were killed in the vapor of 2% osmium tetroxide for 30 minutes before further fixation. A slightly dilated receptor was obtained by cutting the posterior body of the nematodes in a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M phosphate buffer at pH 7.3, 5–10 minutes after the specimens were incubated in the fixative. Fully dilated receptor cavities were achieved by cutting the specimen after 20 minutes in the fixative mixture. Usually all specimens fixed in the 2% glutaraldehyde–2% paraformaldehyde mixture for 30–90 minutes at 22–24 C were transferred to a 10- × 75-mm test tube and rinsed three

Received for publication 30 August 1984.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers University. This work was performed as a part of NJAES project D–11282–8–84. Portion of a dissertation submitted by the senior author in partial fulfillment of the requirement for the Ph.D. degree, Rutgers University.

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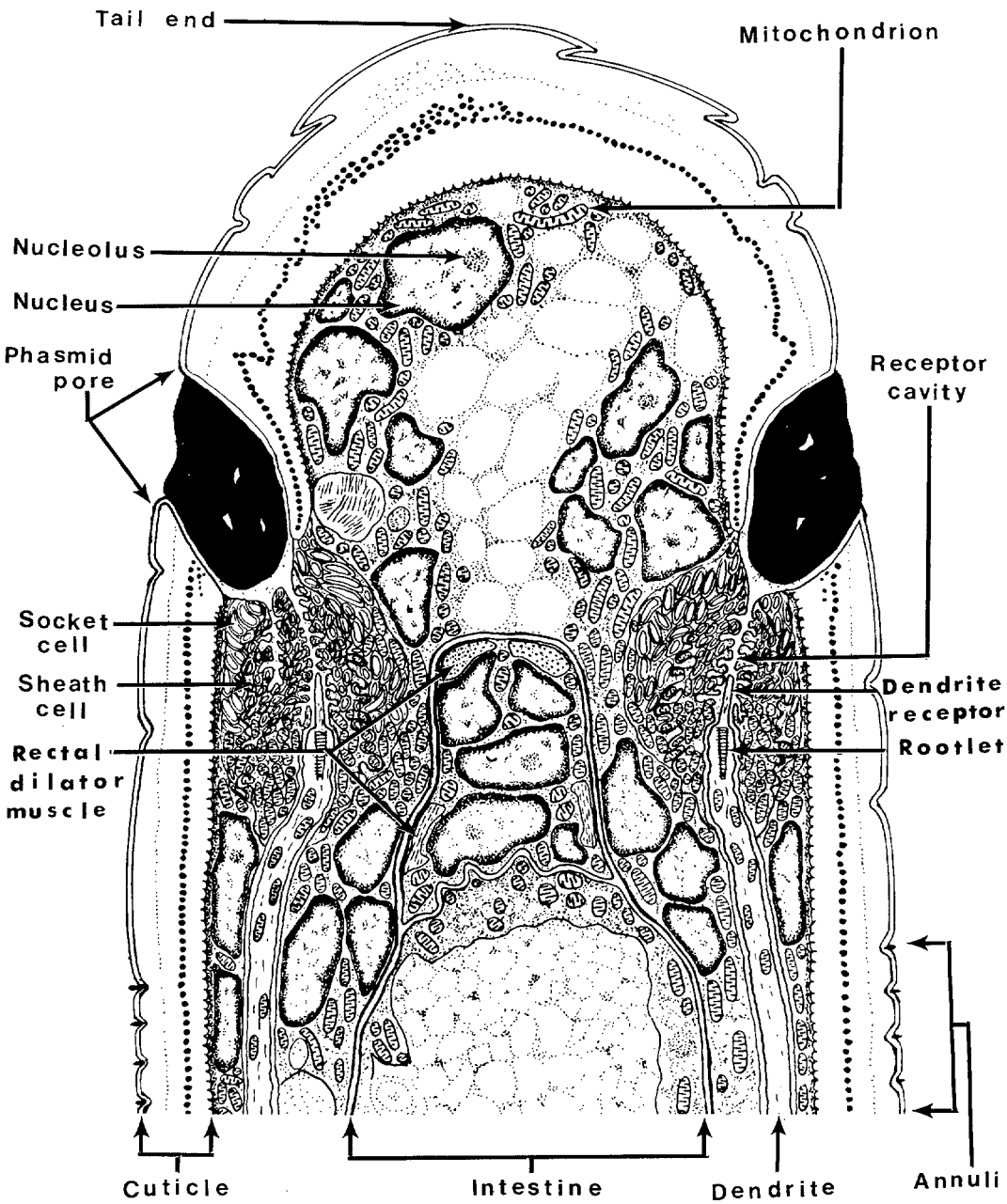

FIG. 1. Composite median dorsoventral view of the tail region of a female *Scutellonema brachyurum* showing phasmids and associated structures.

times with 0.05 M phosphate buffer at 20-minute intervals. The specimens were subsequently fixed in 1% osmium tetroxide in 0.05 M phosphate buffer at pH 7.3 for 2 hours at 22–24 C and then incubated in phosphate buffer for 1 hour. The specimens were then dehydrated with ethanol and infiltrated with Spurr's embedding medium (19). Sections (70–80 nm) were cut with a diamond knife and stained with 4% uranyl acetate in 50% ethanol for 10 minutes. Sections were washed in 50% ethanol and rinsed with distilled water for 3 minutes before staining with lead citrate and examination with an electron microscope at 80 kV.

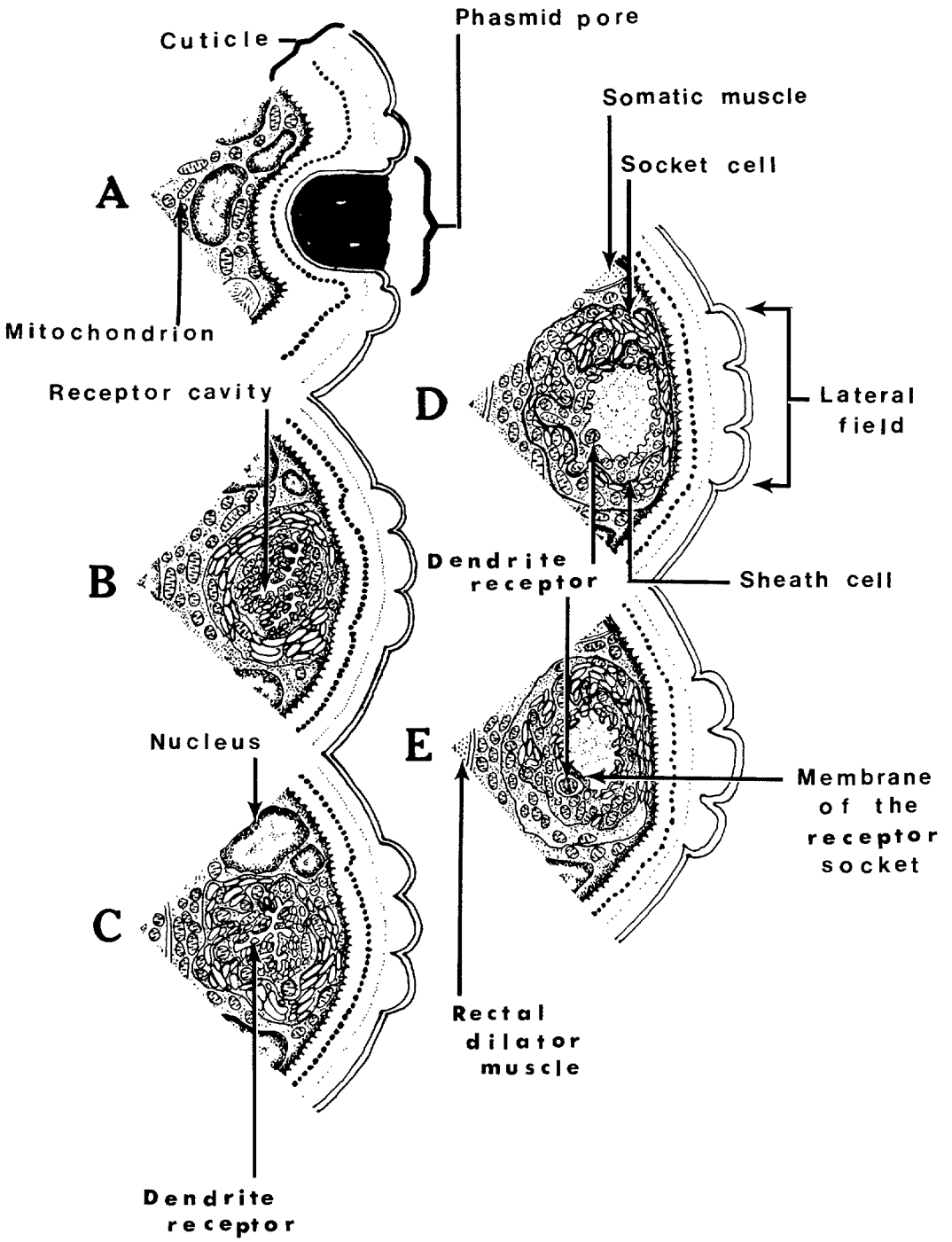

FIG. 2. Diagrams of quadrants of progressive transverse sections through phasmids of a female *Scutellonema brachyurum*. A) Region of phasmid pore. B) Receptor cavity portion to the dendrite receptor. C) Receptor cavity including dendrite receptor. D) Dilated receptor cavity including dendrite receptor. E) Region including receptor socket adjacent to dilated receptor cavity.

RESULTS

The pair of enlarged phasmids of *S. brachyurum* are situated opposite one another in the lateral field of the tail region (Fig. 1). The distance between the tail tip and phasmids is 6–9 μm in females and

about 12 μm in males. Each phasmid consists of an ampulla filled with electron-dense material, a receptor cavity surrounded by a socket cell, a sheath cell, and a dendrite receptor which penetrates the receptor cavity (Figs. 1, 2). The external openings of the phasmid pores are 2–3 μm d in females and about 2 μm d in males. Sometimes round to oval-shaped electron-dense material is present on the surface of the opening (Fig. 12H).

Electron-dense plug: The cup-shaped ampulla, which is formed by an infolding of the cuticle, contains a plug of electron-dense material (Figs. 1, 3–5). A duct leading from the ampulla extends anteriorly at an angle of about 45 degrees to the body surface in females (Figs. 3–5) and about 90 degrees in males (Fig. 6). These cylindroid structures are 1.6–2.9 μm long on the anterior sides and 3–5 μm on the posterior sides in females (Figs. 3–5) and about 1.9 μm on both sides in males (Fig. 6). The electron-dense plug is a complex compact fine net-like material (Figs. 3–6).

Receptor cavity: The receptor cavity is continuous with the ampulla. Each cavity is lateral to the rectal musculature. Its distal end which terminates near the electron-dense plug is surrounded by the socket cell. The rest of the main cavity is enclosed by the sheath cell. Cavity walls are composed of membranous chambers and numerous deeply invaginated membrane lamellae opening into the cavity (Figs. 2–4, 6–8). The distance between the electron-dense plug and the bottom of the cavity is 3–4 μm in females and about 2 μm in males. In somewhat hypertonic environments (e.g., mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M phosphate buffer or 0.34 M NaCl) the receptor cavity distended to varying degrees (Figs. 2D, E, 4, 5, 7–10). When the phasmid is not distended, the cavity is slit-like and enclosed by the sheath cell which in turn is enclosed by the anterior portion of the socket cell. The fully distended receptor cavity may be 3.8–4.1 μm long and 2.7–3.0 μm in diameter.

Dendrite receptor: Within the bottom of the receptor cavity, a dendrite receptor originating from the dendrite of the neurocyte is enclosed by the sheath cell and extends into the cavity (Figs. 1, 5, 6). The plasmalemma of the sheath cell surrounds and creates a tight junction with that of the dendrite to form a receptor socket with a diameter of 0.8 μm when the cavity is fully dilated (Figs. 2E, 5, 10). The dendrite receptor tapers slightly to the distal tip (Fig. 6). The diameter of its base is 0.3–0.4 μm, whereas that of the distal tip is only 0.1–0.2 μm in both females and males. The length of the dendrite receptor is 2.1–2.8 μm in females and about 2.2 μm in males.

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FIG. 3. Longitudinal section through the median region of the undilated phasmids of a female *Scutellonema brachyurum* showing electron-dense plug (EDP), socket cell (So C), sheath cell (Sh C), receptor cavity (Re C), mitochondria (Mi), and rectal dilator muscle (RDM).

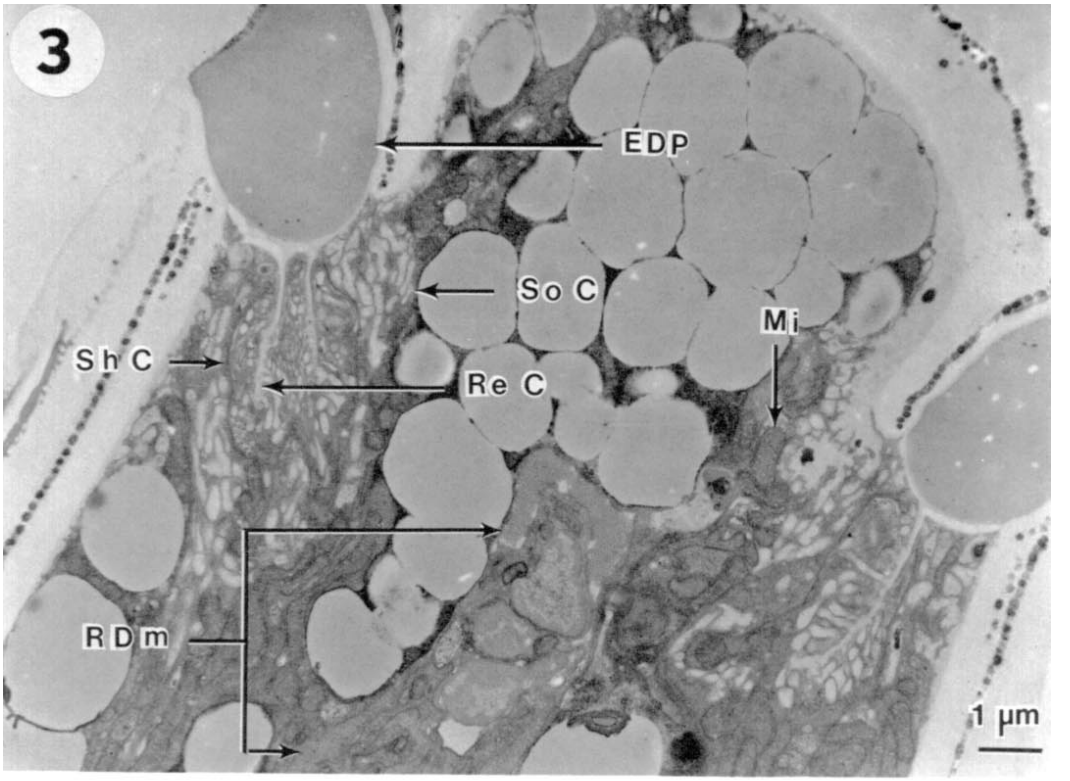
FIG. 4. Longitudinal section through the median region of the slightly dilated phasmids of a female *Scutellonema brachyurum* showing the slightly dilated receptor cavity (Re C), receptor rootlet (Re R), dendrite (De), nucleus (Nu), and electron-dense plug (EDP).

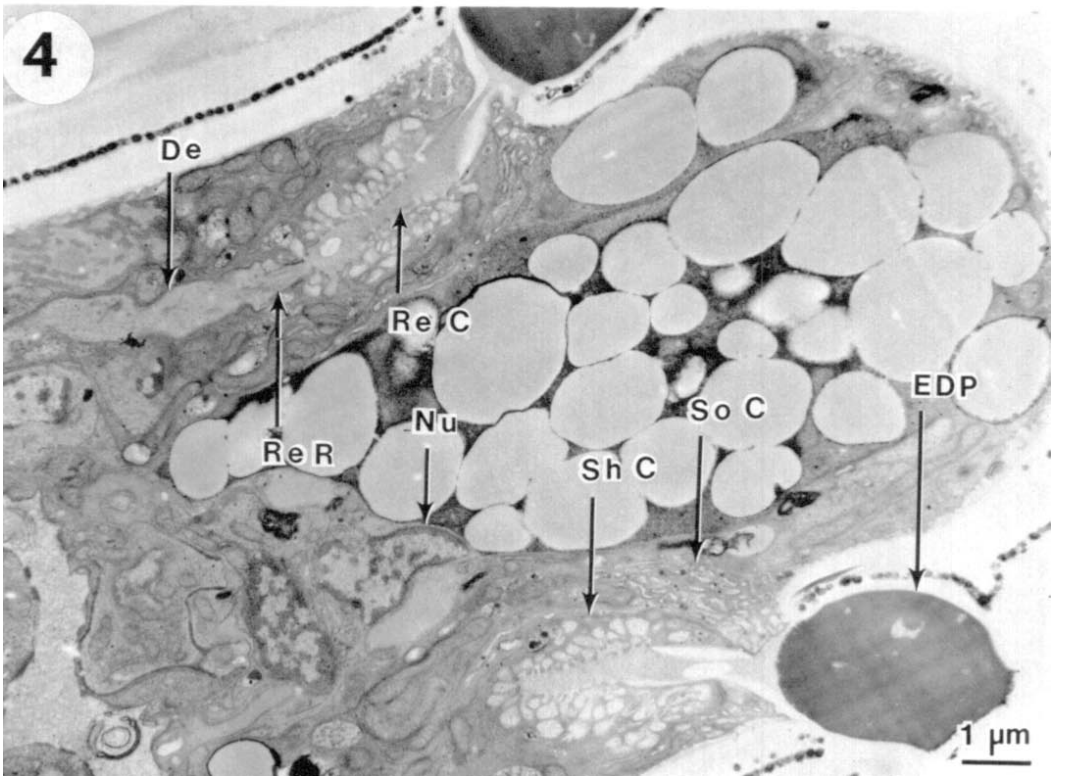
FIG. 5. Slightly oblique section through the receptor socket region of the dilated phasmids of a female *Scutellonema brachyurum* showing phasmid pore (Ph P), cuticle (Cu), socket cell (So C), sheath cell (Sh C), dilated receptor cavity (Re C), dendrite receptor (De R), membrane of the receptor socket (MRS), dendrite (De), rectal dilator muscle (RDM), nucleus (Nu), and intestine (In).

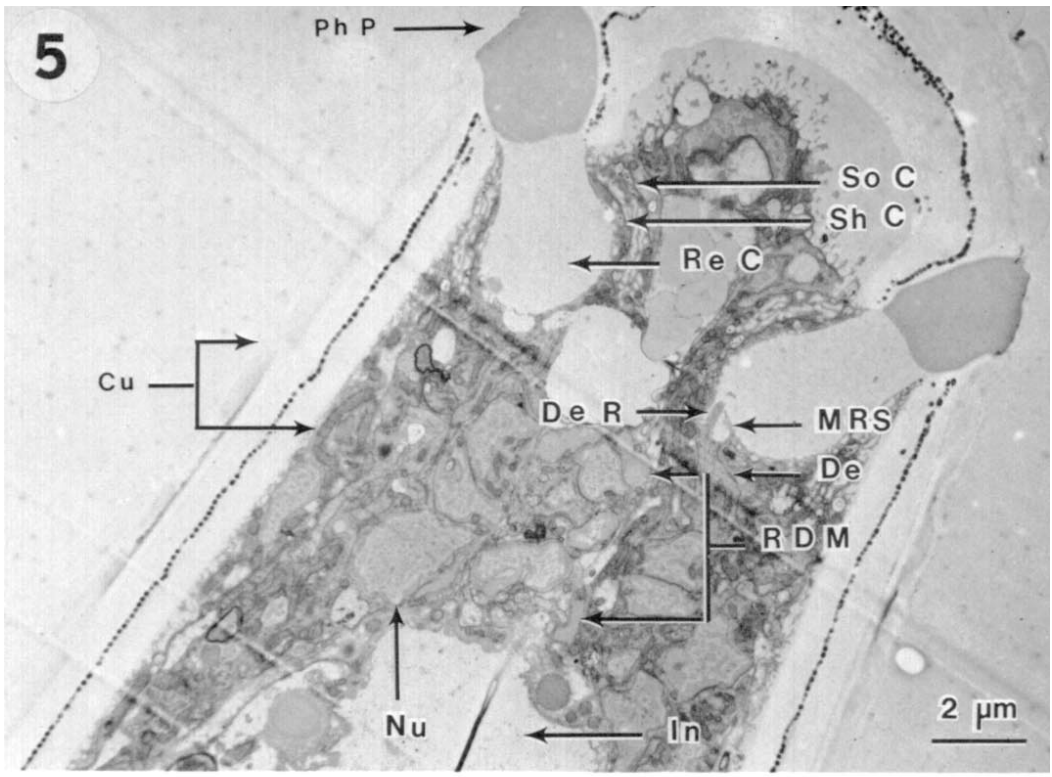
FIG. 6. Longitudinal section through the sublateral region of the middle of the phasmid of a male *Scutellonema brachyurum* showing thick cuticle (Cu), electron-dense plug (EDP), slightly dilated receptor cavity (Re C), dendrite receptor (De R), receptor rootlet (Re R), dendrite (De), and nucleus (Nu).

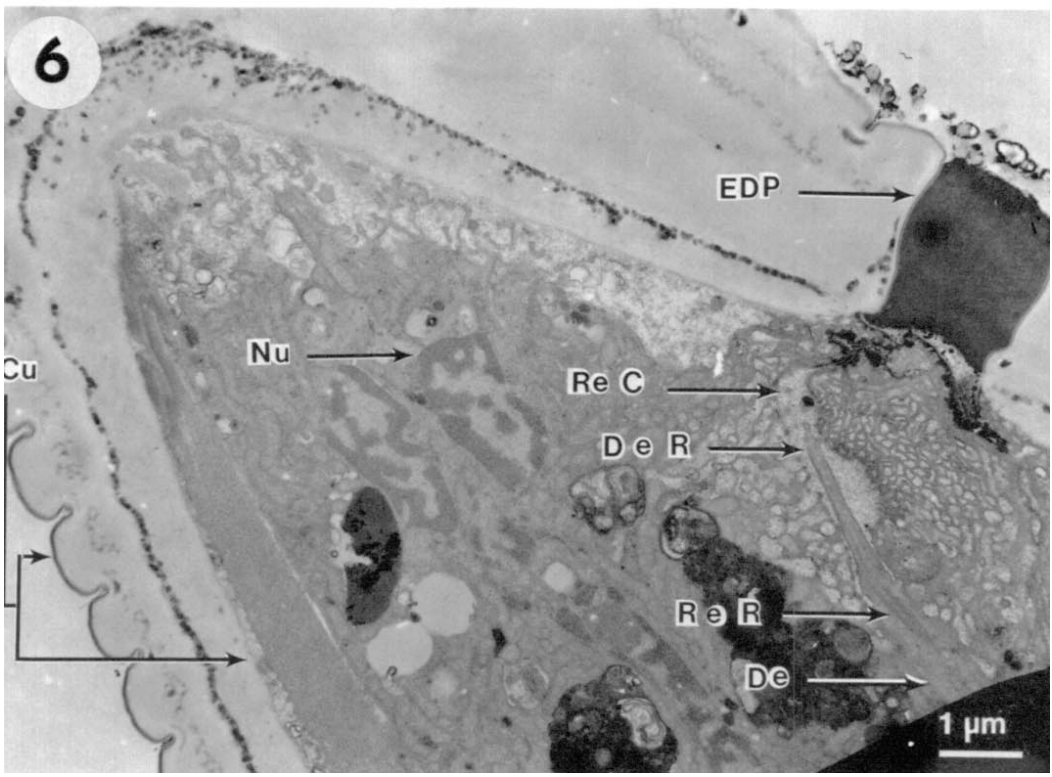
FIG. 7. Longitudinal section through slightly dorsal region of the phasmids of a female *Scutellonema brachyurum* showing two of the four rectal dilator muscle (RDM) groups fused together, socket cell (So C), sheath cell (Sh C), receptor cavity (Re C), receptor rootlet (Re R), mitochondria (Mi), intestine (In), and Nucleus (Nu).

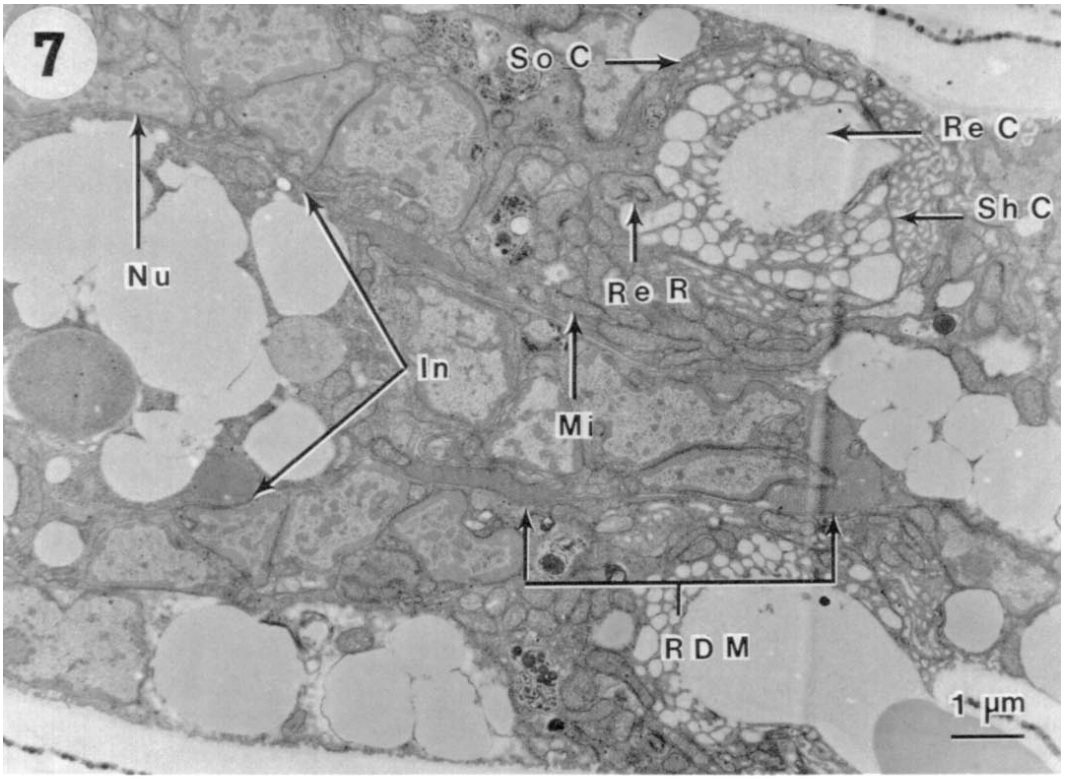
FIG. 8. A close-up view of the phasmid of a longitudinal section through slightly dorsal region of the phasmid of a female *Scutellonema brachyurum* showing rectal dilator muscles (RDM), electron-dense plug (EDP), socket cell (So C), sheath cell (Sh C), receptor cavity (Re C), nucleus (Nu), and nucleolus (Nuc).

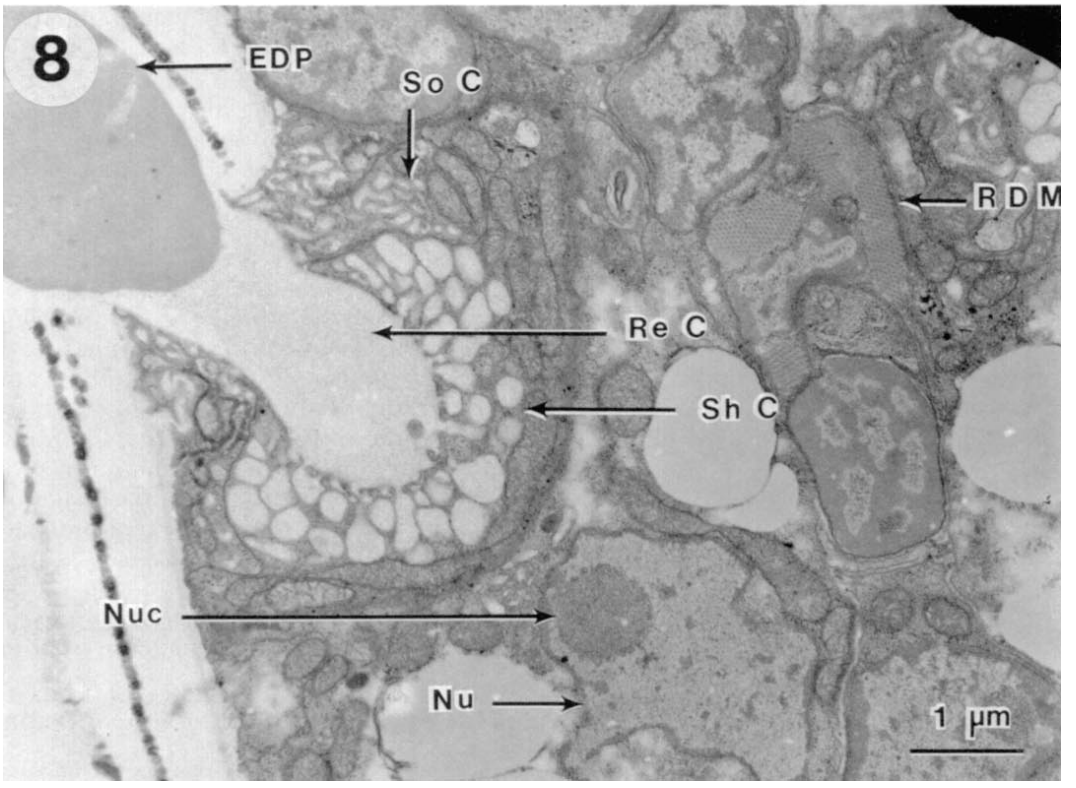

3
EDP
So C
Mi
Sh C
Re C
RDm
1 µm


4
De
Re C
EDP
So C
Nu
ReR
Sh C
1 µm


5
Ph P
So C
Sh C
Re C
Cu
De R
MRS
De
R D M
Nu
In
2 µm


6
EDP
Nu
Cu
Re C
De R
Re R
De
1 µm


7
So C
Re C
Sh C
Nu
Re R
In
Mi
R D M
1 µm


8
EDP
So C
R D M
Re C
Sh C
Nuc
Nu
1 µm

The number and arrangement of dendrite receptor microtubules deviate from the characteristic 9+2 pattern. In fact, singlets seem to increase in number toward the distal end and doublets increase in number toward the base of the receptor. Various combinations of singlets and doublets from the distal end to the base were observed and are illustrated as 1+8, 3+8, 3+7, 3+6, and 8+3 in Figure 12A–F. A pattern of eight peripheral doublets of microtubules is found in the basal region. These doublets are attached to the outside of a central fibrillar ring while three singlets are situated internally and connected to the ring. A fibrillar strand extends outward from the center of the doublets to the receptor membrane (Fig. 12G). No typical basal bodies with triplets have been found. The receptor base is supported by the regularly cross-banded rootlet of the dendrite which extends anteriorly. The transitional regions between the receptor and dendrite are characterized by the presence of tight junctions, rootlets, mitochondria, and vesicles (Figs. 1, 4–6).

Socket cell: The socket cells is elongated and tubular connecting the hypodermis with the rectal dilator muscles. The socket cell lies anteriorly, and terminates somewhere close, to the intestine (Figs. 1, 3, 5, 7). Most organelles seem to be concentrated in the basal region of the socket cell near the posterior tip of the intestine where numerous mitochondria and the nucleus occur. Membranous chambers encircle the tip of the receptor cavity and the sheath cell. Granular material occurs throughout the length of the cell. Some nonmembrane-bounded granules appear to be delivered into the receptor cavity and may contribute to the electron-dense plug (Figs. 3, 4).

Sheath cell: The sheath cell is irregularly shaped and encloses the main receptor cavity, including the small receptor socket. The plasmalemma of the sheath cell is deeply invaginated and interdigitates with that of the socket cell (Figs. 3, 4, 11). These processes of the cells are particularly prominent at the basal portion of the cell. The plasmalemma lining the receptor cavity is deeply invaginated, resulting in a membranous chamber. The sheath cell is darkly stained and thus is easily distinguished from surrounding cells (Figs. 1–3, 8). Organelles include a nucleus, Golgi, mitochondria, and membrane-bound vesicles (Fig. 11).

Discussion

Phasmids of *S. brachyurum* are basically similar to those of animal-parasitic and free-living nematodes. Certain differences—such as number of dendrite receptors, the receptor socket around the base of the receptor, large ampulla, and the interdigitating plasmalemma of the socket and sheath cells—seem to be unique.

The socket cell encircles not only the distal end of the receptor cavity, but also the sheath cell. This is in contrast to earlier reports (3,26) that the socket cell of the nematode sensillium distally surrounds only the sensillar pouch or channel, but not the sheath cell.

The granular materials found throughout the length of the socket cell as well as some nonmembrane-bounded granules indicate that they are being emitted or secreted into the tip of the receptor cavity and may serve to replenish the electron-dense plugs. Thus, the socket cell may be glandular. On the other hand, although no such granules were observed in the sheath cell, there are membranous chambers and numerous deeply invaginated lamellae opening into the receptor cavity. Uniformly fine substances filling the main receptor cavity are identical to those that make up the membranous chambers and lamellae.

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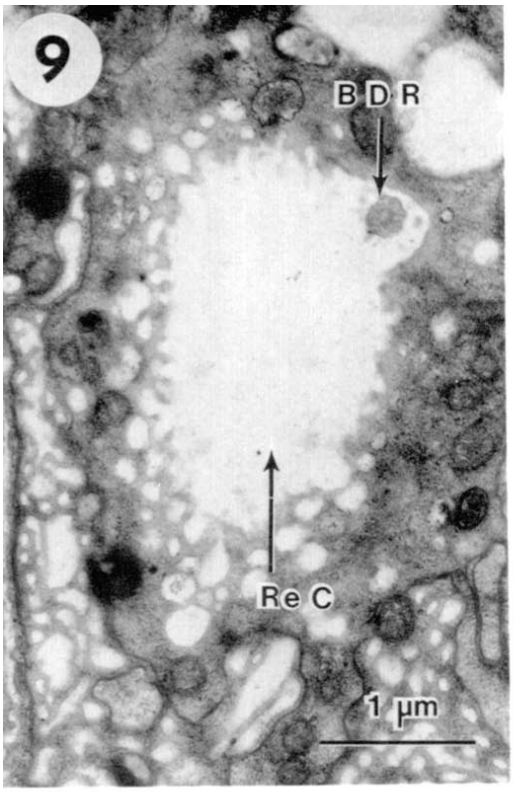
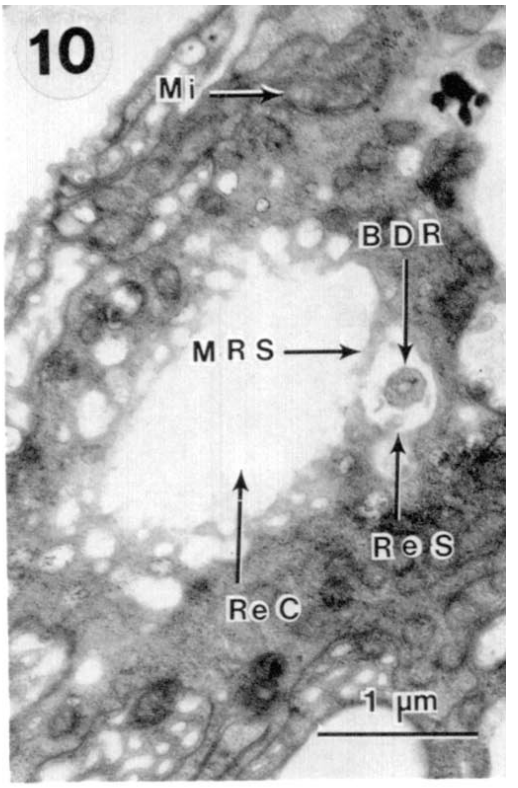
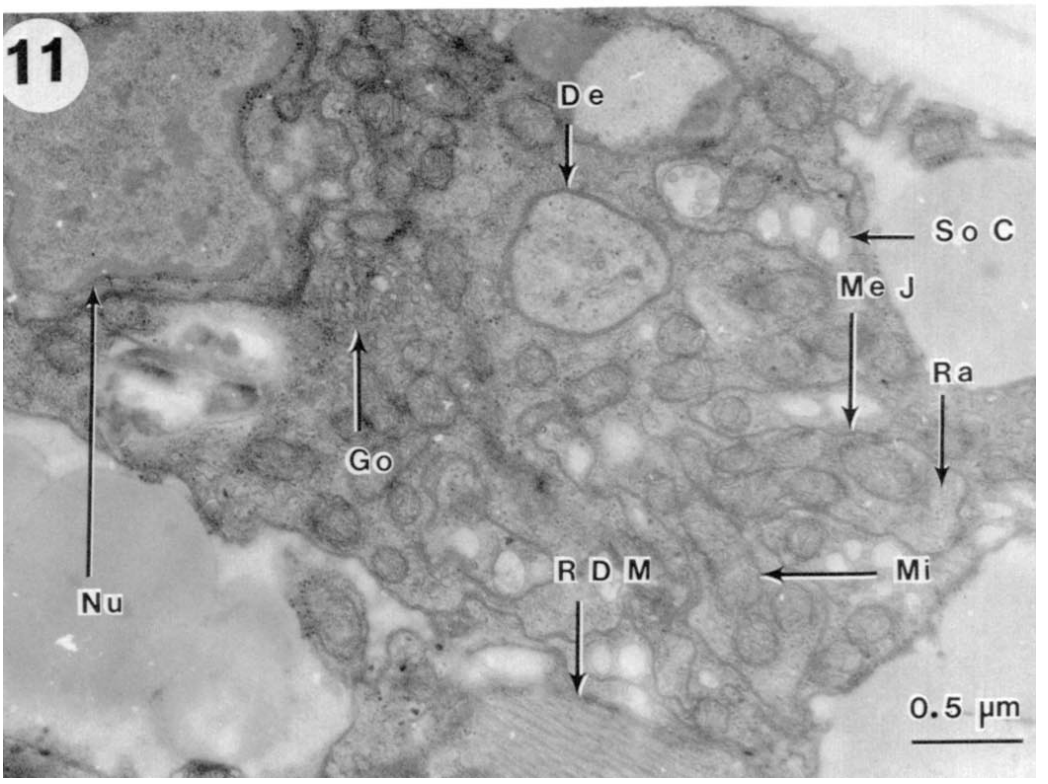
FIG. 9. Transverse section through the basal region of the dendrite receptor (BDR) in the dilated receptor cavity (Re C) of a female *Scutellonema brachyurum* showing the curved wall of the receptor cavity along the base of the dendrite receptor.

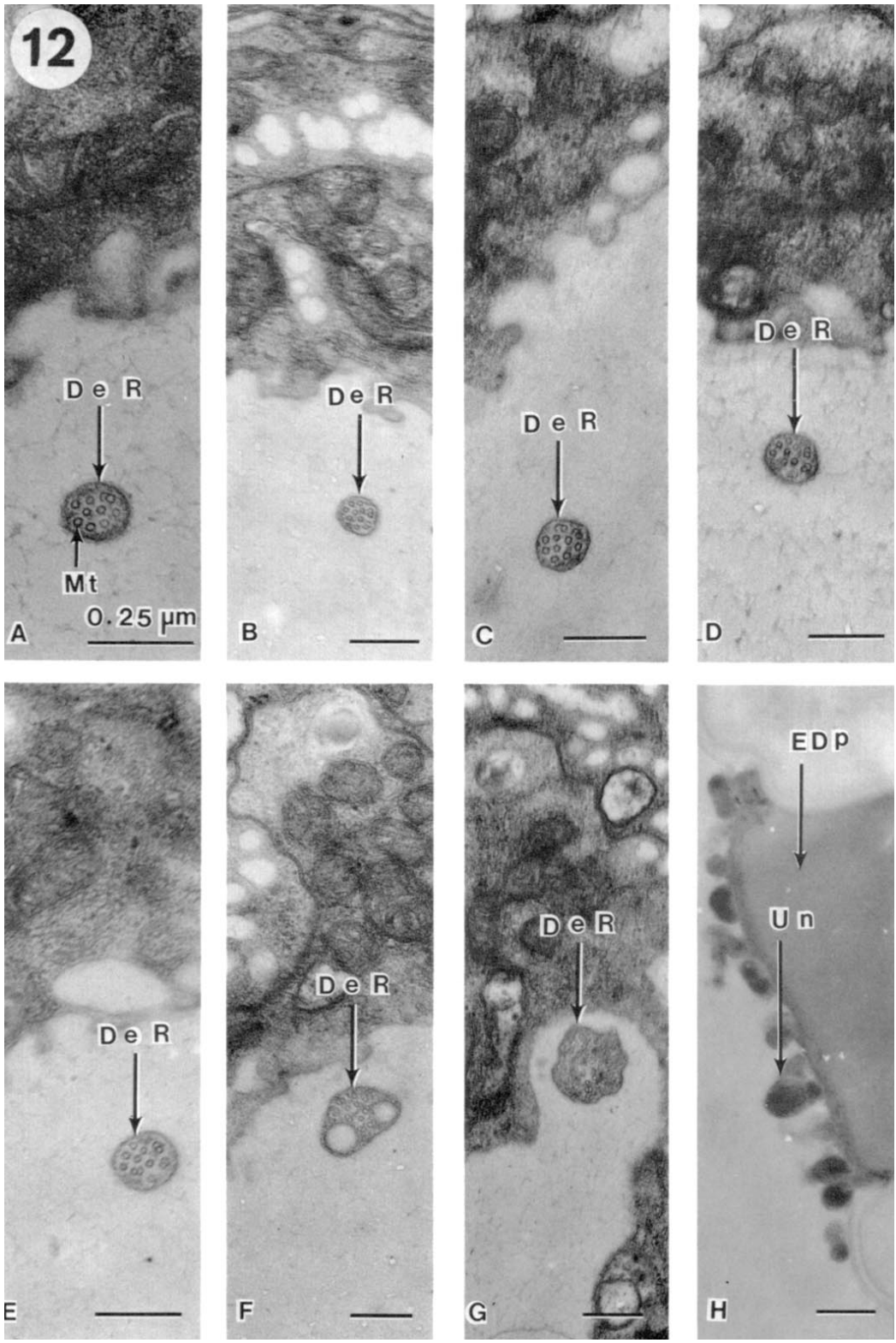
FIG. 10. Transverse section through the receptor socket (Re S) of the dilated receptor cavity (Re C) of a female *Scutellonema brachyurum* showing the membrane of the receptor socket (MRS), receptor base (BDR), and numerous mitochondria (Mi).

FIG. 11. Transverse section through the basal region of the sheath cell of a female *Scutellonema brachyurum* showing the polylobed-like protrusions or rays (Ra) interdigitating with the socket cell (So C), membrane junction (Me J) between the sheath cell and socket cell, Golgi (Go) near the nucleus (Nu), dendrite (De), mitochondria (Mi), and rectal dilator muscles (RDM).


9
BDR
ReC
1 μm


10
Mi
BDR
MRS
ReS
ReC
1 μm


11
De
SoC
MeJ
Ra
Go
Nu
RDM
Mi
0.5 μm


12
DeR
Mt
0.25 µm
A
DeR
B
DeR
C
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D
DeR
E
DeR
F
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EDP
Un
H

In addition, cell organelles like Golgi and vesicles occur in large numbers in the sheath cell (Fig. 11). Thus, the sheath cell may also function as a gland. If such is the case, then the socket and sheath cells may produce different materials serving different purposes. Amalgamation of the rays of the sheath cell with the socket cell suggests that one may influence the activity of the other. Materials produced by the sheath cell in the main receptor cavity may be polyanionic glycoproteins responsible for protecting or nourishing the sensory dendrite receptor, or may be involved in maintaining appropriate osmotic and ionic levels in the receptor cavity (22–26).

The cylindroid electron-dense plug of the phasmids may be lipopolysaccharide and may prevent access of external harmful substances to the receptor cavity and dendrite receptor. It may also serve to filter materials of specific molecular size. Electron-dense materials of various sizes and shapes are occasionally deposited on the external surface of the plug (Fig. 12H), but not on the cuticle surface. Whether these electron-dense materials are old plug or specific receptors is not clear. The complex compact fine net-like structure of the plug is different from the fibrillar material found in the phasmids of *Hoplolaimus* sp. (2). The locations of the phasmids are also quite different between *Hoplolaimus* sp. and *S. brachyurum*. It would be interesting to compare phasmids of these two otherwise similar nematode genera.

Since the volume of the receptor cavity is influenced by the osmolarity of the environment, the receptor socket around the base of the dendrite receptor may serve as a protective sleeve to prevent damage to the receptor base by a sudden dilation of the cavity. The receptor socket in *S. brachyurum* may resemble the electron-dense collar around the basal portion of each cilium of the phasmids of the animal parasite *D. medinensis* (12).

The distension of the receptor cavities of *S. brachyurum* when specimens were placed in hypertonic solutions suggests that the cells around the cavity may serve as osmoregulators. Similar results were obtained with *D. medinensis* (12), *Rhabditis terrestris* (20), and *Pelodera strongyloides* (14).

We observed that the phasmids are intimately located on the lateral sides of the spicules, and the difference of the angle formed by the electron-dense plug with the body surface between the male and female nematodes may morphologically suggest that the phasmids of males function differently from those of females. Thus, one function of the phasmids of *S. brachyurum* may be associated with sexual activity. Although we have no direct evidence, we speculate that the phasmids of males may serve as receptors for sexual attractants secreted by females. Sexual attraction has been reported for *Globodera rostochiensis* (11), *Heterodera schachtii* (3), and *Pelodera teres* (4). In all of these species, male nematodes were attracted to females. Whether the phasmids of the female nematode also serve a sexual function is not clear.

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FIG. 12. Transverse section through different region of the dendrite receptor (De R) (A–G), and longitudinal section through the phasmid pore (H) of a female *Scutellonema brachyurum* showing the microtubule (Mt), doublets and fibrillar ring and strand arrangements, and the round to oval electron-dense material sticking to the external surface of the electron-dense plug (EDP), respectively, A) 1+8, B) 3+8, C) 3+8, D) 3+7, E) 3+6, F) 8+3, G) the doublets and fibrillar ring and strand in the dendrite receptor base, and H) the external surface of the electron-dense plug. All bars = 0.25 μm.

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