

Ultrastructure of Male Sexual Apparatus of *Scutellonema brachyurum*¹

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Abstract: Electron micrographs of serial sections show that the male sexual apparatus of *Scutellonema brachyurum* includes two morphologically identical spicules. Each is composed of a swollen tubular head, crescentic shaft, and leaf-like blade with membranous velum expanded from the central trunk. The spicules are concave and grooved on the ventral side and convex on the dorsal side near the trunk. The trunk is continuous with the shaft and head. Nerve tissue occupies the core of the spicule and includes a dendritic process which gains access to the exterior via a small pore on the lateral side of the spicule tip. Three protractor and two retractor muscles are associated with each spicule. A sensory accessory piece connects with the tip of the gubernaculum and protrudes from the lower side of the opening of the spicular pouch; it protracts and retracts with the muscularized gubernaculum. The gubernaculum varies from bow-shaped in the distal part to boat-shaped in the mid region. A sac exits beneath the accessory piece as a buffer for its movement. A cuticular guiding bar originating from the dorsal wall of the spicular pouch has a tongue. The ventral surface of the tongue is sclerotized to separate the two spicules. It is mobile by muscles of the protractor gubernaculi, retractor gubernaculi, and seductor gubernaculi.

Key words: accessory piece, bar tongue, capitulum, gubernaculum, guiding bar, sensory organ, spicule.

The spicules of nematodes are the major male sex organs of copulation. They vary greatly among taxa in shape and size and are therefore of taxonomic value. For example, the shape of the spicule tip differs between *Heterodera* and *Globodera* (3,10,11).

Bird (1) in 1971 commented on the lack of study of the ultrastructure of nematode spicules. Since then, spicules of *Heterakis gallinarum* (9), *Nippostrongylus brasiliensis* (9), *Syphacia obvelata* (6), *Heterodera rostochiensis* (3), *Aphelenchoides blastophthorus* (4), *Pratylenchus penetrans* (13), and *Rotylenchus robustus* (5) have been studied by transmission electron microscopy, and those of *Syphacia obvelata* (6), *Hoplolaimus galeatus* (7), *Heterodera* spp. (3), and *Aphelenchoides blastophthorus* (4) have been examined by scanning electron microscopy. Many morphological differences have been found among these genera. These studies also suggest that functions of the spicules include dilation of the vulva and vagina (2) and for-

mation of a tube to convey sperm (3,7,13). In addition, a sensory function is inferred from nerve tissue (1,3) located in the central core of the spicule (3,9).

The objectives of this study were to elucidate the fine structure of the spicules and related organs of *Scutellonema brachyurum* and to reconstruct a three-dimensional model of the spicule from serial sections to provide insight into the function of these organs.

MATERIALS AND METHODS

Adult males of *Scutellonema brachyurum* (Steiner, 1938) Andrassy, 1958 were obtained from cultures on jade plants (*Crasula argentea* L.) growing in the greenhouse. Posterior parts of the specimens were cut and fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M phosphate buffer at pH 7.3 for 1.5 hours at 22-24 C. They were transferred to a test tube (10 × 75 mm) and rinsed three 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. Solutions were changed by carefully aspirating the supernatant after the specimens had sunk to the bottom of the test tube. The specimens were then dehydrated with ethanol and infiltrated with Spurr's embedding medium (12). Sections (70-80 nm) were cut with a

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diamond knife and stained with 4% uranyl acetate in 50% ethanol for 10 minutes. They were washed in 50% ethanol and rinsed with distilled water for 3 minutes before staining with lead citrate. Observations were made with a Siemens IA electron microscope at 80 kV.

For reconstruction of the spicule, all cross sections were photographed at 2,900× magnification. Only those serial sections showing a marked shift in shape were selected as the representative portion of the spicule. About 85 negatives were chosen for each of the three specimens examined. The final magnification of each print was 9,062×. Each print was assigned a section number in the order of its sequence, traced on paper, and fastened onto basswood sheets of 3 mm thickness. Basswood discs of various shapes were then cut with a coping saw and labeled with section numbers. The discs were sequentially glued together and reinforced by adjustable iron "C" clamps. The surface of the model was smoothed with a linoleum cutter and dissecting knife after 24 hours.

In reality, it is almost impossible to save every section of the serially cross-sectioned specimen. The model of the spicules was therefore made according to the nearest proportion of the length of the spicules of the live nematode.

RESULTS

Male sexual apparatus of *S. brachyurum* consists of the spicules, spicular pouch, accessory piece, accessory piece sac, gubernaculum, capitulum, cloaca, and copulatory muscles.

Spicules

Two morphologically identical spicules are the copulatory organs; these are enclosed within an invagination, the spicular pouch. They are separated by a cuticular guiding bar and bar tongue. Each spicule may be divided into three parts: a swollen

tubular head, a crescentic shaft, and a blade with two wing-like membranous vela expanded from a central trunk (Figs. 1–4). A concave region is formed on the ventral side of the spicule; it includes the shaft and the basal part of the blade. In addition, two grooves occur on the same ventral side of the spicule, one where each wing-like velum joins the trunk in the blade region (Figs. 2, 3). The spicules are composed of an electron-dense sclerotized material (Figs. 1, 5H–P, 6A–D) with an overlay of less electron-dense material (Fig. 5I–P). This less electron-dense material thickens at the points where the spicules touch or where they slide along the cuticular guiding bar. In the head region of the spicule, the cuticular middle layer is continuous with the cuticular lining of the spicular pouch (Fig. 6C). The base of the head is embedded in the body tissue. The central core of the spicule is filled with nerve tissue (Fig. 6C) extending through the trunk of the blade toward the tip of the spicule. A small pore to the exterior has been located on the lateral side of the tip (Fig. 5H).

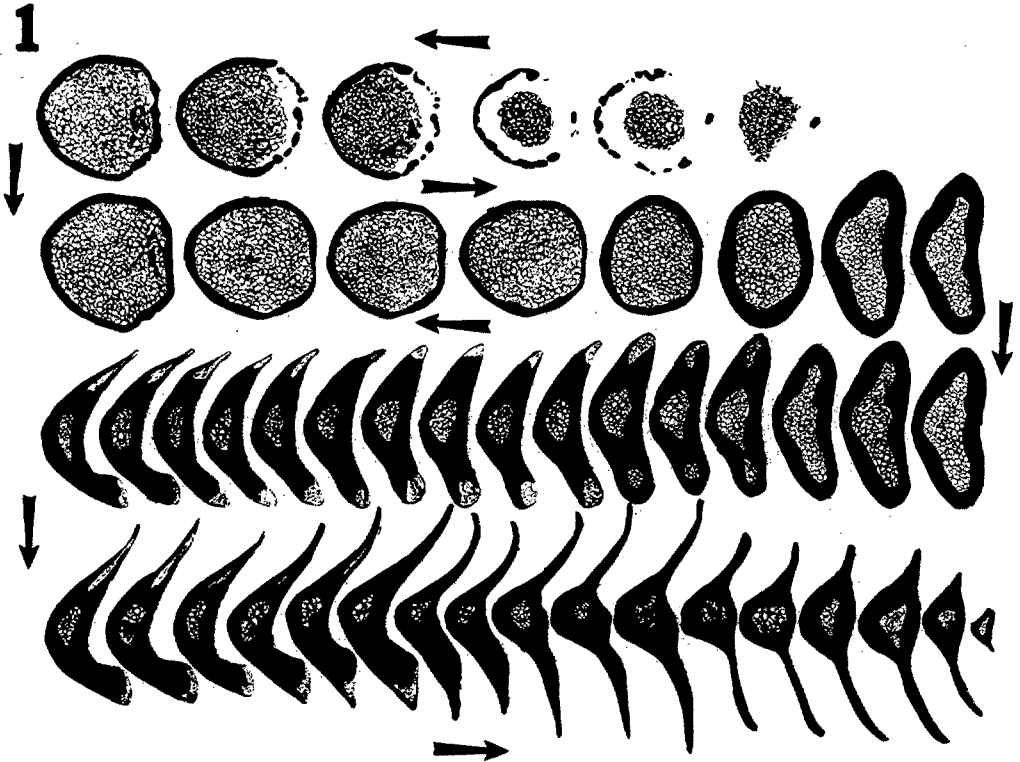
The wing-like membranous vela of the blades are about 220 nm thick at the anterior regions in both the ventral and dorsal sides (Fig. 5I–L). The inner side of the dorsal velum gradually increases in thickness to about 1,020 nm, and its edge changes from a rounded to a cusped shape (Fig. 5M, N). The ventral velum appears shorter than the dorsal velum in the anterior region but becomes equal in length to the dorsal velum in the posterior region (Fig. 5I–N). The widest parts of both ventral and dorsal vela measure about 2.8 μm and 3.06 μm, respectively.

Spicular pouch

The spicules and the cuticular guiding bar with bar tongue lie within a cuticle-lined spicular pouch. The spicular pouch is divided distally into two parts which hold the heads and shafts of the spicules.

FIG. 1. Drawing of serial sections showing 47 representative images of one spicule. Arrows point to the sequential sections from head to the distal tip of spicule.

FIG. 2. Ventral view of spicule model showing head or manubrium (He), shaft or calomus (Sha), and blade or lamina (Bla) with vela (Ve). A concave area formed in shaft and blade regions is situated on the ventral side of the blade.



Accessory piece and accessory piece sac

An accessory piece, composed of a sclerotized trunk covered with thick cuticle, protrudes from the lower side of the opening of the spicular pouch (Fig. 5A-E). This structure is connected to the distal end of the gubernaculum; its ventral surface, covered by thick cuticle (Fig. 5D-F), is concave to hold the dorsal side of the spicules and the bar tongue of the guiding bar. The accessory piece has a wart-like dorsal surface (Fig. 5F). Two cuticular pedunculated titillae extend from the trunk (Fig. 5B, C). The free titilla tip contains a pore to the exterior (Fig. 5B). The pore leads to a duct with a dendritic process that extends distally through the dorsal side of the accessory piece and the tip of the gubernaculum and then laterally to the lateral caudal chord (Figs. 5D, 6B).

Beneath the accessory piece is an accessory piece sac (Figs. 5E, F, 6D) which is lined with thick cuticle and extends posteriorly from the lower side of the opening of the spicular pouch to the junction of the accessory piece and gubernaculum.

Gubernaculum

Proximal to the accessory piece lies the gubernaculum, composed of a thickening of the dorsal wall of the spicular pouch and occurring in the blade region of the spicules (Fig. 5G-M). The gubernaculum is completely embedded in the cuticle lining of the pouch. The electron density of the gubernaculum is similar to that of the spicules. Distally the gubernaculum is bow-shaped (Fig. 5G-H), while the mid region is boat-shaped (Fig. 5I-L), and proximally it becomes a simple plate (Fig. 5M).

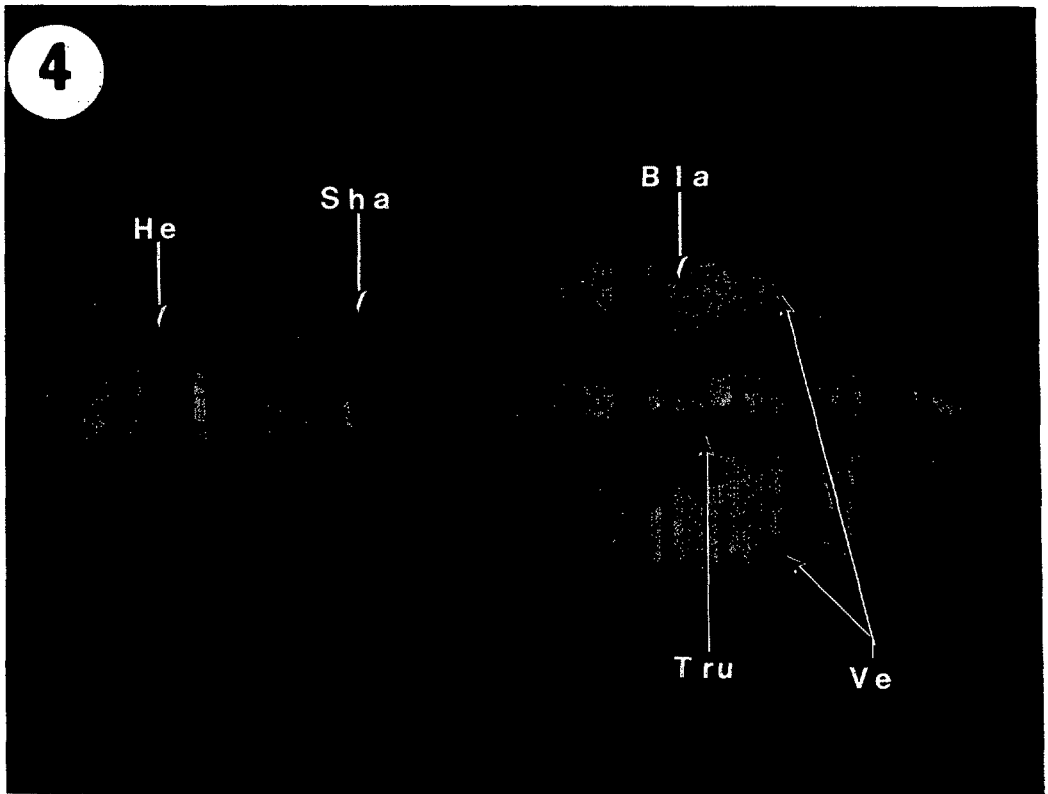
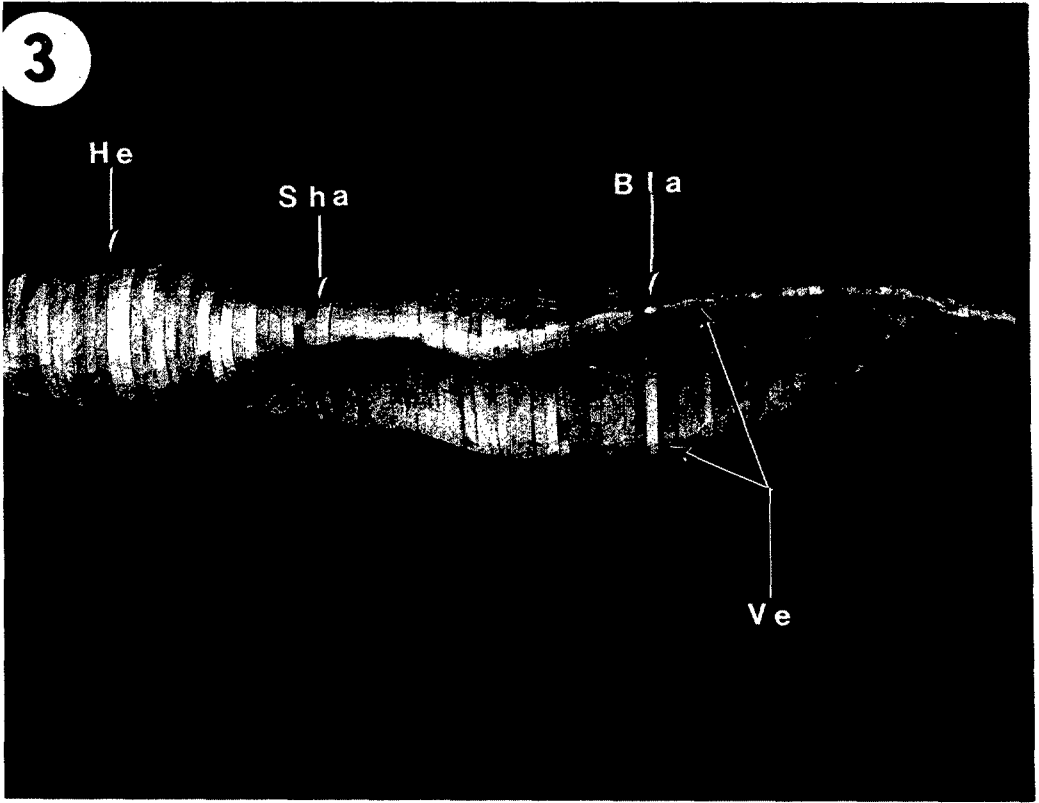
Capitulum

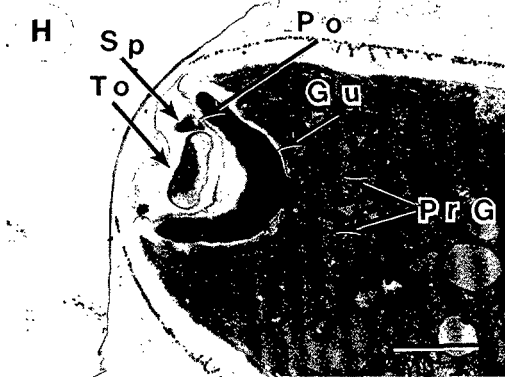
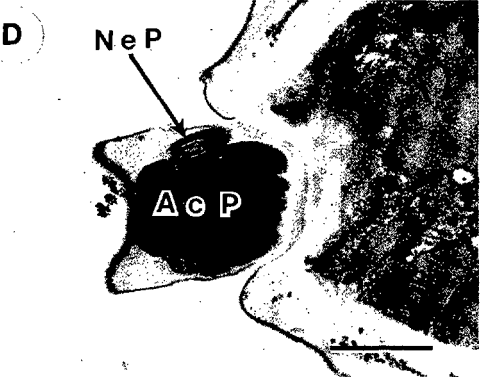
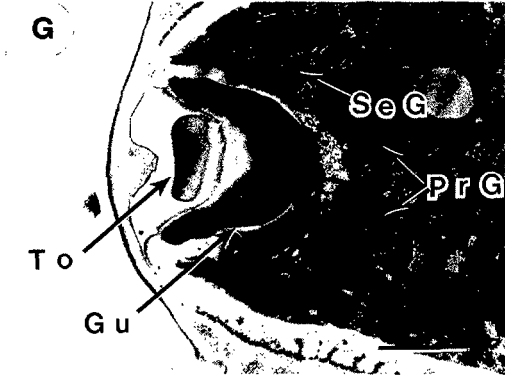
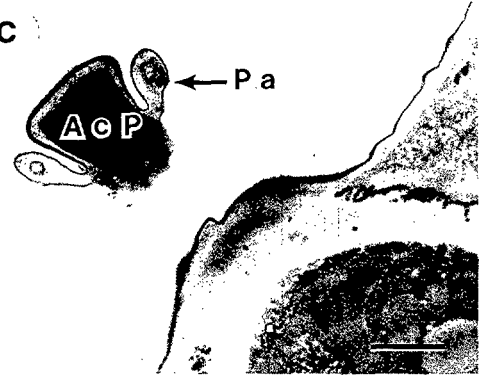
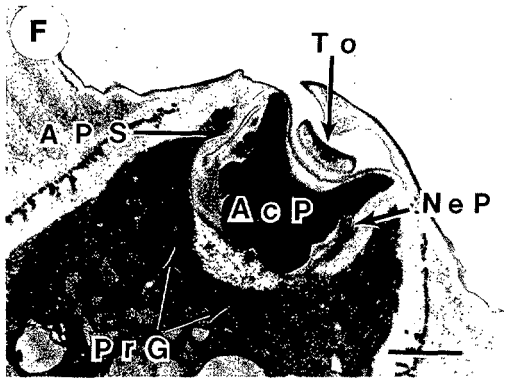
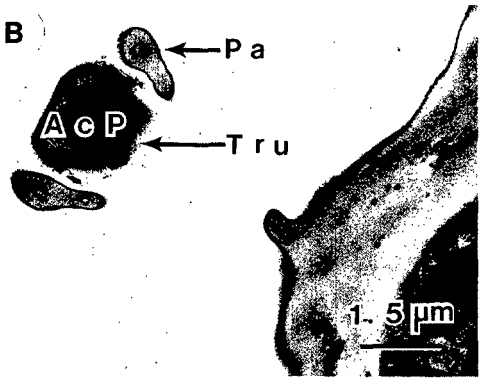
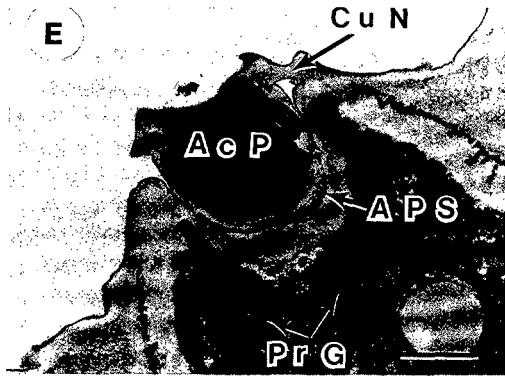
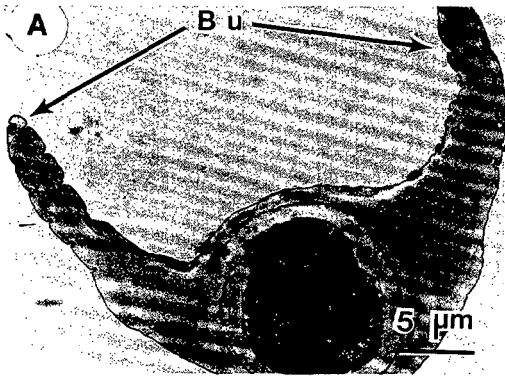
A cuticular guiding bar projects between the spicules from the dorsal wall of the spicular pouch (Fig. 5I-N). The guiding bar extends from the opening of the cloaca which comes from the ventral wall of the spicular pouch to the opening of the spicular pouch (Fig. 5M, N). In the mid region it separates from the dorsal wall of the spicular pouch to form a bar tongue extending distally to the opening of the spicular pouch (Fig. 5F-J). The ventral surface forming

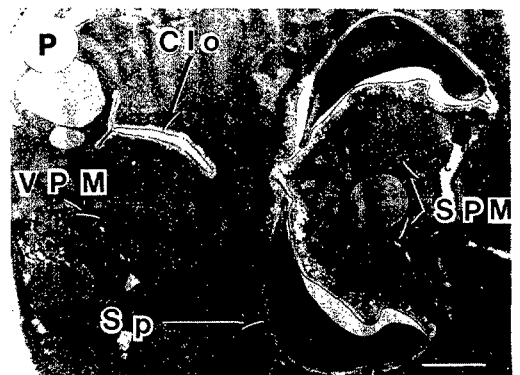
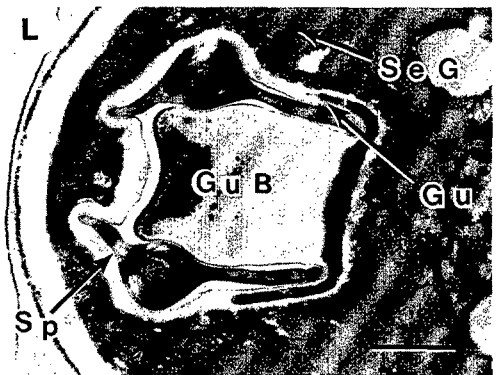
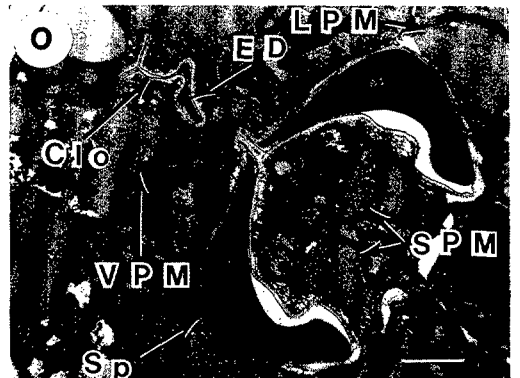
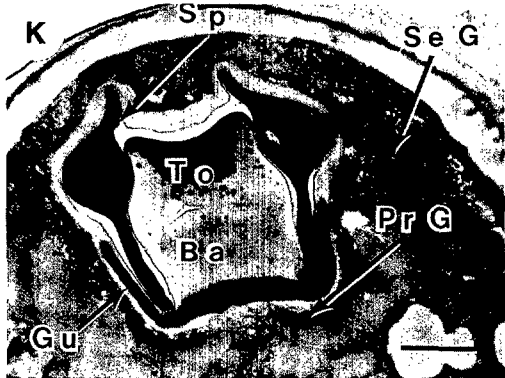
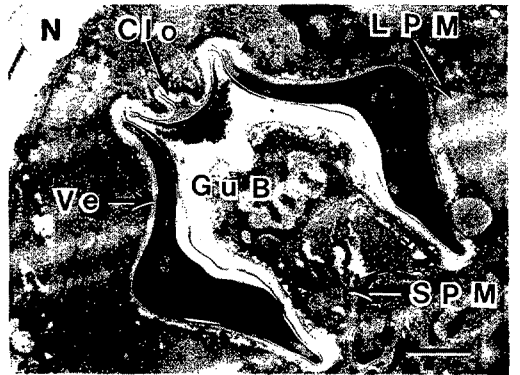
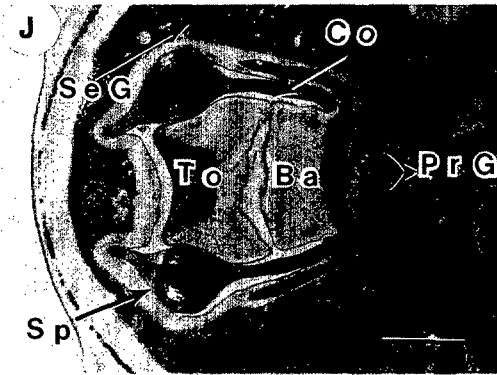
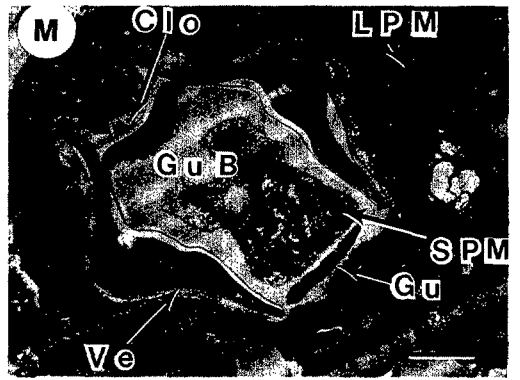
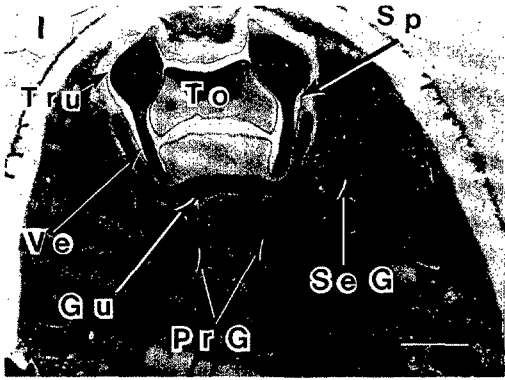
FIG. 3. Side view of spicule model showing wing-like membranous velum (Ve), head (He), shaft (Sha), and blade (Bla).

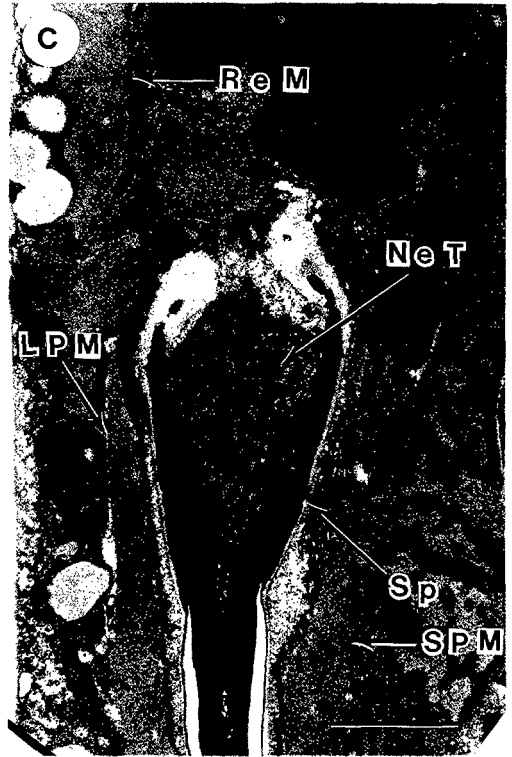
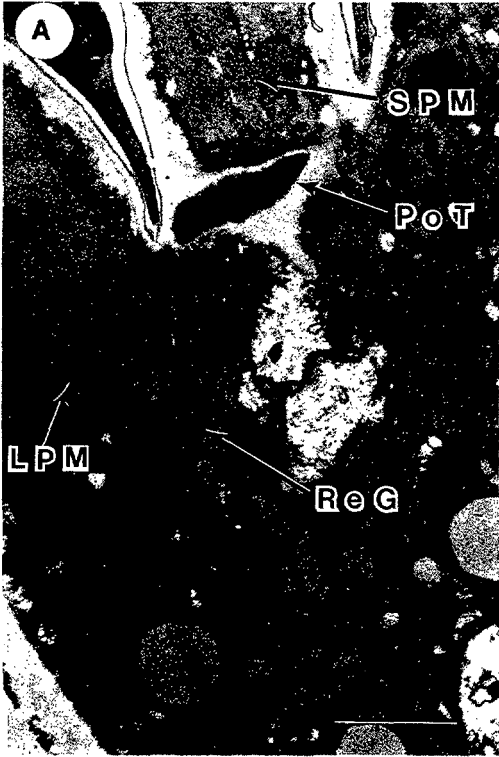
FIG. 4. Dorsal view of spicule model showing trunk (Tru), head (He), shaft (Sha), blade (Bla), and velum (Ve). The dorsal side velum expands wider than the ventral one.

FIG. 5. Transverse sections through different regions of male sexual apparatus. (All bars = 1.5 μ m unless labeled otherwise.) A) Tail region showing bursae (Bu). B) Tip of accessory piece (Ac P) showing free titillae (Pa) on sides of trunk (Tru) of accessory piece. C) Titillae (Pa) attached to sides of trunk of accessory piece (Ac P). D) Posterior region of accessory piece (Ac P) showing innervated dendritic process (Ne P). E) Cuticular connection (Cu N) of accessory piece (Ac P) with opening of accessory sac (APS) showing protractor gubernaculi (Pr G). F) Base of accessory piece (Ac P) showing its wart-like bottom, tip of bar tongue with sclerotized ventral surface (To), protractor gubernaculi (Pr G), nerve process (Ne P), and end of accessory piece sac (APS). G) Anterior region of bow-shaped gubernaculum (Gu) showing bar tongue with sclerotized ventral surface (To), protractor gubernaculi (Pr G), and seductor gubernaculi (Se G). H) Tip of spicules showing small pore (Po), bar tongue with sclerotized ventral surface (To), bow-shaped gubernaculum (Gu), and protractor gubernaculi (Pr G). I) Blade of spicule (Sp) showing boat-shaped gubernaculum (Gu), thick bar tongue with sclerotized ventral surface (To), oval trunks with wing-like membranous velum (Ve), protractor gubernaculi (Pr G), and seductor gubernaculi (Se G). J) Bar tongue base with sclerotized ventral surface (To) showing connection (Co) between tongue and base of guiding bar (Ba), spicules (Sp), protractor gubernaculi (Pr G), and seductor gubernaculi (Se G). K) Tongue region of bar tongue base with sclerotized ventral surface (To) and guiding bar base (Ba) showing spicules (Sp), gubernaculum (Gu), protractor gubernaculi (Pr G), and seductor gubernaculi (Se G). L) Cuticular guiding bar with sclerotized ventral surface (Gu B) showing descended wall of boat-shaped gubernaculum (Gu), spicules (Sp), and seductor gubernaculi (Se G). M) Posterior plate-like gubernaculum (Gu) showing anterior end of opening of thickened dorsal velum (Ve), guiding bar with sclerotized surface (Gu B), lateral protractor muscles (LPM), and subdorsal protractor muscles (SPM). N) Opening of cloaca (Clo) showing thickened dorsal velum (Ve), guiding bar base with sclerotized ventral surface (Gu B), subdorsal protractor muscles (SPM), and lateral protractor muscles (LPM). O) Shaft of spicules (Sp) showing lateral protractor muscles (LPM), subdorsal protractor muscles (SPM), ventral protractor muscles (VPM), and cloaca (Co) with electron-dense material (ED). P) T-shaped cloaca region (Clo) showing ventral protractor muscles (VPM), shaft of spicules (Sp), and subdorsal protractor muscles (SPM).









the capitulum is electron dense and thickly sclerotized (Fig. 5F–N).

Copulatory muscles

Musculature of the spicules: Three protractor and two retractor muscles are attached to the head of each spicule. The three protractor muscles are divided into ventral, lateral, and subdorsal groups. The ventral protractor muscle attaches along the ventral side of the spicule head, passes along the ventral side of the spicule and lateral side of the cloaca, and attaches to the basal lamina of the body wall cuticle on the ventral surface near the opening of the spicular pouch (Fig. 5O, P). The lateral protractor muscle attaches along the lateral sides of the spicule head, extends along the lateral side of the spicule, and attaches to the basal lamina of the body wall cuticle on the lateral side of the tail (Figs. 5M–O, 6C). The subdorsal protractor muscle attaches along the inner dorsal side of the head, passes along the inner dorsal side of the spicule, and attaches to the base of the guiding bar (Figs. 5M–P, 6C). The two retractor muscles attach to the swollen head of the spicules and extend anteriorly to the basal lamina of the body wall cuticle on either side of the lateral chord.

Musculature of the gubernaculum: Three types of muscles are involved with the gubernaculum and accessory piece: protractor gubernaculi, retractor gubernaculi, and seductor gubernaculi. Two protractor gubernaculi attach to the dorsal wall of the mid region of the gubernaculum and to the basal lamina of the ventral body wall cuticle posterior to the spicular pouch (Fig. 5E–L). The retractor gubernaculi attach to the dorsal wall of the proximal region of the gubernaculum and to the basal lamina of the lateral body wall cuticle (Fig. 6A). The seductor gubernaculi attach to the lateral wall of the gubernaculum and acces-

sory piece and to the basal lamina of the lateral body wall cuticle (Fig. 5G, I–L).

DISCUSSION

A tactile sensory function for the spicules of nematodes was postulated (9), and detection of cholinesterase activity supports the sensory function of the spicules of *Ascaris lumbricoides* (8), *Heterakis gallinarum* (9), and *Nippostrongylus brasiliensis* (9). After a pore opening to the exterior at the tips of spicules of *Heterodera* spp. (3), *Hoplolaimus galeatus* (7), *Aphelenchoides blastophthorus* (4), and *Pratylenchus penetrans* (13) was observed a chemosensory function for nematode spicules was considered. Our observation of a small pore on each spicule tip and each titilla of the accessory piece of *S. brachyurum* males strengthens the hypothesis that spicules possess a chemosensory function.

Titillae may also be chemosensory. In *S. brachyurum* a titilla occurring on each side of the tip of the accessory piece is innervated by a dendritic process opening to the exterior by a pore. These observations on *S. brachyurum* support the sensory function speculated for the accessory piece of *Syphacia obvelata* (6).

A variety of sensory organs are located around the tail region of male nematodes. Each may serve a specific sensory purpose. For example, the sensory function of the caudal papillae of *A. blastophthorus* may be to locate the vulva of the female (4). No sensory papillae have been found in males of *S. brachyurum*. On the other hand, the titillae are exposed even when the spicules are retracted and therefore titillae may be the sensory organs responsible for locating the vulva of females. The position of the accessory piece, its sclerotized tissue, and wart-like bottom seem similar to the "crura" described by Chitwood and Chitwood (2), the posterior lips of *P. penetrans*

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FIG. 6. Tangential sections through different regions of secondary male sex organs. (All bars = 1.5 μ m.) A) Proximal tip of gubernaculum (Po T) showing subdorsal protractor muscles (SPM), retractor gubernaculi (Re G), and lateral protractor muscles (LPM). B) Tip of spicule (Sp) showing accessory piece (Ac P), dendritic process (Ne P), and bar tongue (To). C) Head of spicule (Sp) showing nerve tissue (Ne T) in core of spicule, retractor muscles (Re M), subdorsal protractor muscles (SPM), and lateral protractor muscles (LPM). D) Opening of cloaca (Clo) into spicular pouch showing guiding bar (Gu B), spicules (Sp), accessory piece (Ac P), and accessory piece sac (APS).

(13), and the distal region of the gubernaculum of *H. galeatus* (7) and *R. robustus* (5).

The association of the accessory piece and gubernaculum with well-developed musculature indicates that they move simultaneously. Since the subdorsal protractor muscles attach to the head of the spicule at one end and to the posterior plate region of the gubernaculum at the other end, retraction of these muscles results in protraction of the spicules while retracting the gubernaculum and accessory piece. These movements are assisted by the lateral protractor and ventral protractor muscles of the spicule and retractor gubernaculi. The cuticular accessory piece sac beneath the accessory piece may act as a buffer as the latter moves. The accessory piece sac may be equivalent to the W-shaped space behind the posterior lips of the cloaca of *P. penetrans* (13) and the cuticular space beneath the gubernaculum in the region of cloacal opening of *R. robustus* (5).

The bar tongue is similar in shape and position to the anterior ends of the cuticular guiding bars of *Heterodera* spp. (3), *P. penetrans* (13), and *A. blastophthorus* (4). This tongue may function as a plate to seal leakage from the proximal end of the posterior channel formed by the two spicules to help transmission of sperm.

The spicules of *H. galeatus* (7) and *P. penetrans* (13), while different in some respects, in both species serve as a sperm channel. It was suggested that the channel is formed by the velum of the outer spicule overlapping the inner one. The concave region between the shaft and the blade joining the concave part of the correspond-

ing spicule forms a holder that may store the sperm as they come from the cloaca.

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