Ultrastructure of the Spicules of Pratylenchus penetrans[1]

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Abstract: The two spicules of *Pratylenchus penetrans* are not morphologically identical. The outer spicule is slightly larger and longer than the inner spicule. Each spicule is composed of a blade, shaft, and base. The spicules originate from the gradual thickening and invagination of the posterior walls of the spicular pouch. At the tip of each spicule are two small pores with one dendritic process associated with each pore. At the shaft portion, the spicule has a thick body which has a central core filled with nerve tissue. Dorsal and ventral wings project from the body. When protruded, the two wings of a given spicule curve toward the corresponding wings of the other spicule to form a complete tube for the conduction of sperm. The base of each spicule is composed of an electron-dense, sclerotized ring with nerve tissue in the central core. Each spicule is mobilized by two protractor and two retractor muscles. The posterior lips(=hypoptygma) of the cloaca are prominent and innervated. The vas deferens and rectum join near the cloacal opening. *Key Words:* gubernaculum, vas deferens, rectum and cloacal lips.

Among free-living nematodes, most males have two spicules which are approximately equal in size and shape, but in many animal parasites and some plant parasites, the spicules are not equal (1, 2, 3, 6). Each spicule is tubular and consists of a sclerotized cuticular covering and a central cytoplasmic core (2). Observation made by Högger and Bird (6) showed that spicules of *Hoplolaimus galeatus* (Cobb) Thorne differed slightly in both morphology and orientation. The outer spicule was enveloped by a distinct velum. The gubernacula of *Isolaimium californicum* Timm and *I. stictochroum* Timm are movable since these two nematodes have subventral and subdorsal gubernacular muscles attached to the gubernaculum and extending to their tail tips (8). Although spicular structures of 11 species of *Heterodera* were observed with the scanning electron microscope by Clark et al. (3), only *H. rostochiensis* Wollenweber was sectioned and observed with the transmission electron microscope. Our paper describes the detailed ultrastructure of male copulative organs in *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa.

MATERIALS AND METHODS

Males of *Pratylenchus penetrans* were obtained from monoxenic alfalfa (*Medicago sativa* L. 'Du Puits') callus tissue cultures. They were killed by exposure to the vapor of 2% OsO_4 for 2-3 h (This process also straightened out their tails for longitudinal sections). Additional fixation was obtained by immersion in fresh, cold 2.5% glutaraldehyde in 0.1 M Sodium Cacodylate buffer at pH 7.3 for 1.5 h, and followed by 2 h in 1% OsO_4 in 0.1 M phosphate buffer at pH 7.3. The nematodes were then rinsed with buffered solution for 2-3 min and embedded in 1.5% water agar for orientation (4, 9). After they were dehydrated with a graduated ethanol series, specimens were infiltrated with Spurr's medium (7). Polymerization was carried out at 70 C for 24 h. Sections were cut at 70-80 nm (silver interference color) with a diamond knife and stained with 2% uranyl acetate for 25 min and lead citrate for 5 min. Observations were made with JEM-120 EM operated at 80 KV.

OBSERVATION AND RESULTS

Males of *P. penetrans* have two morphologically similar spicules. When the spicules protrude, one embraces the other. The outer spicule is slightly larger and longer than the inner spicule. Each spicule is composed of a blade, shaft, and base (Fig. 1, 10, 11). The outer coverings of the spicules are electron-dense, sclerotized substances. Their central cores consist primarily of nerve tissues extending toward the spicular tips, which are bifid (Fig. 10. The numbers 2-9, indicated in Fig. 1, show the approximate level of each section observed in Fig. 2-9. At the tip of each spicule are two small pores, each of which has one

Received for publication 16 September 1974.

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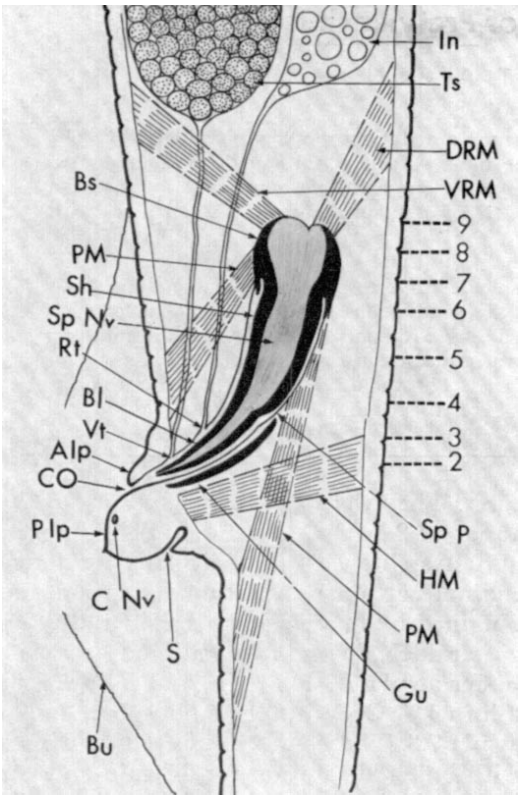


FIG. 1. Schematic drawing of the posterior end of *Pratylenchus penetrans* male. The numbers 2-9 indicate the approximate location of each section of Figs 2-9.

dendritic process which is perhaps modified as a cilium associated with each pore (Fig. 2).

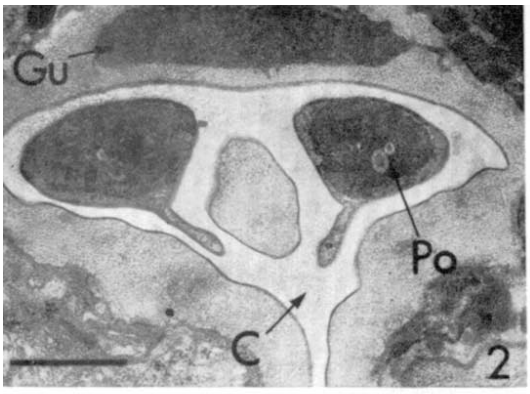
The size and shape of the spicule change gradually toward the base. A cross section through the shaft shows that each spicule has a central body and a dorsal, sclerotized wing as well as a longer and thinner ventral wing (Fig. 4, 5). The spicular wings gradually decrease in size until they terminate (Fig. 4-9). The two wings of a given spicule curve toward the corresponding wings of the other spicule and form a tube-like structure when the spicules are protruded (Fig. 12, 14).

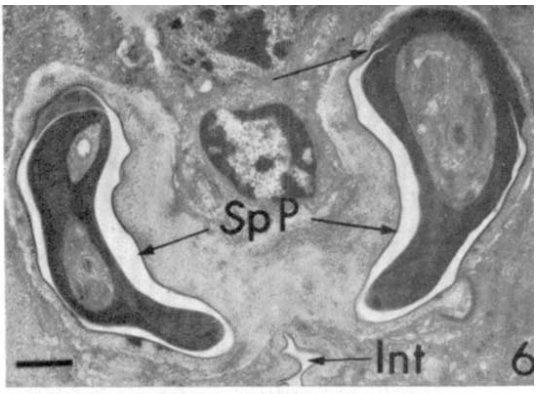
The two spicules are separated by a cuticular guiding bar which extends from the bottom of the spicular pouch to a point near the end of the cloacal opening (Fig. 1, 10). At its base, each spicule is composed of an electron-dense, sclerotized ring with nerve tissues in its central core (Fig. 8, 9). The base is embedded in a knob-like structure, and attached to the knob are two protractor and two retractor muscles (Fig. 1). The protractor muscles extend ventrally and are attached to the hypodermis and cloaca both anteriorly and posteriorly (Fig. 1). The ventral retractor muscle extends anteriorly to the ventral hypodermis, whereas the dorsal retractor muscle extends anteriorly to the dorsal hypodermis.

The gubernaculum is a simple, sclerotized plate located in the posterior of the spicules (Fig. 2, 3, 10, 11). It is embedded beneath the cuticularized wall of the spicular pouch. There is no muscle attachment to the gubernaculum. Two dendritic elements are located beneath the gubernaculum and extend separately to the surface of each of the two posterior lips (hypoptygma) of the cloaca (Fig. 10, 14, 15). The posterior lips are prominent and about three times larger than the anterior lips in which no nerves were found.

The testis and seminal vesicle are on the ventral side, whereas the intestine is on the dorsal side (Fig. 1). The vas deferens and rectum join to form the cloaca just before it opens externally as a transverse slit

FIG. 2-9. Progressive cross-sections of the spicules show that the size and shape of the spicules change gradually from the tip toward the base. Bar represents 0.5 μm. *2)* Two pores (Po) at the tip of each spicule. The spicules are located in cloaca (C) with no direct connection between the cloaca and gubernaculum. *3)* Spicules located in spicular pouch (Sp P), but separated by guiding bar (GB). Gubernaculum (Gu) appears to be a supporting plate embedded beneath the spicular pouch. *4)* Spicules separated by guiding bar. The ventral wings (VW) of the spicules are longer and thinner than their dorsal wings (DW). *5)* Dorsal wings of the spicules change their shape, but they are still thicker than their ventral wings. The central core (CC) of the spicule is filled with nerve tissues. The cloaca no longer exists in this section since the spicular pouch and the intestine (Int) have separated. *6)* The dorsal wings of the spicules almost terminate. The arrow indicates the direct connection between the spicule and spicular pouch. It further shows the gradual thickening of the spicular pouch for the formation of spicules. *7)* The ventral wings of the spicules almost terminate. *8)* Both dorsal and ventral wings of the spicules terminate at this level of the section. The base (Bs) of the spicule is also filled with nerve tissues (Sp Nv). *9)* The spicular nerve enters the spicule through its base.


Gu
Po
C
2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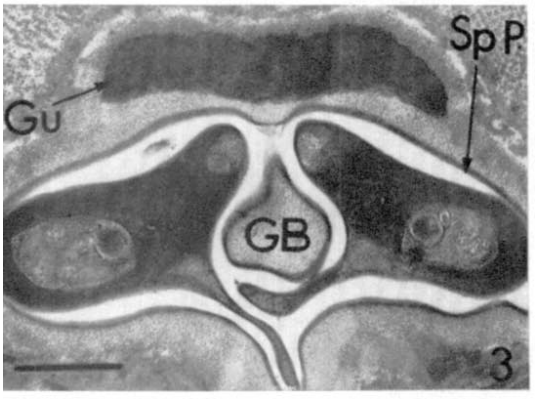
6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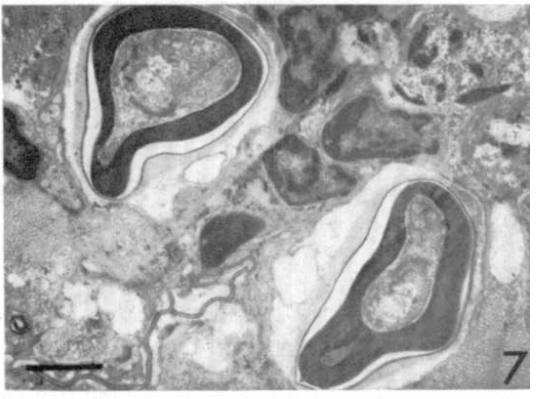
Gu
GB
3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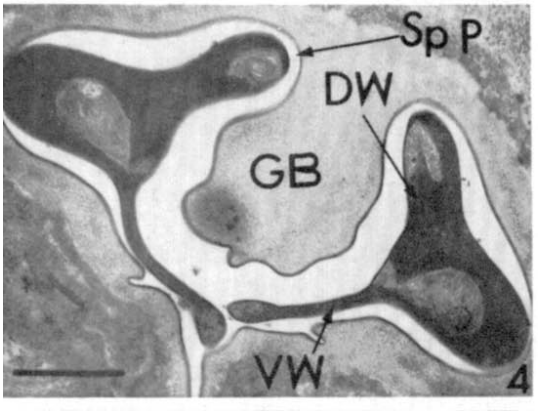
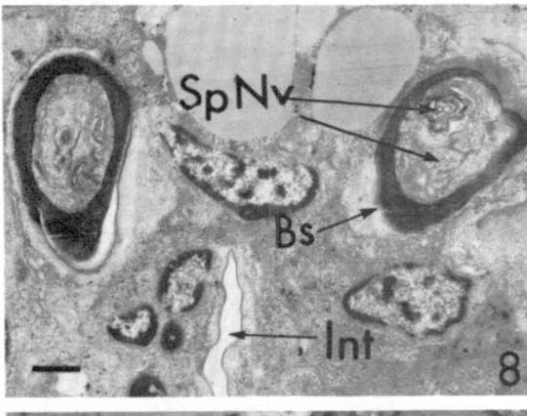
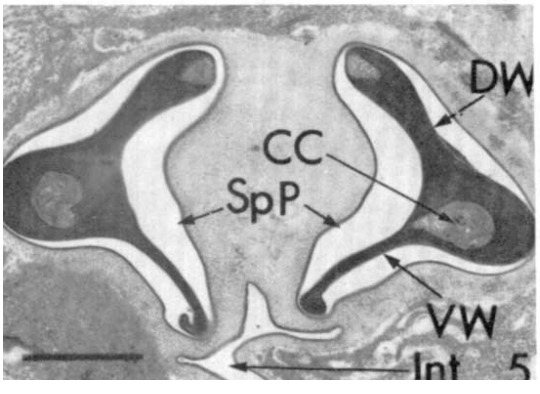

Sp P
DW
GB
VW
4


Sp Nv
Bs
Int
8


DW
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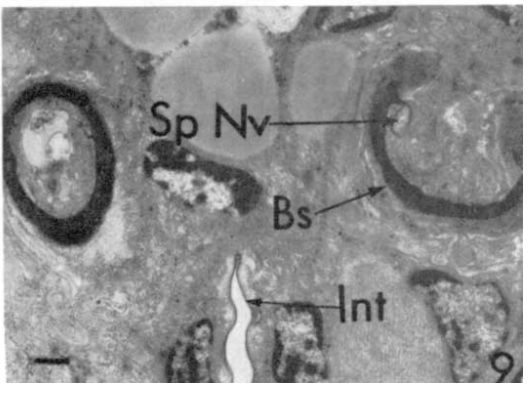
5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Bs
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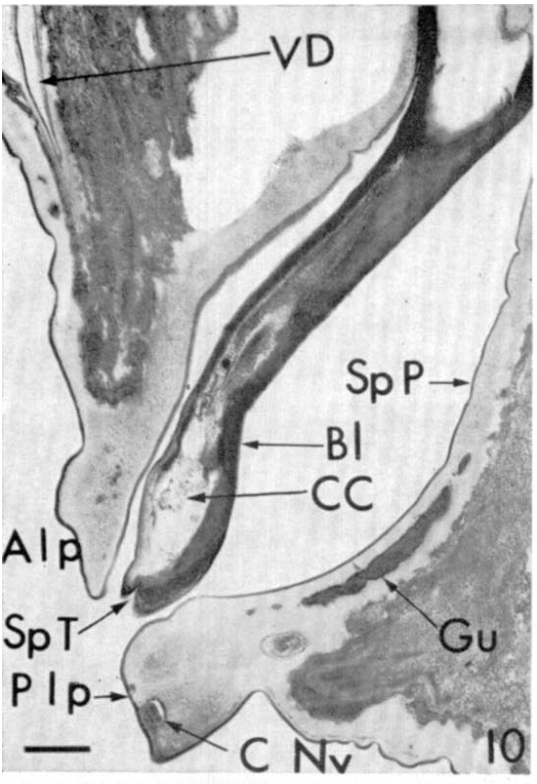
9


VD
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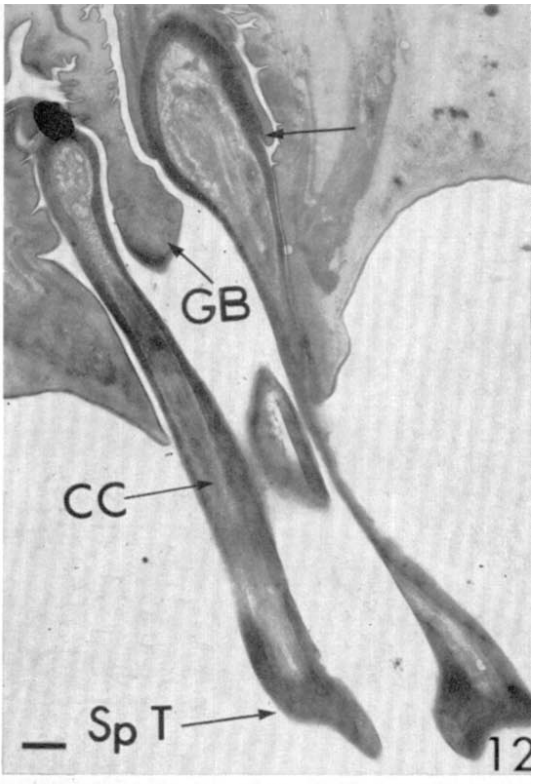
C Nv
10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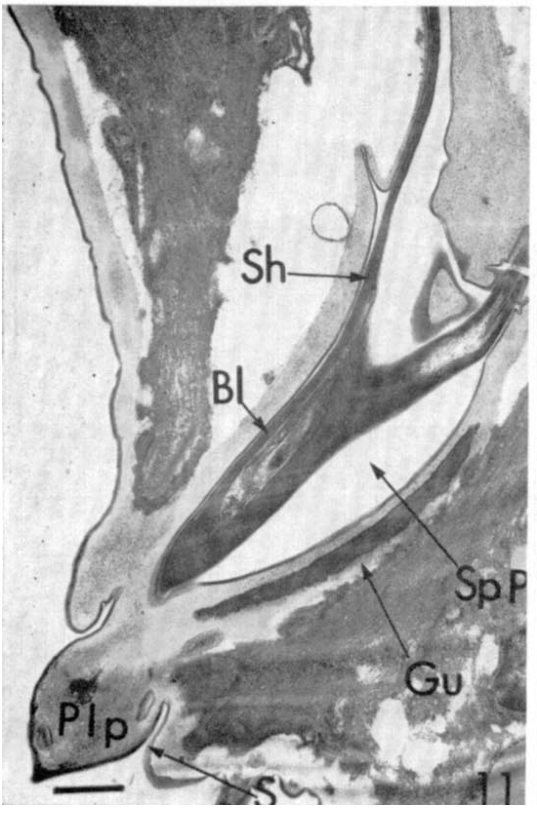
CC
Sp T
12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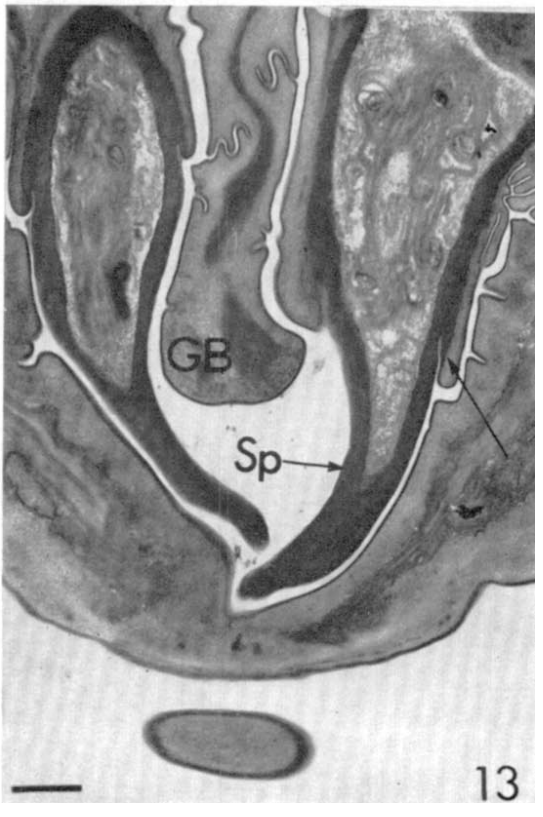
Gu
P lp
11


GB
Sp
13

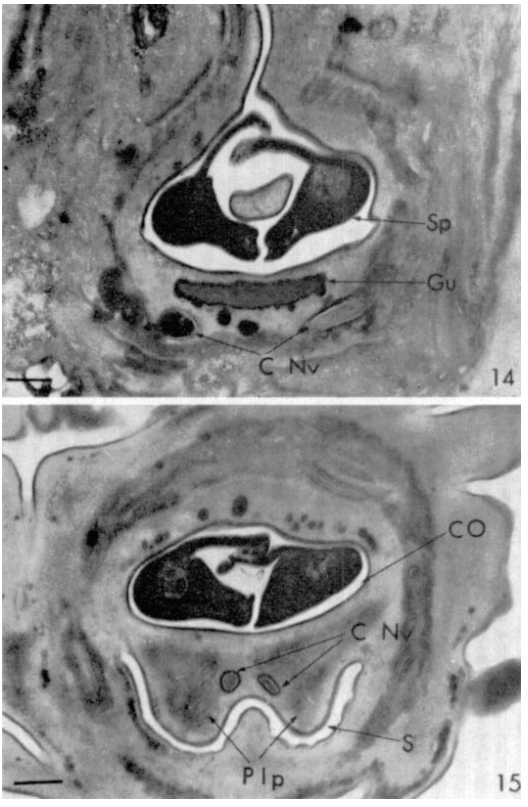


FIG. 14-15. Tangential sections, through the cloacal opening (CO), showing the cloacal nerve (C Nv), gubernaculum (Gu), and the W-shaped space (S) behind the posterior lips of the cloaca. The two spicules form a tube when they protrude. Bar represents 0.5 μm.

(Fig. 15). H-shaped muscle connects the dorsal wall of the cloaca and lateral side of the nematode.

DISCUSSION

Chitwood and Chitwood (2) stated that the spicules and gubernaculum are formed from the wall of the spicular pouch. Hirschmann (5) further stated that the spicules arise from primordia in the rectal epithelium and not from the gonad. Our observations indicate that the cuticular walls of the spicular pouch thicken at their bases (Fig. 6, 12, 13) and continously extend to form the coverings of the spicules. Nerve processes may enter the central core of the spicules during the process of invagination and the development of the spicular pouch wall. Therefore, we believe that the extension of nerve tissue, along with the thickening and invagination of the bottom walls of the spicular pouch, lead to spicule formation.

The gubernaculum has an electron density similar to that of the spicules. It has no direct contact with the spicular pouch since it is completely embedded in the pouch cuticle. Thus, the gubernaculum and the spicules may not have the same origin. Because no muscles attach to it, the gubernaculum of *P. penetrans* is an immovable plate which serves as a supporting and guiding structure for the spicules.

The velum present in the outer spicule of *H. galeatus* (6) and the telamon present in some other nematodes (1) were not observed in *P. penetrans,* but it is quite possible that these structures are equivalent to the ventral wings in spicules of this nematode. The two spicules are separated by a cuticular guiding bar which extends from the bottom of the spicular pouch to a point near the end of the cloacal opening. When the two spicules protrude beyond the end of this guiding bar, they join to form a complete tube which conducts sperm from the vas deferens.

The spicules are highly innervated sensory organs since their central cores are filled with dendritic elements which extend to the two small pores at the tip of each spicule. They may function as chemosensors that locate females by a female sex attractant. The posterior lips of the cloaca may play an important role in the sexual behavior of nematodes since they are prominent and innervated. Contraction of the H-shaped muscles will elevate the dorsal wall of the cloaca and posterior lips of the cloaca during defecation.

FIG. 10-13. *10-11)* Longitudinal sections of spicules at cloacal region showing the bifid tip of the spicule, opening of cloaca, anterior (A lp) and posterior lips (P lp) of cloaca, and the cloacal nerves. Bar represents 0.5 μm. *12-13)* Cross sections of spicules showing that they form a tube when they protrude. Also shown is the thickening and continuation of the wall of spicular pouch to the covering of spicules. Bar represents 0.5 μm.

Abbreviations used in the figures

A lp, Anterior lip of cloaca; Bl, Blade; Bs, Base; Bu, Bursa; C, Cloaca; CC, Central core; C Nv, Cloacal nerve; CO, Cloacal opening; DRM, Dorsal retractor muscle; DW, Dorsal wing; GB, Guiding bar; Gu, Gubernaculum; HM, H-shaped muscle; Int, Intestine; P lp, Posterior lip of cloaca; PM, Protractor muscle; Po, Pore on the tip of spicule; Rt, Rectal terminal; S, Space behind the posterior lips of cloaca; Sh, Shaft; Sp, Spicules; Sp Nv, Spicular nerve; Sp P, Spicular pouch; Sp T, Spicular tip; Ts, Testes; VD, Vas deferens; VRM, Ventral retractor muscle; Vt, Vas deferens terminal; VW, Ventral wing. Arrows in Fig. 6, 12 and 13 indicate the thickening areas of spicular pouch for the formation of spicules.

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