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Morphological Comparison and Taxonomic Utility of Copulatory Structures of Selected Nematode Species[1]

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Abstract: Spicules of 9 *Meloidogyne*, 2 *Heterodera*, 3 *Globodera*, and 12 other plant-parasitic, insect-parasitic, and free-living nematodes were excised and examined using scanning electron microscopy (SEM). Gubernacula of some of the species were also excised, and their structure was determined. The two spicules of all species examined were symmetrically identical in morphology. The spicule typically consisted of three parts: head, shaft, and blade with dorsal and ventral vela. The spicular nerve entered through the cytoplasmic core opening on the lateral outer surface of the spicule head and generally communicated with the exterior through one or two pores at the spicule tip. Spicules of *Xiphinema* sp. and *Aporcelaimellus* sp. were not composed of three typical parts, were less sclerotized, and lacked a cytoplasmic core opening and distal pores. Spicules of *Aphelenchoides* spp. had heads expanded into apex and rostrum and had very arcuate blades with thick dorsal and ventral edges (limbs). Gubernaculum shapes were stable within a species, but differed among species examined. The accessory structures of *Hoplolaimus galeatus* consisted of a tongue-shaped gubernaculum with two titillae at its distal end and a plate-like capitulum terminating distally in two flat, wing-like structures. A comparison of spicules of several species of *Meloidogyne* by SEM and light microscopy revealed no striking morphological differences.

Key words: spicule, gubernaculum, capitulum, titillae, scanning electron microscopy, light microscopy, *Aphelenchoides, Aporcelaimellus, Belonolaimus, Dolichodorus, Globodera, Heterorhabditis, Heterodera, Hoplolaimus, Meloidogyne, Mesorhabditis, Panagrellus, Tylenchorhynchus, Xiphinema.*

Spicules, a pair of copulatory structures in the tail region of the male, vary considerably in shape and size in different nematode groups. Within each group their form shows little variation. Although spicule length has often been used in nematode identification, few species descriptions have considered spicule structure or shape. The spicule has been used as a taxonomic character at both generic and specific levels in some aphelenchids and cephalobids (11,16), and spicule form and dimensions have been considered important in certain tylenchid genera (10).

Spicule structure is sometimes difficult to interpret in whole nematode mounts because details are often obscured or are beyond the light microscope resolving power.

Efforts have been made to elucidate the ultrastructure of spicules of free-living, animal-parasitic, and plant-parasitic nematodes (3,4,14,15,17–19). Detailed work by transmission electron microscopy (TEM) showed that spicules are innervated, function as sense receptors, and generally are instrumental as intromittent organs to pass sperm from the male to the female (3,4,14, 15,18,19). Three-dimensional reconstruction of spicules has been based on light microscope (LM) observations, on TEM serial cross sections, and on scanning electron microscopy (SEM) of exposed parts of protruded spicules.

To date, few observations have been reported on spicules completely isolated from the nematode body (7). The objective of this study was to make a detailed comparison of excised spicules using SEM in order to define precisely the three-dimensional aspects of spicule structure in several taxa. A light microscope comparison of spicules of the four common species of *Meloidogyne* was undertaken concurrently with SEM observations to determine whether any features revealed by SEM can also be observed in LM, and thus can be used in rou-

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tine taxonomic work. In addition, gubernacula of a few species examined were excised in order to determine their exact shape. Like the spicules, these accessory copulatory structures appear to differ in shape and size among taxa and, therefore, can be useful diagnostically.

Materials and Methods

Males of three populations each of the four most common species of *Meloidogyne*—*M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood—were selected. Males of one population each of *M. spartinae* (Rau and Fassuliotis) Whitehead, *M. microcephala* Cliff and Hirschmann, *M. microtyla* Mulvey, Townshend and Potter, *M. naasi* Franklin, and *M. hispanica* Hirschmann were also examined to obtain additional information on spicule structure in the family Meloidogynidae.

Males of *Heterodera glycines* Ichinohe, *H. schachtii* Schmidt, *Globodera rostochiensis* (Wollenweber) Mulvey and Stone, *G. tabacum virginiae* (Miller and Gray) Stone, and *Globodera* sp. were used to compare spicule morphology within the family Heteroderidae.

In addition, one population each of the following species of free-living, plant-parasitic, and insect-parasitic nematodes was examined for morphological features of spicules and gubernacula: *Aphelenchoides fragariae* (Ritzema Bos) Christie, *Aphelenchoides* sp., *Belonolaimus longicaudatus* Rau, *Dolichodorus heterocephalus* Cobb, *Hoplolaimus galeatus* (Cobb) Sher, *Tylenchorhynchus claytoni* Steiner, *Xiphinema* sp., *Aporcelaimellus* sp., *Mesorhabditis spiculigera* (Steiner) Dougherty, *Mesodiplogaster lheritieri* (Maupas) Goodey, *Panagrellus redivivus* (Linn.) Goodey, *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann) Poinar.

For excision of spicules, males were used either live or fixed and preserved in glycerin. They were obtained from appropriate host plants reared in the greenhouse, from soil samples, agar cultures, and in the case of *Heterorhabditis,* from *Heliothis zea* (Boddie) larvae. Live males of Meloidogynidae were obtained by incubating washed root systems in moist chambers at room temperature.

Spicules were excised by employing the same method used previously for nematode stylets (8). Five live males were transferred to a drop of 40% lactic acid (50% for glycerin-preserved males) on a small, round, ringed coverslip which was attached to a microscope slide. After 5 minutes, the posterior end of each male was cut off using an eye knife or sharp dental root canal file under a stereoscope at 60× magnification. The spicules were pushed out of the spicule pouches and cleaned of attached muscle tissues inside the body cuticle before they were transferred to the coverslip, where further cleaning was done as necessary. Each spicule was adhered to the coverslip by pressing down on the spicule head. The lactic acid was removed with a very fine micropipette, and 2% formalin was added several times to eliminate any traces of lactic acid. Excision of the two spicules without their separation was done by lowering the concentration of lactic acid of live or glycerin-preserved specimens. After 10–15 minutes, the formalin was absorbed with filter paper and the spicules were air dried and marked by glass rods. The coverslip was removed from the slide and was attached to a stub. After coating with 250 Å of gold or gold-palladium, the spicules were viewed and photographed using a JEOL T 200 SEM at 25 kV. Observations were made with the specimens perpendicular to the electron beam to avoid foreshortening of the image. At least 20 spicules from each population were observed and photographed at different positions for comparison.

The same technique was used to excise the gubernacula of some of the species.

Observations

Excised spicules usually lie laterally on their inner or outer surface. Only spicules lying in the same position were compared

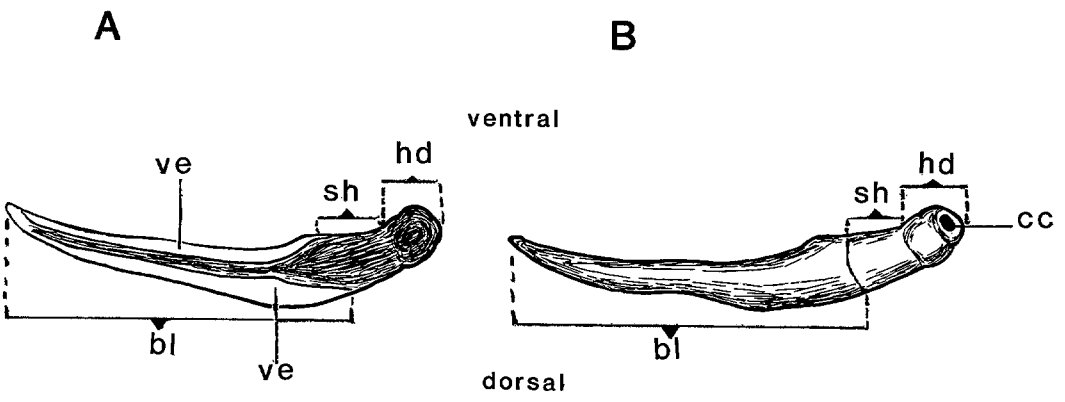

FIG. 1. Generalized lateral diagrams of tylenchid spicules. A) Inner surface. B) Outer surface. cc = cytoplasmic core opening; bl = blade; hd = head; sh = shaft; ve = velum.

in all SEM studies. No morphological differences were noticed between spicules excised from live or glycerin-preserved males. Also, in all taxa examined, the two spicules of each male were morphologically identical exhibiting a mirror image relationship.

Basic spicule morphology was similar in all nematodes examined (Fig. 1) except Dorylaimida and Aphelenchida. In general, each spicule is a tubular structure consisting of a sclerotized cuticular covering and a central cytoplasmic core containing nerve tissues. Three parts can generally be distinguished: a cylindrical head, a cylindrical shaft, and a more flattened blade with two sclerotized, wing-like projections (the vela), one extending toward the dorsal and the other toward the ventral side. The vela are more conspicuous when the spicule is observed from the inner surface. The cytoplasmic core opening is usually situated on the lateral outer surface of the spicule head. There may be one or two small pores at the distal tip of each spicule.

Meloidogyne spp. (Fig. 2): Each spicule is composed of a short, cylindrical, distinctly demarcated head with circular cytoplasmic core opening, a broad shaft, and a tapering blade. Variations of head size and shape were observed between and within species. The cytoplasmic core opening is oriented slightly laterally on the outer surface. The shaft can be well defined (Fig. 2G, H). It starts with a constriction immediately below the head and widens towards the blade. The blade is arcuate and curved ventrally. Wide where it merges with the shaft, it narrows considerably after a short distance posteriorly and tapers progressively towards the tip. The two wing-like vela can be clearly observed on the inner surface of the spicule blade (Fig. 2E, G, I). The inward curvature of the vela and lateral outward curvature of the blade result in a canal-like shape of the spicule. The blade tip is simple, with two pores to the exterior. When the two spicules of a male are excised as a whole in their natural position, the dorsal and ventral vela overlap, forming a channel that insures sperm transmission during copulation (Fig. 2A, B).

SEM observation of spicules of the most common species of root-knot nematodes—*M. incognita, M. javanica, M. arenaria,* and *M. hapla* (Fig. 2C–F)—revealed no major morphological differences useful in routine species identification. Slight differences in shape and size of the head and in blade length and curvature are difficult to observe with LM. Spicules of *M. spartinae,* however, can be distinguished from those of other species using SEM as well as LM. They have a more ventrally curved and markedly set off head and a broad shaft ending in a curved tip (Fig. 2H).

Heterodera and Globodera spp. (Fig. 3): The spicules of *H. schachtii* (Fig. 3A, B) and *H. glycines* (Fig. 3C, D) are morphologically similar. The short, cylindrical head is swol-

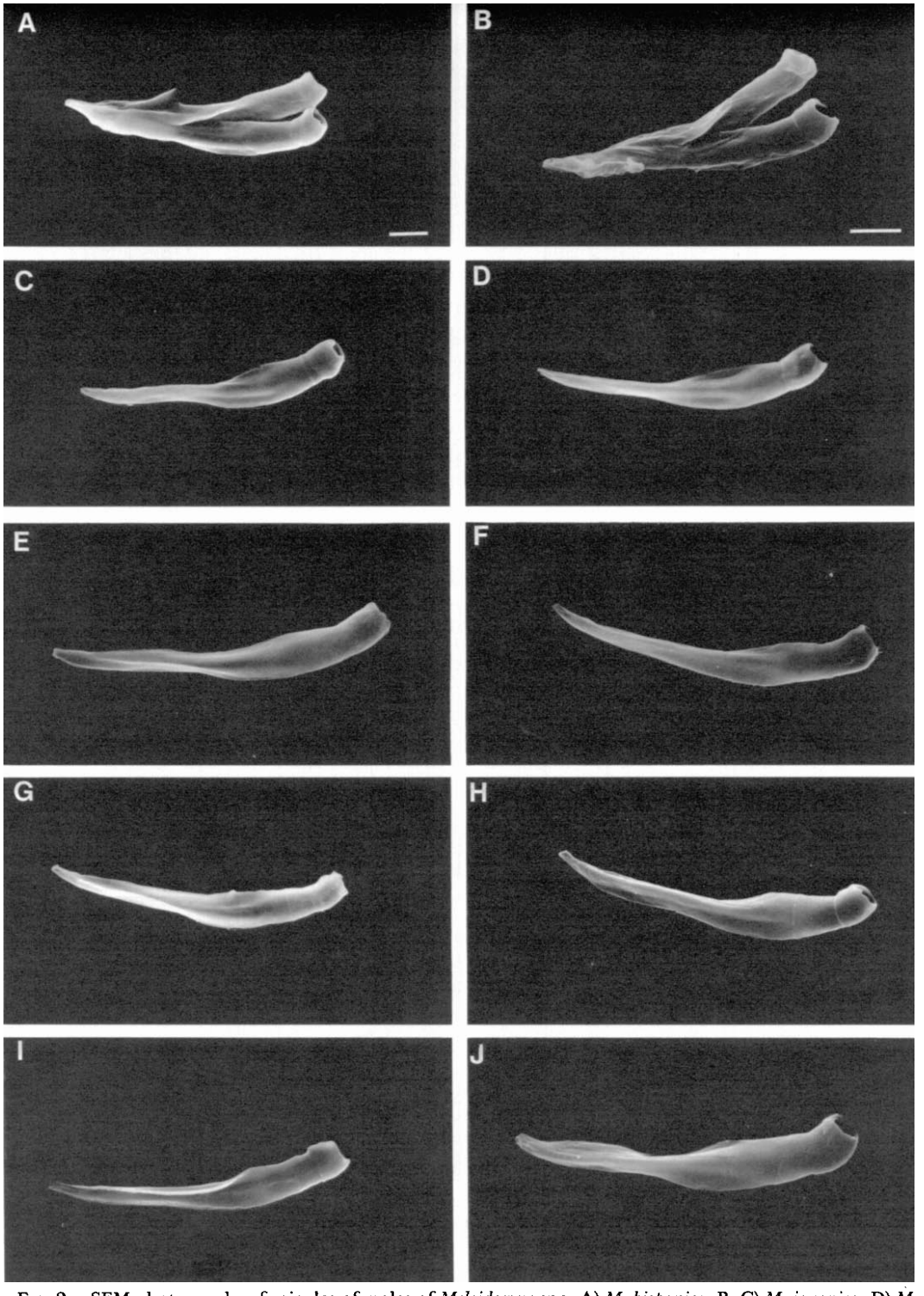

FIG. 2. SEM photographs of spicules of males of *Meloidogyne* spp. A) *M. hispanica.* B, C) *M. javanica.* D) *M. incognita.* E) *M. hapla.* F) *M. arenaria.* G) *M. naasi.* H) *M. spartinae.* I) *M. microtyla.* J) *M. microcephala.* A, B) Spicules not separated. C, D, F, H, J) Outer aspect. E, G, I) Inner aspect. All scales 5 μm (D same scale as A; C, E–J same scale as B).

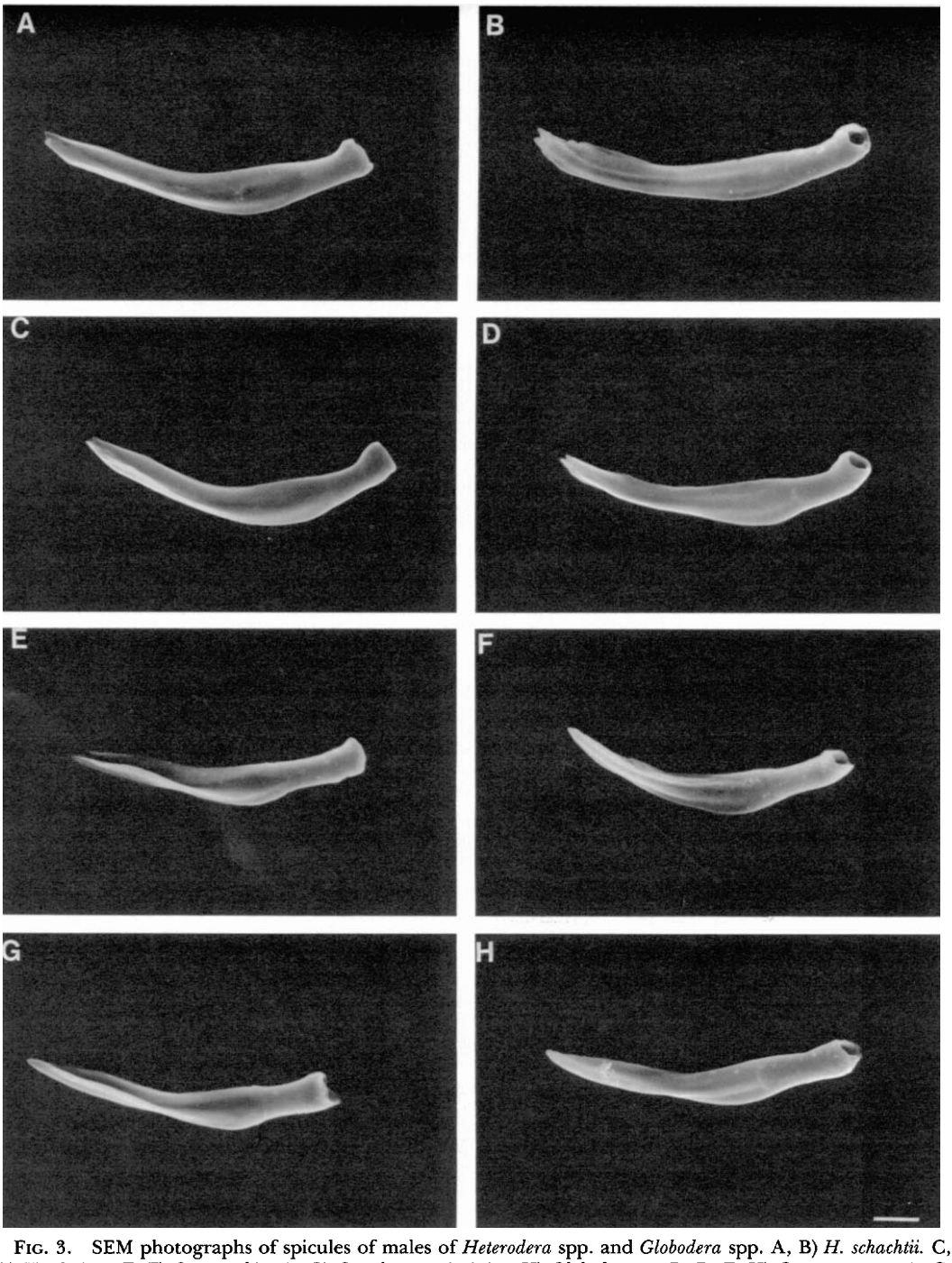

FIG. 3. SEM photographs of spicules of males of *Heterodera* spp. and *Globodera* spp. A, B) *H. schachtii.* C, D) *H. glycines.* E, F) *G. rostochiensis.* G) *G. tabacum virginiae.* H) *Globodera* sp. B, D, F, H) Outer aspect. A, C, E, G) Inner aspect. Scale 5 μm.

len and distinctly bent ventrally. The cytoplasmic core opening is situated slightly outward on the lateral side of the head. The shaft is cylindrical and distinct from the other parts. The blade with its vela is smoothly curved ventrally and terminates with a bifid tip. The spicules of *G. rostochiensis* (Fig. 3E, F), *G. tabacum virginiae* (Fig.

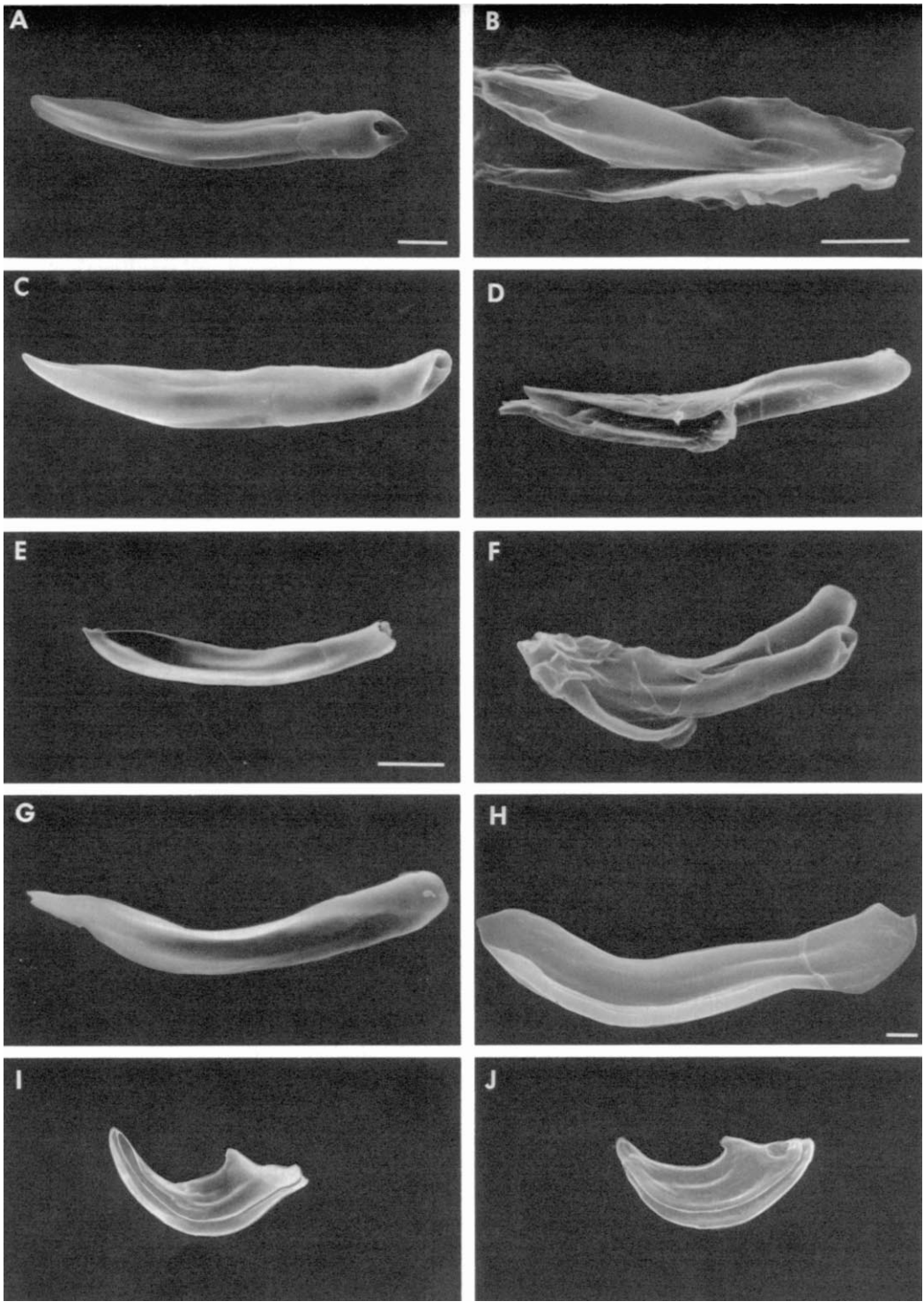

FIG. 4. SEM photographs of spicules and gubernacula of males of selected plant-parasitic nematodes. A) Spicule of *Hoplolaimus galeatus.* B) Gubernaculum and capitulum of *H. galeatus.* C, D) *Dolichodorus heterocephalus.* E, F) *Tylenchorhynchus claytoni.* F) Spicules and gubernaculum. G) *Belonolaimus longicaudatus.* H) *Xiphinema* sp. I) *Aphelenchoides fragariae.* J) *Aphelenchoides* sp. A, C, J) Outer aspect. D, E, G, H) Inner aspect. F, I, J) Spicules not separated. All scales 5 μm (C, D, G same scale as A; F, J same scale as E; I same scale as B).

3G), and *Globodera* sp. (Fig. 3H) are identical and show gross morphology similar to that of *Heterodera* spp., except that the head is not as much bent ventrally and the blade tapers progressively toward a single tip.

Hoplolaimus galeatus (Fig. 4A, B): The two spicules are morphologically similar. Each spicule has a tubular head with cytoplasmic core opening (Fig. 4A). The shaft is cylindrical and not clearly separated from the head. The slightly arcuate blade has two well-expanded vela. The ventral velum widens immediately anterior to the rounded tip.

The gubernaculum (Fig. 4B) is tongue shaped and centrally concave with two curved sides expanding laterally. Its proximal part is simple with slightly curved sides. Distally it terminates in a thick, rectangular plate with one horn-like titilla projecting from each side. The proximal part of the capitulum is a simple plate that tapers distally and terminates in two small flat wings which fit into the middle of the concave center of the gubernaculum.

Dolichodorus heterocephalus (Fig. 4C, D): The slightly arcuate spicule has a tubular head that is continuous with a long shaft (Fig. 4C). The distal half of the blade curves toward a sharply pointed tip. The ventral velum appears to be long and considerably curved inward (Fig. 4D). The distal plate of the gubernaculum is parallel to the chord of the spicule and bears two titillae (Fig. 4D).

Tylenchorhynchus claytoni (Fig. 4E, F): The spicule consists of a slightly globular head, a tubular, distinct shaft, and a crescent-shaped blade ending in an acute tip (Fig. 4E). The broad ventral velum becomes very thin distally. The large gubernaculum is enlarged distally, with raised sides, and is deeply curved proximally (Fig. 4F).

Belonolaimus longicaudatus (Fig. 4G): The spicule is arcuate and deeply curved medially. The swollen head merges into a short shaft continuous with a blade provided with two pronounced vela and ending in a bifid tip. The distal one-fifth is narrow and slightly bent dorsally.

Xiphinema sp. (Fig. 4H): Only two parts are distinct in this spicule, the head and boat-shaped blade. This spicule seems to be less sclerotized than tylenchid spicules. The edges appear more solid than other parts, and longitudinal grooves run throughout the spicule length. No cytoplasmic core opening was observed.

Aphelenchoides spp. (Fig. 4I, J): Spicules of *Aphelenchoides fragariae* and *Aphelenchoides* sp. are thorn shaped and smaller than spicules of all other species studied. *A. fragariae* has spicules with pronounced apex and rostrum (Fig. 4I) and a markedly curved blade. The blade comprises the solidly sclerotized dorsal and ventral limbs connected by a thin bridge of less sclerotized material. The dorsal limb is longer than the ventral, and its tip is hooked ventrally. The spicules of *Aphelenchoides* sp. (Fig. 4J) are similar to those of *A. fragariae* but less curved and sclerotized. The cytoplasmic core opening located at the outer surface of the apex is distinctly visible (Fig. 4J).

Mesorhabditis spiculigera (Fig. 5A, B): Ventrolateral and dorsal views of the spicules show that they are completely fused at their distal ends (Fig. 5A) and connected over much of the dorsal velum length (Fig. 5B). The small, globular head is set off and slightly curved laterodorsally. The short shaft is well demarcated from the blade whose ventral velum is curved inward. The tip is long and pointed. The gubernaculum (Fig. 5A), which becomes dislodged from its original position when the spicules are excised, is boat shaped and about two-thirds of the spicule length.

Mesodiplogaster lheritieri (Fig. 5C, D): The inside aspect of the spicule shows a short, globular head, constricted shaft, and arcuate blade with two vela (Fig. 5D). The head is markedly set off from the short shaft by a deep constriction and has a large circular cytoplasmic core opening which is situated ventrolaterally (Fig. 5C). The spicule tip is pointed. The gubernaculum, triangular with oblique dorsal projection, distally encloses the spicule tips (Fig. 5D).

Panagrellus redivivus (Fig. 5E, F): Spicules of this nematode are peculiar in shape (Fig. 5E). The head is curved and bends

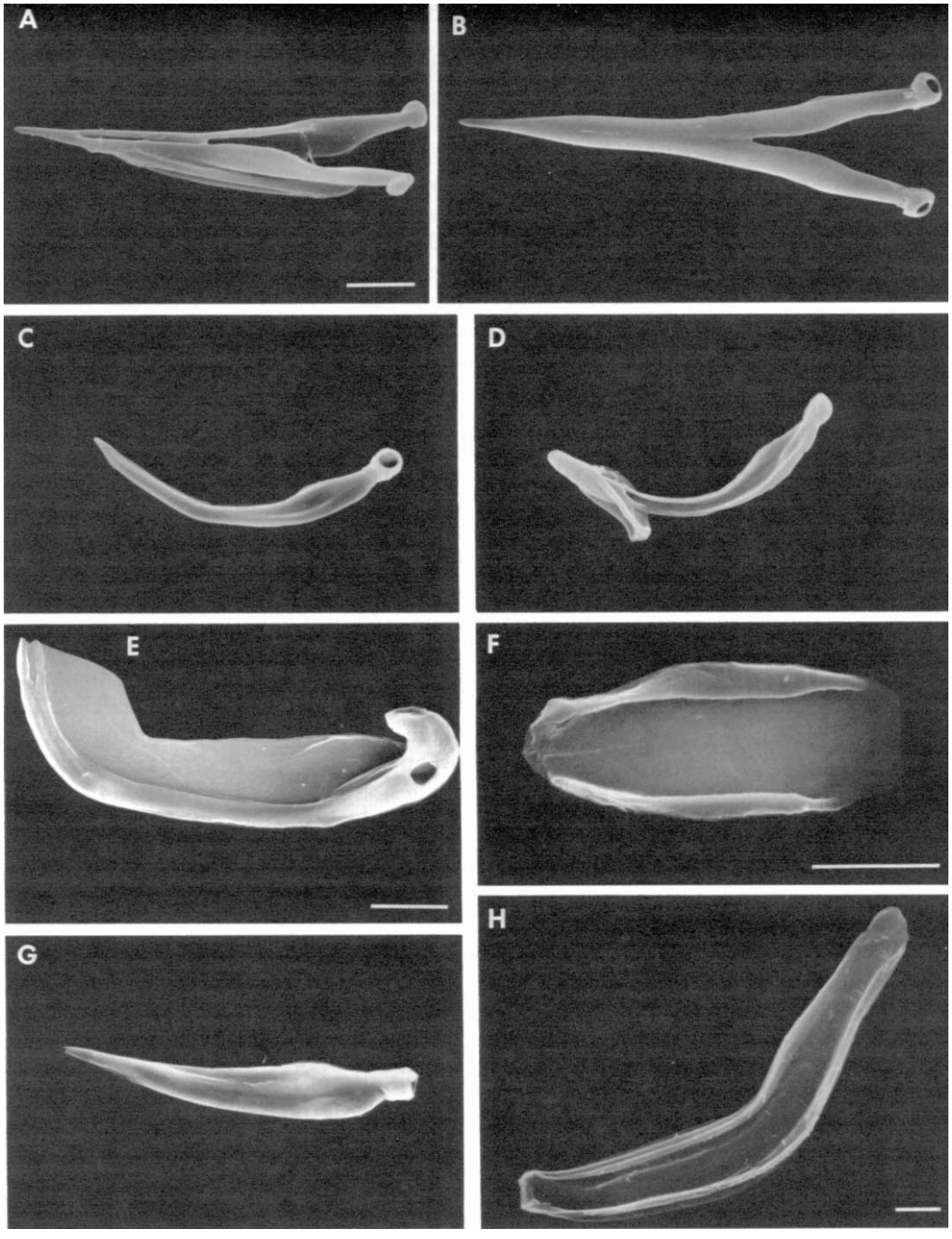

FIG. 5. SEM photographs of spicules and gubernacula of free-living and insect-parasitic nematodes. A, B) *Mesorhabditis spiculigera.* A) Fused spicules, ventrolateral, and gubernaculum. B) Fused spicules, dorsal. C, D) *Mesodiplogaster lheritieri.* D) Spicule and gubernaculum. E, F) *Panagrellus redivivus.* F) Gubernaculum, ventral. G) *Heterorhabditis heliothidis.* H) *Aporcelaimellus* sp. C, E, G) Outer aspect. D, H) Inner aspect. All scales 10 μm (B–D same scale as A; G same scale as E).

ventrally, forming a hook. A large cytoplasmic core opening is situated laterodorsally on the outer lower part of the head. The blade is curved, with its distal one-third bent almost to a 90-degree angle and terminating in a bifid tip. The dorsal edge of the blade appears thick; the nerve tissue probably passes through it. The large ventral velum is membranous and soft and flexible. The large, shallow trough-shaped gubernaculum has thick incurved edges (Fig. 5F).

Heterorhabditis heliothidis (Fig. 5G): The spine-shaped spicule is straight to slightly curved, with small head and pointed tip. The shaft is short and continuous with the blade tapering progressively toward the tip. The cytoplasmic core opening is located terminally at the proximal end in the direction of the spicule axis.

Aporcelaimellus sp. (Fig. 5H): The different parts of this large, slightly sclerotized spicule cannot be distinguished easily. No cytoplasmic core opening was observed. The edges extend the length of the spicule and become more solid and thick at its distal end. The tip is truncate.

Discussion

Scanning electron microscopy has recently become an important tool in nematology for revealing relatively stable morphological characters, some of which have subsequently been observed with LM and thus shown to be of practical value for species identification. Such characteristics include in particular the head and stylet morphology in plant-parasitic Tylenchida and Aphelenchida (8,9,13).

Few studies have used TEM and SEM to clarify spicule morphology and function (1,3–5,10,14,15,17–19), and SEM studies concerned only the protruded parts of spicules (3,4,6,14,17). The three-dimensional aspect of spicules has been reconstructed from TEM serial sections (18) which is a time-consuming method and less precise. The method employed here has been shown to be very satisfactory for SEM observations. Spicules of different nematode species have been successfully dissected from fresh and preserved material without creating unacceptable artifacts.

Spicules are variable among taxa in size and shape and have been shown to be useful in identifying some members of Rhabditida (11), Aphelenchida (16), and a few genera of Tylenchida (10). The original illustrations of Chitwood (2) depict some differences in spicule size between males of the four most common species of root-knot nematodes. The SEM and LM observations of spicules in our studies, however, showed no striking morphological differences that could be used to distinguish these four *Meloidogyne* species. Also, the morphology of spicules of other amphimictic and parthenogenetic species seemed to be very similar. Only *M. spartinae* had slightly different spicules exhibiting a more curved head and blade tip. We also found that the morphology of spicules of *H. glycines* and *H. schachtii* is similar and cannot be used as a differentiating character. In addition, no morphological differences were found between the selected *Globodera* species. The blade tip can be used to distinguish between males of *Heterodera* and *Globodera* species, as reported previously (4).

Individual spicules of *Hoplolaimus galeatus, Pratylenchus penetrans,* and *Tylenchulus semipenetrans* have been reported to be unequal in length (14,15,19). This dissimilarity may be observed only in the position of the protruded, exposed parts of spicules, not when they are retracted. Our study showed that males of all species examined have morphologically identical spicules that exhibit mirror image relationships.

Spicules of the *Aphelenchoides* species examined consist of a head with apex and rostrum and a blade with thick dorsal and ventral edges (limbs). Our SEM observations support earlier TEM observations (3) that the spicular nerve enters through the apex of the spicule head and proceeds to the tip along the outer dorsal surface.

Spicules have been described as tubular structures formed of sclerotized cuticle enclosing a cytoplasmic core (12). Our observations confirmed that spicules of all species examined, except those of the

dorylaimids *Xiphinema* sp. and *Aporcelaimellus* sp., are solid and hard sclerotized structures. Dorylaimid spicules appear to be formed of less sclerotized material, and a cytoplasmic core opening was not detected. The presence of a cytoplasmic core opening in most nematodes studied indicates the sensory nature of spicules and their probable function as tactile or chemoreceptive organs. In the case of Dorylaimida, the supplementary organs on the ventral side of the male tail may play the role of finding females and locating the vulva.

The excised gubernacula are of similar hard, sclerotized material as the spicules. The three-dimensional aspect of the gubernaculum of *H. galeatus* and its morphological features are shown here for the first time. The gubernacula of *H. galeatus* and *D. heterocephalus* have similar gross morphology with respect to the presence of a capitulum. The space existing between the distal part of the capitulum and the gubernaculum may allow for the sliding of the dorsal vela of the spicules when they are protruded. The capitulum is probably attached to the gubernaculum on its dorsal side and plays a role in directing sperm to the channel formed by the spicules during copulation.

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