External Morphology of the Spicules of some Trichodoridae

R. RODRIGUEZ-M. and A. H. BELL[1]

Abstract: External morphology of spicules in several species of Trichodoridae was studied by scanning electron and light microscopy. The bristles on the spicules observed in the light microscope in several species were revealed as small scales forming a sheath which covers most of the spicule body. Some species have smooth spicules, whereas other species exhibited complicated structures formed by projections. In all of the species studied, either a ventro-terminal or terminal opening of the spicules was observed. *Key Words:* spicules, morphology, scanning electron microscope, *Trichodorus, Paratrichodorus, Monotrichodorus.*

Investigation of spicule morphology provides information regarding trichodorid taxonomic characters and insight into functional differences of various spicule structures. The anatomy of the posterior end of trichodorid males has been discussed in detail by several authors (3, 5, 9). The spicules of *Trichodorus* Cobb were defined (9) as "arcuate, irregularly tapering, with or without transverse striations," and those of *Paratrichodorus* Siddiqi as "straight to arcuate, regularly tapering, transversely striated." The spicules of certain *Trichodorus* species, however, have a stout appearance, with the distal half quite wide, and sometimes with a ventral flange as in *T. velatus* Hooper (3). Also in some species, the spicules have bristles. These so-called bristles were first mentioned by Sczcygiel in *T. sparsus* Sczcygiel (10). Loof (4) confirmed the setose condition of the spicules of *T. sparsus* and described these structures in *T. primitivus* (de Man) Micoletzky, *T. velatus,* and *T. cylindricus* Hooper. He pointed out that the bristles of *T. cylindricus* spicules have the appearance of coarse setae.

Usually, the morphology of spicules has been described from glycerin-mounted specimens with retracted spicules; under those conditions, the external morphology is very difficult to see. Loof (4) mentioned the serrated outline of the spicules as an indication of the presence of bristles. On protruded spicules, external structures are sometimes overlooked or not well understood, because of optical limitations of the light microscope.

During the past 2 years, trichodorid specimens with protruding spicules have been obtained and their external anatomy studied by scanning electron microscopy.

Received for publication 8 June 1977.

[1]Department of Nematology, University of California, Riverside, CA 92521. Current Address (Senior Author): Colegio de Postgraduados, Escuela Nacional de Agricultura, Chapingo, Mexico.

MATERIALS AND METHODS

Specimens studied came from the nematode collections of the University of California, Riverside and Davis. For SEM observation, the specimens were prepared following the technique of Sher and Bell (8). Observations of glycerin-mounted specimens were made by light microscopy prior to SEM examination.

RESULTS

Trichodorus californicus Allen: As viewed with the light microscope (LM), the spicules of this species are slightly curved ventrally, tapering regularly toward the distal end with the proximal end slightly cephalated. The inner core appears to be formed of spongy tissue, interrupted by very fine transverse striations. This inner core is surrounded by a thick cuticle, also showing transverse striae, which never reach the inner tissue and are not present near the distal end. Ornamentation is suggested by the slightly wavy outline of the spicule.

With the SEM, the external cuticle of the protruded spicule exhibits very fine lines forming a regular pattern resembling the scales of snakeskin (Fig. 1-A). These small overlapping scales (Fig. 1-F) are oriented with their tips towards the distal end of the spicule, and form a sheath that surrounds the spicule shaft. The distal end of the spicule is smooth and has a ventral longitudinal groove that ends at the truncate spicule tip.

Trichodorus intermedius Rodriguez-M and *Bell* (6): With LM, the spicules appear similar to those of *T. californicus*. They have the same ventral curvature and slightly cephalated proximal end, but the distal end appears slightly more expanded. The transverse striae of the cuticle are widely spaced. The shaft seems to be covered by bristles, oriented towards the distal end, but away from the spicule's longitudinal axis. The outline of retracted spicules appears to be serrated along the shaft.

The SEM reveals large membranous scales with a strong thickening in the middle (Fig. 1-C, D). Ventrally, the distal end shows a small invagination (Fig. 1-D) of the same shape as that observed in *T. californicus* (Fig. 1-A).

Trichodorus cylindricus Hooper: Complete transverse striae were observed by Hooper (2) only on the proximal half of the spicule; however, the thick cuticle exhibits transverse striations almost to the spicule tip. An inner vacuole occurs in the distal half, and the proximal one-third of the spicule has two or three coarse setae obliquely oriented towards the distal end (Fig. 2-D).

In the two specimens examined, no evidence of setae was seen under the SEM; instead, a complicated structure was found (Fig. 2-E), formed by what appears to be projections of the external cuticle of the spicule (Fig. 2-F). These cuticular projections form pockets which overlap each other and surround the spicule shaft (Fig. 2-E). The spicules of *T. cylindricus* lack the ventral groove on the tip as seen in *T. californicus* and *T. intermedius*, but have two dorso-lateral invaginations, one at each side, which appear connected at the spicule tip, and enclose a terminal opening (Fig. 2-F).

Trichodorus aequalis Allen and *Trichodorus proximus Allen:* When observed with the SEM, the spicules of *T. aequalis* have a terminal opening and slightly expanded tips but no scales or similar structures (Fig. 2-H). A single specimen of *T. proximus* was examined by SEM and LM. The spicules of this species lack setae and appear similar to those of *T. aequalis* (Fig. 2-I).

Paratrichodorus (Nanidorus) minor (Colbran), Siddiqi: Males are extremely rare, and only one male was found with one protruding spicule. This spicule had several flexures as observed by LM, but when later dehydrated, it straightened. The inner core seemed of the same texture observed in previous species and appeared interrupted, almost at regular intervals, by transverse striations. The external cuticle appeared thinner than in spicules of *Trichodorus*. An inner longitudinal septum began about the middle portion of the spicule and ended before reaching the spicule tip.

With the SEM, this spicule exhibited irregular transverse marks, which corresponded with the transverse striae seen under LM (Fig. 1-B). Dorsally and at the proximal end, a longitudinal invagination was observed (Fig. 1-E).

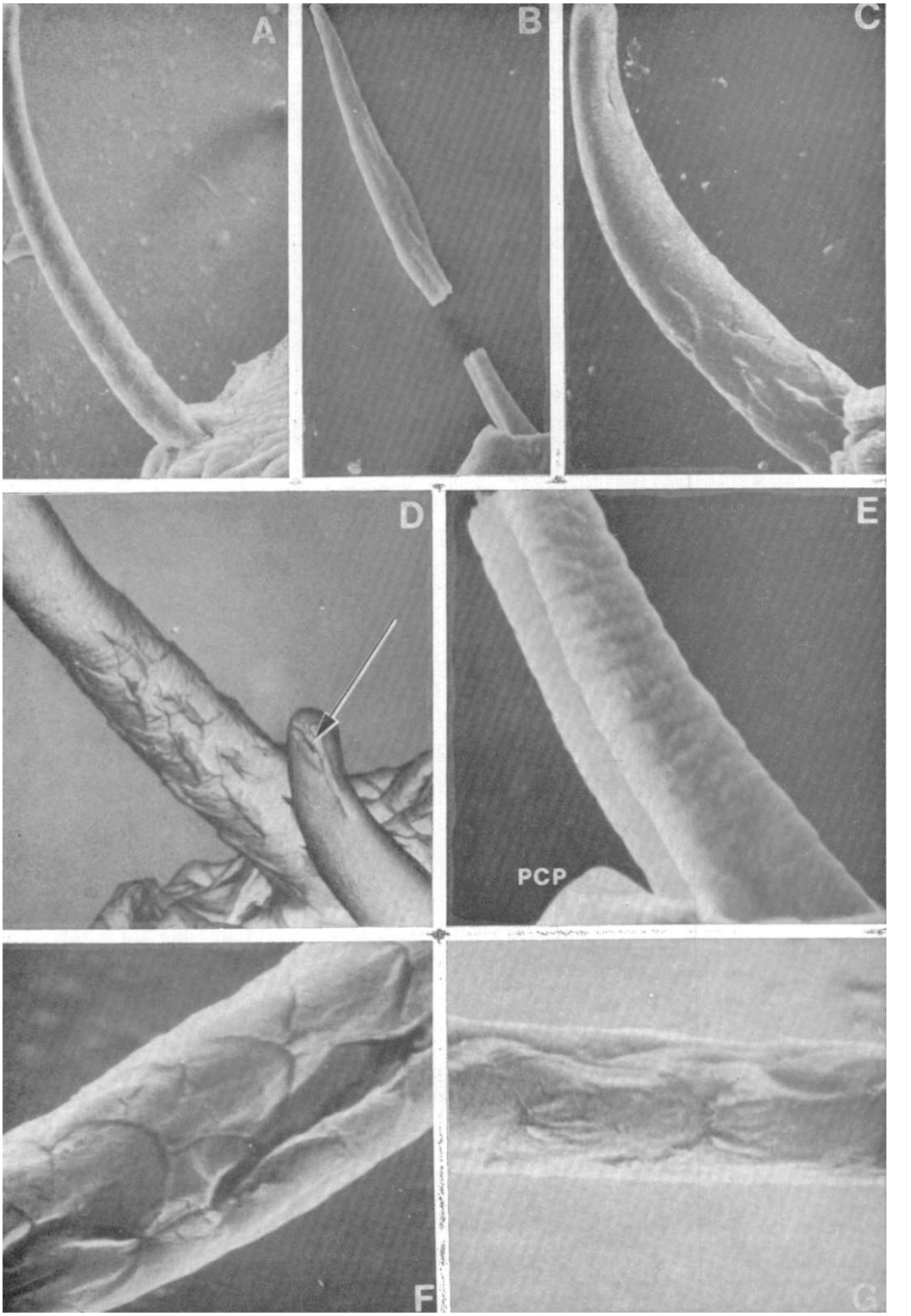
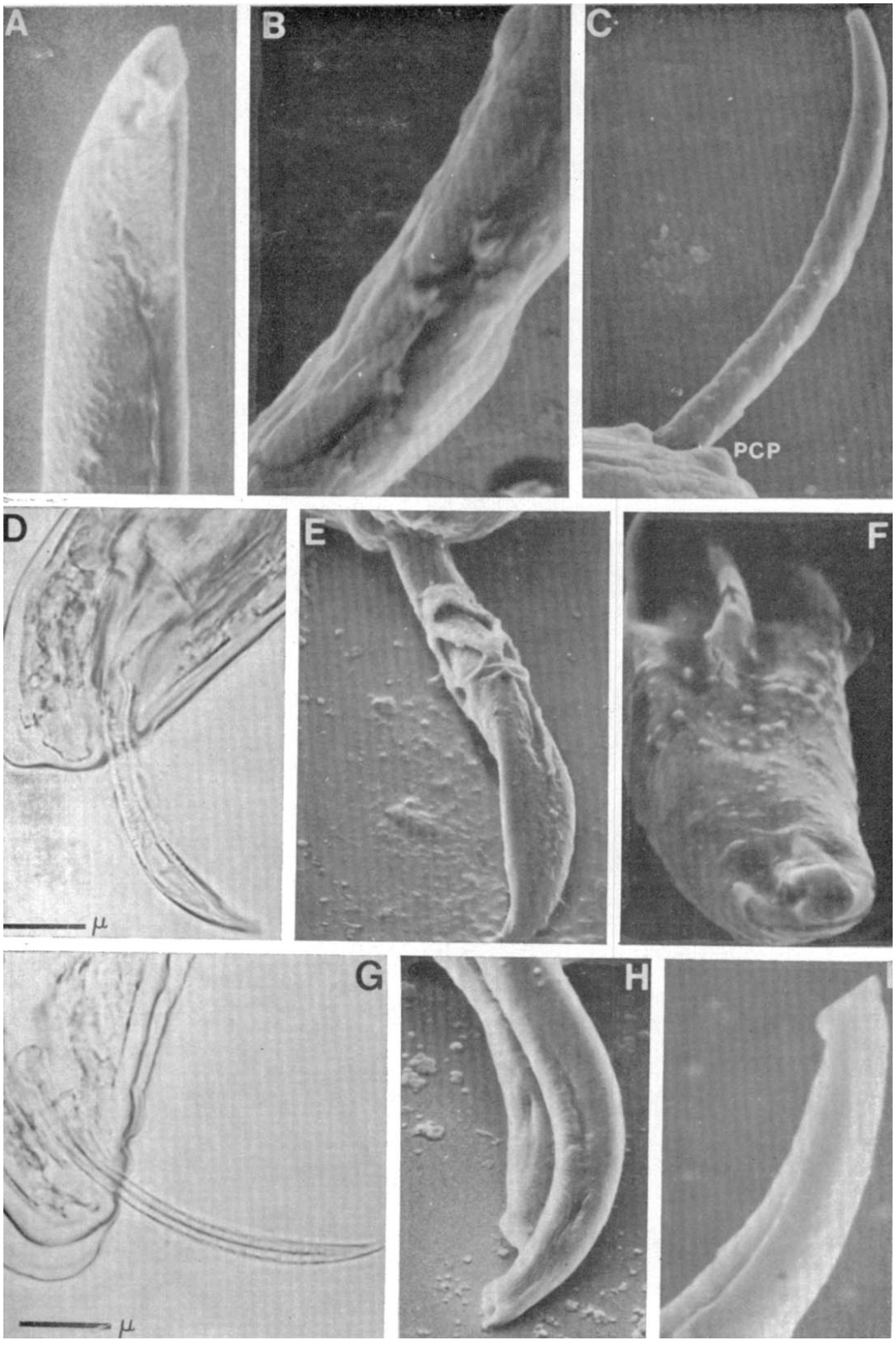


FIG. 1-(A-G). *A) Trichodorus californicus.* Almost completely protruded spicule X3,000. *B) Paratrichodorus (Nanidorus) minor.* Partially protruding broken spicule X4,200. *C. Trichodorus intermedius.* Partially protruded spicule X4,200. *D) T. intermedius.* Partially protruding spicules, one shows the ventroterminal opening (arrow) X5,000. *E) P. (N.) minor.* Detail of the spicule surface X12,000. PCP = Postcloacal papilla. *F) T. californicus.* Detail of spicule surface X10,000. *G) Monotrichodorus vangundyi.* Detail of spicule surface X10,000.


A
B
C
PCP
D
μ
E
F
G
μ
H

Monotrichodorus vangundyi Rodriguez-M, Sher, and *Siddiqi:* This species has long and slender spicules, with a small, well-defined head. With LM, the shaft is almost uniform in width from the head to near the tip, where it expands slightly. Very fine transverse striae are present along the shaft. An inner longitudinal septum extends from the head-shaft junction and terminates near the distal end. The outline of the spicule shaft appears to be serrated with very fine bristles (Fig. 2-G).

As determined by SEM, the spicule has a sheath formed by comparatively strong setae (Fig. 2-B, C), similar to those of *T. intermedius* (Fig. 1-C, D), but wider spaced (Fig. 1-G). The tip of this spicule has a terminal opening with two small dorso-lateral invaginations which appear to be continuous with a ventral suture (Fig. 2-A).

DISCUSSION

We have found that trichodorid spicules have ornamentations never well understood when observed under the light microscope and even less understood when the spicules are observed within the nematode, where they are covered by several layers of tissue.

The structures observed on spicules of *T. cylindricus,* which appeared as coarse setae, were most striking. The bifid condition of the spicule (2) can be explained by the dorso-lateral invaginations which appear to cut the spicule tip. The absence of ornamentations in the spicules of *T. aequalis* agrees with previous observations (4).

Trichodorus californicus and *T. intermedius* have spicules with what appears to be a ventroterminal opening, as opposed to the other spicules studied, in which the opening appears terminal.

The ornamentations observed on the spicules of *T. californicus, T. intermedius,* and *M. vangundyi* appear to be scales or derivations of scales. The fine transverse striations observed under the SEM appear to be the origin or suture point of scales with the spicule body. The scales are arranged in a regular fashion, overlapping each other, and thus forming a sheath that covers most of the spicule body, leaving the distal portion of the spicule smooth. In all instances, scales are oriented towards the distal end of the spicule. When observed under the LM, the distal ends of the scales sometimes appear separated from the spicule body, being obliquely oriented. In partially protruded spicules, it was possible to see that scales maintain the same orientation inside and outside the body of the nematode; therefore, they do not interfere with protrusion and retraction of the spicules. This observation suggests that the scales maintain this orientation during copulation and may function as a mechanical barrier against complete penetration of the spicule because of the resistance presented by the cuticularized ring of the vagina. Possibly, scales may also help maintain the opening of the vulvar lips during transference of sperm. The complicated structure observed on the spicules of *T. cylindricus* could also perform the same function. In the other species with stout, short, and smooth spicules, the shape of the spicule itself may interfere with deep penetration.

The spicules of *P. (N.) minor* differ from spicules of the other species studied. They exhibit transverse striae which give a segmented appearance to the spicule. The deformations observed under the LM and SEM suggest that these spicules are of soft consistency. The fact that males of this species are rare and that reproduction occurs without males may suggest that spicules in this species are nonfunctional.

Trichodorid spicules are generally composed of an inner core of spongy material surrounded by a thick cuticle, which in some species is surrounded by an additional sheath formed by the scales. A vacuole or chamber is sometimes observed in the inner core, which may indicate that the spongy

FIG. 2-(A-I). *A) Monotrichodorus vangundyi.* Spicule tip X10,000. *B) M. Vangundyi.* Detail of spicule surface X10,000. *C) M. vangundyi.* Spicule X3,000. PCP = postcloacal papilla. *D) Trichodorus cylindricus.* Tail and spicule as seen with light microscope. *E) T. cylindricus.* Spicule as seen with SEM, X3,000. *F) T. cylindricus.* Spicule tip X8,000. *G) M. vangundyi.* Spicule as seen with light microscope. *H) Trichodorus aequalis.* Partially protruding spicules X5,000. *I) Trichodorus proximus.* Spicule tip in lateral view X8,000. Bar in D and G represents 10 μm.

material is a residue of secretions present in the spicules. Because of no visible vas deferens, and because spicules appear hollow with a terminal or ventro-terminal opening or pore, it is possible to speculate that sperms pass through the spicule body.

LITERATURE CITED

1. ALLEN, M. W. 1975. A review of the nematode genus Trichodorus with descriptions of ten new species. Nematologica 2:32-62.
2. HOOPER, D. J. 1962. Three new species of Trichodorus (Nematoda: Dorylaimoidea) and observations on T. minor Colbran, 1956. Nematologica 7:273-280.
3. HOOPER, D. J. 1975. Morphology of Trichodorid Nematodes. Pages 91-101. *in* F. Lamberti, C. E. Taylor, and J. W. Seinhorst, eds. Nematode vectors of plant viruses. Plenum Press, London and New York.
4. LOOF, P. A. A. 1973. Taxonomy of Trichodorus aequalis-complex (Diphtherophorina). Nematologica 19:49-61.
5. LOOF, P. A. A. 1975. Taxonomy of Trichodoridae. Pages 103-127. *in* F. Lamberti, C. E. Taylor, and J. W. Seinhorst, eds. Nematode vectors of plant viruses. Plenum Press, London and New York.
6. RODRIGUEZ-M, R., and A. H. BELL. 1978. Three new species of Trichodoridae (Nematoda: Diphtherophorina) with observations on the vulva in Paratrichodorus Siddiqi, 1973. J. Nematol. 10:132-141.
7. RODRIGUEZ-M, R., S. A. SHER, and M. R. SIDDIQI. 1978. Systematics of the monodelphic species of Trichodoridae (Nematoda: Diphtherophorina) with descriptions of a new genus and four new species. J. Nematol. 10: 141-152.
8. SHER, S. A. and A. H. BELL. 1975. Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida: Nematoda). J. Nematol. 7:69-83.
9. SIDDIQI, M. R. 1973. Systematics of the genus Trichodorus Cobb, 1913: (Nematoda: Doylaimida) with descriptions of three new species. Nematologica 19:259-278.
10. SCZYGIEL, A. 1968. Trichodorus sparsus sp. n. (Nematoda: Trichodoridae). Bull. Ac. Pol. Sci. 16:695-698.