On the Morphology and Ultrastructure of the Esophageal Region of Trichodorus allius Jensen

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The search by electron microscopy for plant-pathogenic viruses in nematode vectors began with Xiphinema index, the vector of the fanleaf strain of arabis mosaic virus. No evidence of the virus was found in X. index but the study did yield valuable information on the ultrastructure of the nematode vector (Wright, 1966; Roggen, Raski and Jones, 1966, 1967).

More recently, the search has been directed to Trichodorus allius, a vector of tobacco rattle virus, in a similar study of nematode-virus relationships. One advantage of this virus is the rod-shape of its particles which might be easier to detect in animal tissue than the polyhedral particles of fanleaf virus.

A study of ultrathin sections of T. allius has not been successful in locating virus particles but has been fruitful in developing a better understanding of the odontostyle and mechanism of feeding and of the ultrastructure of the esophagus.

Materials and Methods

Nematodes used in these studies were collected originally from soil about the roots of sugarbeet (Beta vulgaris) and alfalfa (Medicago sativa) near Salinas, California. Populations were reared at Davis, California, under greenhouse conditions feeding on the roots of alfalfa, tobacco (Nicotiana tabacum), but principally on sweetpea (Lathyrus odoratus). No males of T. allius were seen in any of these populations.

Female and larval nematodes were wet-sieved from the soil then hand-picked into tap water. The nematodes then were transferred to 0.9% NaCl and frozen by dry ice in a fume-chamber with 2% OsO₄ in distilled water. After being allowed to return slowly to room temperature they were fumed overnight. This treatment was followed by 2 minutes immersion in 15% DMSO (dimethylsulfoxide) in distilled water and 20 minutes in 2% OsO₄ in distilled water. This procedure was used unless otherwise indicated in plate text. The nematodes then were handled according to Wright and Jones (1965). Embedded worms were cut by diamond knives on an LKB Ultrotome and examined on an RCA EMU-3G or modified EMU-3E electron microscope each having a 50 μ objective aperture and operating mostly at 50 kv, occasionally at 100 kv.

Observations

Cuticle

The cuticle of the adult female of T. allius (Plate 1, A–D; Fig. 1, A) appears to be composed of eight layers as follows:

L₁. A triple-layered, thin (0.025 μ) osmophilic membrane.

L₂. A thick (0.16 μ) layer, uniform in texture. There may be two sub-layers making up L₂ because in one section (OsO₄ fixative plus Pb(OH)₂ postsectioning stain for 15 minutes) a fine, outer, whitish band and dark inner band
were observed. These were not consistently or certainly set apart in other sections.

Fine cross-striations (cs) (Plate I, A–C), approximately 3 \( \mu \) apart, were present on most of the body and extended almost to the terminus. The striations were formed by an infolding of L' and L'' and at most created a slight indent in the outer surface of L'.

L'. A layer, varying in thickness, from 0.48–1.50 \( \mu \) with an average of about 0.64 \( \mu \). This layer also appeared to have two components, different in texture and somewhat in color, but there was no sharp demarcation between the two. The outer portion (L'a) comprised 74–82% of the total and was coarser-grained in appearance than the inner portion. In cross-section there was only a vague suggestion of structure as concentric lines but without regularity. In longitudinal section (Plate I, C) there were many lines running longitudinally which appeared to set off sublayers, 18–20 in number, whitish with dark borders. The inner 14 or 15 were broader, narrowing (or doubling) near L'. The inner portion (L'b) comprised 18–25% of the total of L', was darker in color and of finer grain than L'a. In longitudinal section there were 11 or 12 sublayers similar to those in L'a but dark bands with light borders.

L''. A layer variable in thickness from minimal or nonexistent to 0.68 \( \mu \), spongy or amorphous in texture.

L'''-.. The next three layers were granular with no apparent structure and about equal in thickness (0.09 \( \mu \) each). The innermost (L'') had an irregular inner surface projecting in rough, lumpy protuberances.

L''. Underlying L'' was an irregular and light gray layer the nature of which is unknown. It was intimately connected with the epidermis which, on the outer margin, formed an irregular edge with polyp like protuberances intermingled with L''.

**Oral Aperture**

The most anteriad section shows the oral opening to be triradiate (each arm about 0.5–0.7 \( \mu \) long). These arms are almost straight but slightly posteriorly they become irregular with several short side branches, the distal branches on two of the arms give an almost forked appearance (Plate II, A). Lining the oral opening is the thin, dark osmiophilic layer (L') which is continuous with the outermost layer of the body cuticle.

The opening is set in L'' of the cuticle which forms a roughly hexagonal shape, each side about 0.7–0.9 \( \mu \) long. The six angles of the hexagon are directed each between two inner labial papillae (IL) and anteriorly these angles extend outward joining a similar layer of tissue outside the papillae. The triradiate oral opening also is oriented with each arm directed between two inner labial papillae. Each papilla is surrounded by more coarsely granular L''.

About 0.6–0.8 \( \mu \) posterior from the anterior surface (Plate I, D) is a crease or folding of the oral aperture extending parallel with the anterior surface. This fold is about 0.6 \( \mu \) long in both directions perpendicular to the longitudinal aperture. The osmiophilic layer lines this fold and is continuous with the lining of the oral aperture.

**Stoma**

The first evidence of the stomatal wall (Plate II, B) appears just below the thick anterior cuticle and is composed of:

L'. The osmiophilic layer forms a very irregular outline of the stomatal lumen and is grossly convoluted or jagged. This lasts only a short distance and soon becomes more simple, triradiate with a single bulge at the midpoint of each side (Plate II, C). Where the lining is convoluted (Plate II, B) there are some membranelike folds inside the lumen of the stoma.

L''. This layer is relatively thick (0.1–0.2 \( \mu \)), at first uniform in texture, and definitely set off from the adjacent L' by a dark line. Later a well-defined membrane appears around L'' (Plate II, C) and soon afterwards tissue appears with the membrane. This tissue probably is the most anterior projection of pharyngeal tissue (ph).

At the same level where the above membrane first appears there is also the first evidence of the lining of the pharyngeal lumen (pII) inside L''. The innermost lining of the pII seems to be similar to, if not identical with, L' and continuous with it. As the pII expands the L'' diminishes to a thin concentric band around it, finally becoming an incomplete band (Plate II, D) and later disappears.

The double-layered L'' surrounds L''. Outside of L'' is a membrane which broadens slightly to a thin line of epidermal tissue.
rounding the epidermal layer is another which appears to be L1 and it in turn is surrounded by another layer of epidermis.

L2 retains its double-layered appearance (Plate II, C) through level 1 (Fig. 1, A–B) where the pharyngeal tissue appears between it and L1. The pharyngeal tissue continues to expand through succeeding levels further separating off L1. As this happens L2 becomes homogeneous in color and evenly granular. Posteriorly L2 disappears (Plate II, E). This seems to be the inner limit of the external cuticle and probably marks the posterior end of the stoma. This is supported by the fact that molted cuticles of larvae show only a very short stomatal cuticular lining which is cast off.

**Pharynx**

Coincident with the very first appearance of the pll there are light colored areas (Plate II, C; level 1, Fig. 1, A–B) which seem to be strengthening rods or bands (Pr) running longitudinally. These may serve as muscle attachments and are located centrally near the osmiophilic layer. Similar refractive bands soon appear (Plate II, D) at the ends of the triradiate lumen. The 3 inner bands are shorter than the distal ones and disappear just before the stylet tip is seen (Plate II, E). The three distal bands divide into three each—one at the tip of the angles of the lumen and one slightly larger on each side.

The next change in these bands is their disappearance in the apical area on all three rays of the lumen accompanied by a lengthening of the dorsal wall which is gently arched (Plate IV, A). On the ventral ray of the lumen the refractive bands divide to form two pairs. These refractive longitudinal bands persist some distance until finally the two pairs on the ventral ray disappear (Plate II, F) and soon afterwards the pairs on the two subdorsal rays disappear.

Posteriorly the next development is a smooth, grayish thickening of L2 lining the two sides of the ventral ray of the lumen (Plate III, G). These thickenings extend posteriorly through the folds of the guide ring then disappear. The rest of the pll outside the L2 becomes restricted to small narrow areas on both walls of the ventral ray on each side of the swelling and finally the entire lumen wall becomes a uniform, gray texture.

**Odontostyle**

The odontostyle is first seen (Plate II, E) as a simple circular body about 0.2 μ across, free in the lumen. It has a uniform gray color with a small central darker dot and a fine, slightly darker, outer border. Posteriorly it en-

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Plate I. Cuticular layers and markings. A, Longitudinal section, 12,000 ×; B, Longitudinal section, 38,000 ×; C, Cross section, 20,800 ×; D, Longitudinal section in stomatal region, (IL) innervations to intero-labial papillae, 43,700 ×.

Plate II. Cross sections of stoma and pharynx including the odontostyle and its extension. A, Near oral aperture, 14,000 ×; B, Stoma, 23,100 ×; C, Transition from stoma to pharynx and first evidence of pharyngeal lumen lining ( pll ), 19,500 ×; D, Transition slightly posterior to D, 18,200 ×; E, Pharynx with first section of odontostyle ( Od ), 30,400 ×; F, Pharynx posterior to E, 20,000 ×.

Plate III. Cross sections of stoma and pharynx (cont’d). G, Odontostyle near attachment to dorsal wall, 19,400 ×; H, Fusion of odontostyle with dorsal wall and guide ring, 13,400 ×; I, Stylet extension posterior to H, 12,500 ×; J, Stylet extension posterior to I, 13,800 ×; K, Attachment of odontostyle protractor muscles to stylet extension, 22,000 ×; L, Transition of stylet extension with wall of pharyngeal lumen, 12,500 ×.

Plate IV. Esophageal muscles. A, Cross section at collar of ( Ap ) amphid, (PPm) perpendicular pharyngeal muscles, (LPm) longitudinal pharyngeal muscles, 19,800 ×; B, Cross section near base of stylet extension, (OPm) odontostyle protractor muscles, 23,100 ×; C, Cross section of esophagus in narrow region posterior to stylet, (ELm) esophageal lumen muscles, 20,200 ×.

Plate V. A–B. Longitudinal sections of amphid ( P = amphidial pouch; C = collar; → indicates basal plates of cilia). A, Near base of amphidial pouch, 9,500 ×; B, Junction (S) of cilia and nerve (N), 15,500 ×; C, Longitudinal section of esophageal gland orifice into lumen of esophagus cut across two rays of lumen (Lu), 30,400 ×; D, Longitudinal section between two rays of lumen (Lu), 31,200 ×.
larges to about 0.3 μ across and shows two layers, a darker band (about 0.05 μ wide) as a concentric ring surrounding the solid inner core with a central black dot (Plate II, F). Later the inner core and the outer band expand and the central dot enlarges, becoming more complex and eventually developing an outer membrane surrounding tissue which includes endoplasmic reticulum and mitochondria.

The outer portion of the odontostyle reaches a maximum thickness of about 0.1 μ when the total diameter is 0.5 μ. Posteriad this outer portion becomes more narrow as the intermediate whitish layer expands to 0.8–1.0 μ in diameter. At the same time the central core enlarges with a membrane surrounding its tissue. When the outer band has disappeared (Plate III, G) the odontostyle consists of a circular, uniformly light, cuticular material surrounded by a thin, dark line with a maximum diameter about 1.1 μ and with an inner tissue core about 0.3 μ in diameter. The cuticular lining of the odontostyle is similar in texture to the odontostyle extension and continuous with it.

Posteriad the odontostyle begins its attachment to the dorsal wall and the “guide ring” appears (level 5, Fig. 1, A). The attachment begins first toward the left side of the dorsal wall (Plate III, H) and is completed in a short distance. Where the odontostyle joins the dorsal wall (Plate III, H) the pII folds anteriorly then again posteriorly, still showing the thickenings of the L'. In the region of the odontostyle extension posterior to the “guide ring” the lumen of the esophagus (Lu) is small, narrow and immediately ventral to the extension (Plate III, I, J).

In the odontostyle extension the inner core of tissue with membrane moves dorsal (Plate III, I) and finally merges (Plate III, J) with the nearby tissue (epidermal?) of the pharynx and the odontostyle extension thus forms a U-shape in cross-section with the open end to the dorsal side. By the time the core of tissue is no longer part of the extension, the latter, in cross-section, is flattened ventrally where it is part of the lumen wall and rounded dorsally. As the extension begins to diminish it becomes irregular in dorsal outline and mottled in texture (Plate III, K). Finally as the last of the whitish extension tissue disappears it becomes banded diagonally across the dorsal wall (Plate III, L). The lumen, as it courses through the narrow esophagus to the glandular bulb, has the familiar osmophilic membrane lining it -with simple rounded ends on the three radiating arms of the lumen.

Musculature

The somatic muscles possess two kinds of elements (myofilaments), fine and large, similar to those in Xiphinema index. These form identifiable areas in the cells where only large ones occur (near the midline of the cell), or where both occur (these areas surround the former). Finally the fine elements seem to be free of the larger elements in the outer margins of each cell. Neither striae nor any regular repeating markings were seen in longitudinal sections.

In the esophageal region there appear to be six or seven sets of muscles.

1. Labial muscles (LM) (Fig. 1, A). For want of a better term these are referred to as labial because they are the first to appear at the anterior end. There seem to be four (two each subventral and sublateral), quite large, extending from the outer stomatal cuticular lining to the body wall and are almost as broad as long at their maximum development. They each occur laterad to their respective dorsal or ventral inner labial papillary innervation processes.

It is not certain just where they attach. These are not long muscles and seem to disappear before the stylet tip is found in the buccal cavity. It is possible these represent the anterior extremity of the pharyngeal muscles (PPm).

2. Pharyngeal muscles (LPM) longitudinal. Four smaller muscles (LPM) appear at about the same level at which the labial muscles disappear. These four are closer in near the stomatal region with two each between the subventral and subdorsal inner labial papilla innervations (Plates II, D; IV, A; Fig. 1, A). The muscles are narrow and longitudinally parallel with but outside the pharyngeal tissue. The muscles seem to diverge and change location, one continuing on the left lateral side, the others as a single subdorsal and two subventral in location. The left lateral muscle persists longest but not quite to the end of the odontostyle proper.
Figure 1. Schematic drawing at head region and esophagus of *T. allius.*
3. Pharyngeal muscles (PPm) perpendicular to longitudinals (LPm). A set of three muscles are present in the area near the anterior tip of the odontostyle when in retracted position. One is about midventral in position attaching to the ventral apex of the triradiate stoma (Plate IV, A; Fig. 1, A). The other two are subdorsal attaching to the two subdorsal apices of the stoma. The muscles extend distally and proceed between somatic muscles to the body wall, possibly to the innermost layer of the epidermis. Internally the muscles end at a perpendicular line darkened in color and adjacent to the basement membrane of the epidermal tissue surrounding the pharynx.

4. Odontostyle protractors (OPm). These are strongly developed muscles which begin at a level anterior to the base of the odontostyle. Here, there seem to be three, one dorsal and two subventral, near the cuticular lining of the pharyngeal lumen. Nearer the end of the odontostyle these move to the outer edge of this pharyngeal tissue and appear as four or five large, irregularly shaped muscles. At the beginning of the odontostyle extension the muscles divide into elements (Plate IV, B), 15 or more in number almost lining the entire outer limit of the pharyngeal tissue. Some are dark staining while others are similar to the muscles anterior to this level. These muscles attach ventrally, two being slightly subventral leaving a corridor of epidermal tissue directly ventrad to that ray of the triradiate lumen. Each of these two becomes bifurcate as they approach the outer edge of the pharyngeal tissue. Where the core of the extension emerges, the muscles shift toward the center of the pharynx. Here mitochondria are large and numerous. Muscle attachments to the extension occur where the sclerotized wall begins to reduce in size and then almost cover the outer dorsal wall of that organ (Plate II, K).

5. Somatic musculature (Sm) (Fig. 1, A). These muscles begin at about the level where the amphidial pouches enter the body, appearing first dorsad and ventrad to the pouches. As the pouches move inward leaving a space laterad to them, the muscles appear between the pouches and the outer wall. Compared to Xiphinema index, the somatic musculature is not very high in cross-section.

6. Esophageal lumen muscles (ELm). Some sections of the narrow part of the esophagus posterior to the odontostyle and also of the enlarged glandular region, show some muscles radiating outward from the angles of the lumen. These appear to attach at the angles of the lumen or on the swellings on the arms of the lumen (Plate IV, C). Some sections show evidence of attachments of muscles at the ends of the rays of the lumen.

Sensory organs

1. Inner labial papillae. The six inner papillae when first seen (Plate II, A) are arranged symmetrically about 1 μ from the center of the oral aperture. The papillae show a scattered arrangement of microtubular elements. Posteriorly these range into four ciliary elements in each papilla (Plate II, B–C).

2. Outer labial and cephalic papillae. Six outer labial and four (two subdorsal and two subventral) cephalic papillae are present with two or three ciliary elements in each.

3. Amphids. These are quite similar to those found in X. index and are formed by invaginations of the cuticle. The inner lining is a continuation of the osmiophilic layer. Adjacent to the osmiophilic layer and surrounding it was cuticular material apparently similar to L' of the outer cuticle but also perhaps some remnants of L''.

The sensillar (amphidial) pouch is evenly granular in texture near the aperture but immediately posteriad is provided with some elements. At first the pouch (P) is irregular in outline then assumes a shape roughly rectangular in outline. The shape then narrows into a circular form with a thick ring of cuticle (C) surrounding it (Plate V, A). In the longitudinal section the amphid has a goblet-shape with a concentration of ciliary elements in the narrow collar (Plate V, A). Posteriorly the amphid widens again as numerous ciliary elements with basal plates (arrows) connect (?) with nerve cells (N) (Plate V, B). Ciliary structures are similar to those in X. index, differing only in number of elements in individual sensory organs. The cuticular structures of the amphids are molted by larvae and persist as prominent remnants in the shed cuticles.

4. Nerve ring. First seen at the level near the posterior end of the odontostyle extension the nerve ring forms a U-shaped arc of nerves on the ventral side but not entirely encircling the esophagus. In the region of the nerve ring
Figure 2. Schematic drawings illustrating feeding mechanism of T. allius. a–c, stylet protraction; d–e, stylet retraction; 1, labial muscles (Lm); 2, perpendicular pharyngeal muscles (PPm); 3, longitudinal pharyngeal muscles (LPm); 4, somatic muscles (Sm); 5, odontostyle protractor muscles (OPm).

and posteriorly around the esophagus are many nuclei with dense chromatin arranged around the periphery of and also scattered throughout the nucleus.

Esophageal glands. There are five nuclei visible in the esophagus of T. allius (Fig. 1, B). One is the large dorsal gland nucleus located in the dorsal sector of the esophagus about midway in the posterior bulb or glandular region. Two large subventral gland nuclei are more posterior in the glandular region. Slightly anterior to the dorsal gland nucleus are two more subventral nuclei, smaller in size and less noticeable.

The dorsal gland extends almost the full length of the dorsal sector but has its orifice into the lumen of the esophagus in the anterior end of the glandular region. In many specimens the narrow part of the esophagus between the stylet and the posterior bulb forms a distinct loop or S shape.

The two posterior subventral glands connect with the lumen of the esophagus in the posterior bulb region not far from the nuclei themselves. However, the orifices of the two smaller anterior subventral glands were not certainly established.

The orifices of the dorsal and two larger subventral glands are provided with minute, complex structures which appear to serve as collection mechanisms. These measure about 2.5 μ long, 1 μ in width and height and are located next to the lumen and each is between two rays of the lumen. The structure seems to be a sinus with radiating and branching arms numbering 20 or more. It is completely surrounded by a triple-layered membrane. A sagittal section crossing both rays of the lumen shows a kidney- or oval-shaped outline (Plate V, C), becoming a common sinus in the center. Sagittal sections bisecting two rays shows three or four separated arms of the sinus or 5–10 or more fingerlike radiating arms becoming fan-shaped and converging to the orifice entering the esophageal lumen (Plate V, D).

Discussion

Cuticle. The report on X. index (Roggen et al., 1967) concluded that the structure of the cuticle of nematodes is too variable and too few species have been studied to permit generalizations. It was not possible to homologize the layers of the cuticle of X. index with those of other nematodes.
Here the same appears to be true and aside from the outer osmiophilic layer the cuticular components of *T. allius* show no certain homology with those of *X. index*. The second layer in *T. allius* may be the cortical layer but it does not appear to be at all similar to the second layer of *X. index*.

Layer four is interesting in that it varies so much in thickness and is very spongy in nature. The cuticle of most *Trichodorus* species is known to be very loose and tends to fold or wrinkle during movement. In some fixatives it swells, sometimes ballooning out and forward beyond the anterior end of the body. The sections seen here indicate most of this change takes place in L'. This suggests osmotic regulation may be active in this layer.

**Feeding Process.** The feeding process of *T. allius* is accomplished by protraction of the stylet to puncture plant cells and to ingest contents of the cells through the triradiate lumen of the pharynx and esophagus. The structure of the odontostyle being toothlike with a solid tip (Plate II, E) precludes the ingestion of food through the odontostyle even though it appears to be hollow in sections just prior to the tip. Anterior to the "guide ring" the stylet is located inside the triradiate lumen of the esophagus. Posteriorly the stylet lumen is ventral to the extension. The muscle structure of the pharynx, as determined by their sections, suggests its function in the protraction and retraction of the stylet to be as follows (Fig. 2): (a) rest position with all muscles relaxed; (b) by contraction of the somatic muscles, labial muscles and perpendicular pharyngeal muscles, the stoma is everted with a slight protraction of the stylet; (c) contraction of the odontostyle protractor muscles results in protraction of the stylet and increased turgor pressure in the pharyngeal wall; (d) upon relaxation of the protractor muscles, turgor pressure effects stylet retraction; (e) relaxation of somatic, labial and perpendicular pharyngeal muscles results in reinvagination of the stoma.

The functioning of the pharyngeal lumen appears to be by the classical mechanism found in most nematodes: opening of the lumen by muscular action, closing by turgor pressure.

Eversion of the stoma during feeding may possibly account for the fine folds in the inner stomatal lining or osmiophilic layer (Plate II, B). If eversion does take place the cuticular lining would be stretched and the layers closest to the lumen would be stretched most. A reasonable adaptation would be to provide folds in the cuticle which would disappear on stretching. The folds would have to be most pronounced in the innermost layers and less in the outer layers of the stoma wall. Furthermore, it would be expected that thinner membranes could not withstand as much stress as the thicker ones and should be more folded. The folded osmiophilic layer fits this concept.

**Summary**

Observations are reported here on the ultrastructure of the cuticle, pharynx, musculature, sensory organs and esophagus of females of *Trichodorus allius*. The feeding process is also discussed.

**Literature Cited**


