

# Ultrastructure of the Feeding Apparatus of *Pratylenchus penetrans*<sup>1</sup>

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**Abstract:** The feeding apparatus of *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa was studied by means of the electron microscope. The stomatostylet is composed of tooth, shaft, and basal knobs. The tooth and shaft are closely interlocked. The tooth forms the outer covering and the inner lining of the anterior half of the stylet, whereas the shaft extends from the basal knobs to the level of the stylet opening. It is proposed that the shaft and knobs are living tissues, whereas the tooth is the product of sclerotized secretion. Stylet protractor muscles are attached to the basal knobs, and they become forked and split into 10 bundles as they extend anteriorly. Two types of secretory granules are observed in the dorsal esophageal gland, and they are different from those produced in the subventral glands. **Key words:** stomatostylet, protractor muscle, esophageal gland.

The stomatostylet of tylenchids was studied by Goodey (4). However, due to the limitations of the light microscope, the anatomy of the stylet has not been clearly demonstrated. Yuen (10, 11) made morphological observations with an electron microscope on the stylet of *Ditylenchus dipsaci* Kuhn. Preliminary investigations were also carried out by Hirumi *et al.* (7) with *P. penetrans*. This paper reports additional detailed studies on the ultrastructure of the feeding apparatus of *P. penetrans*.

## MATERIALS AND METHODS

*Pratylenchus penetrans* was cultivated monoxenically at 25 C on alfalfa callus tissue. The adults were first killed with diffused fumes of osmic acid, then double fixed with glutaraldehyde and osmic acid. After dehydration, the specimens were embedded in Spurr's embedding medium (8). Sections showing silver interference color were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a JEM-120 electron microscope operated at 80 kv.

## OBSERVATIONS AND RESULTS

The stomatostylet of *P. penetrans*, consisting of a tooth, shaft, and the basal knobs (Fig. 1), averaged  $9.2\ \mu$  in length. The tooth, which averaged  $4.5\ \mu$ , appears to be more heavily sclerotized and showed a much greater electron density than that of the shaft and knobs. It forms a partial outer covering of the

shaft as well as lining the stylet lumen (Fig. 3-9).

The total length of the shaft averaged  $7.5\ \mu$ . The exposed cylindrical posterior portion,

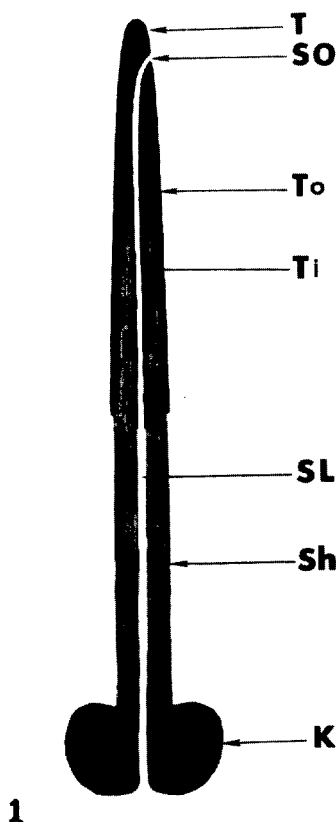


FIG. 1. Schematic drawing of the stomatostylet of *Pratylenchus penetrans*, showing the interlocking of the tooth (T) and shaft (Sh). The tooth actually covers the outer (To) as well as forming the inner lining (Ti) of the anterior half of the stylet. SL, stylet lumen; SO, stylet opening; K, knobs.

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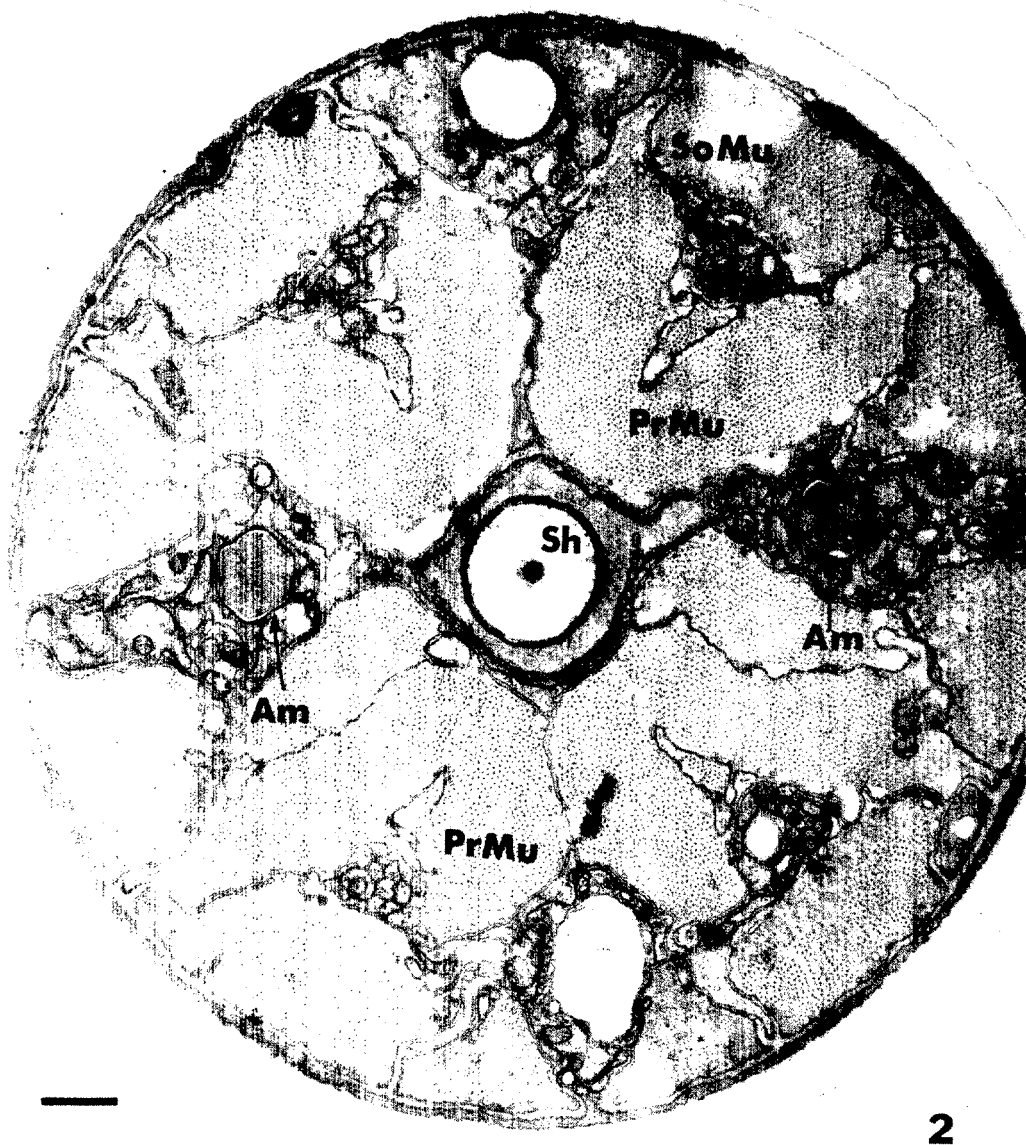
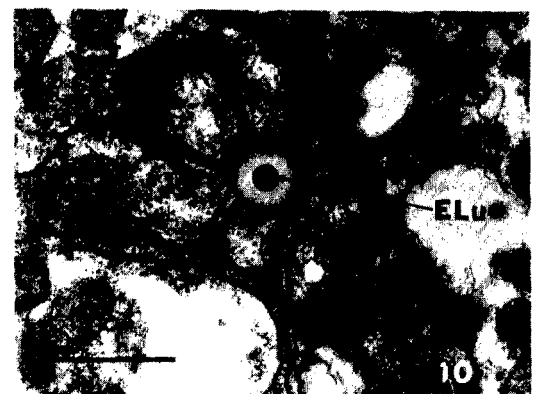
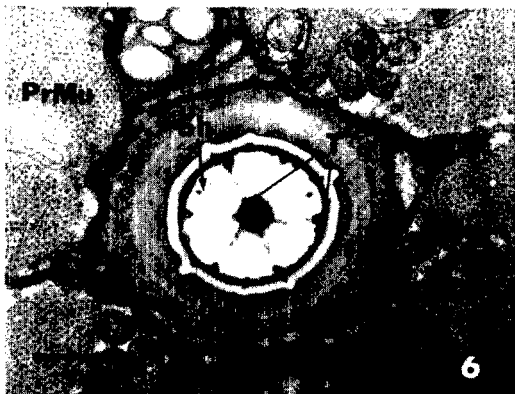
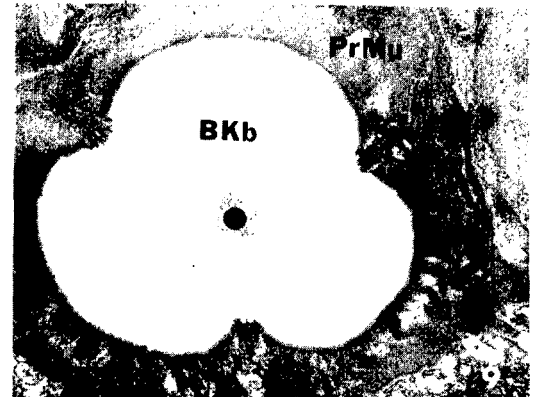
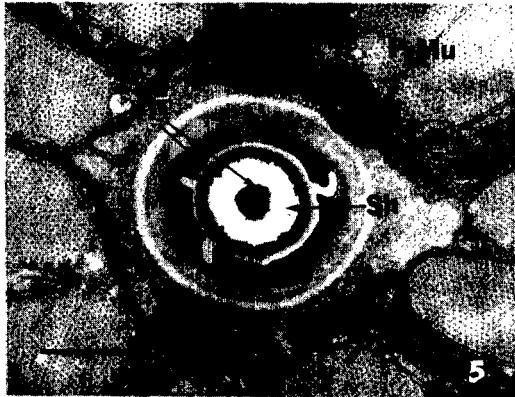
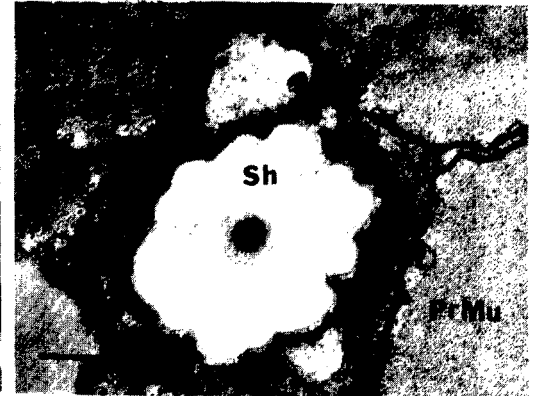
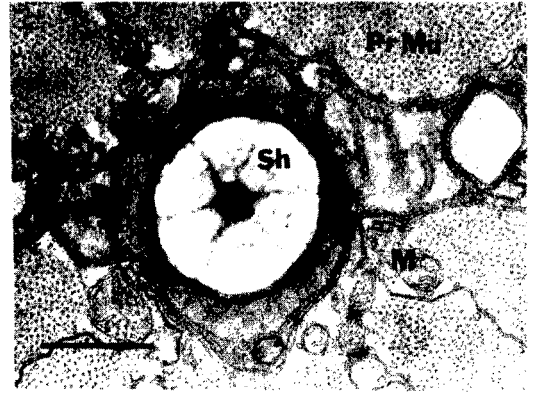
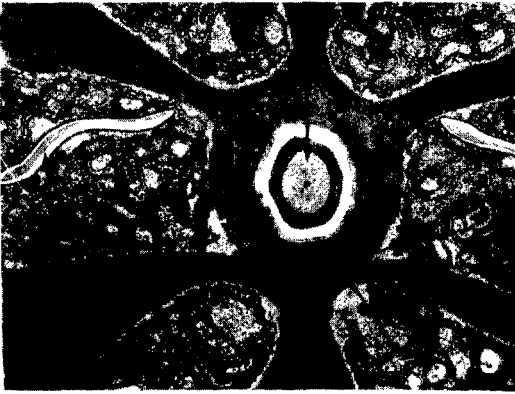


FIG. 2. Cross section of the cephalic region showing the forked protractor muscles (Pr Mu), the somatic muscles (So Mu), the Amphidial nerves (AM), and the shaft (Sh) portion of the stylet. Bar represents 0.5  $\mu$ .

FIG. 3-10. 3-9. Cross section of the stylet progressively from anterior portion to the basal knobs. T, Tooth; Am, Amphid; HSK, Head skeleton; PaN, Papillary nerve; Sh, shaft; GuA, Guiding apparatus; Pr Mu, Protractor muscle; M, mitochondrion; BKb, basal knobs. Bar represents 0.5  $\mu$ . 10. Cross section of the anterior region of the esophagus showing membranes surrounding the lumen (ELu). Bar represents 0.5  $\mu$ .



which measured  $3.5\ \mu$ , is connected and fused to the basal knob structure. The anterior portion is closely interlocked with the tooth. Longitudinal sections reveal that the shaft appears to split the tooth into four parts, but as the stylet narrows anteriorly these parts come closer together and are finally joined at the tip. In cross sections, the shaft at the stylet tip appears as an irregular electron-light ring interrupting the otherwise solid tooth (Fig. 4). It gradually enlarges posteriorly, being sandwiched between two rings of the more electron-dense tooth structure (Fig. 5, 6). There are 6 electron-dense rays, extending radially from the inner to the outer tooth ring and dividing the shaft into 6 sections (Fig. 6, 7). The shaft portion adjacent to the basal knobs instead of being circular has 12 longitudinal ridges (Fig. 8).

The basal knob of the stylet is trifold (Fig. 9). In cross section, it resembles that in *Ditylenchus dipsaci* (10). Three protractor muscles connect to the stylet knobs and split anteriorly into 10 bundles which attach directly to the cuticular skeleton of the head (Fig. 2).

Six cells enclosed by a basement membrane, and running longitudinally from the base of the stylet knobs to the median bulb, form the procorpus portion of the esophagus. Each cell is heavily compacted with mitochondria (Fig. 11). The lumen of the procorpus is cylindrical, with a cuticular lining which has the same electron density as that in the stylet shaft and the basal knobs. In cross section, complex membranes associated with the esophageal lumen have been observed (Fig. 10). Whether these membranes serve to divide procorpus tissue into sectors is not clear.

The dorsal esophageal gland connects with the lumen of the procorpus by a simple short duct located  $2\text{--}3\ \mu$  behind the stylet knobs (Fig. 11, 12). Two different types of glandular granules are observed inside the ampulla; the less electron-dense granules averaging  $0.22\ \mu$  in diam with network-like texture, and the darker granules, which are smaller, with an average

diameter of  $0.18\ \mu$ . Both of these are enclosed by a thin membrane.

The median bulb is enclosed by the same basement membrane that separates procorpus from pseudocoelom. The lumen of the median bulb is cylindrical in the anterior portion. At the level of the median valve, the cuticle lining is thickened and the lumen becomes triradiate (Fig. 15, 16). In cross section, six muscle cells can be seen attached to the dorsal and lateral sides of the lumen wall. The myofilaments fan out radially to the periphery of the bulb and attach to the inner layer of the basement membrane. Interspaced with these muscle cells are six cells which are characterized by the presence of many mitochondria and large nuclei.

At the level just below the posterior end of the median valve, two ducts branch out from the esophageal lumen, and each connects with the ampulla of a subventral esophageal gland (Fig. 13, 14). Only one type of secretory granules is observed in the subventral glands. They are smaller than those produced in the dorsal esophageal glands. There are also network-like textures within the granules, but their electron density is between those of the dark and light granules observed in the dorsal gland.

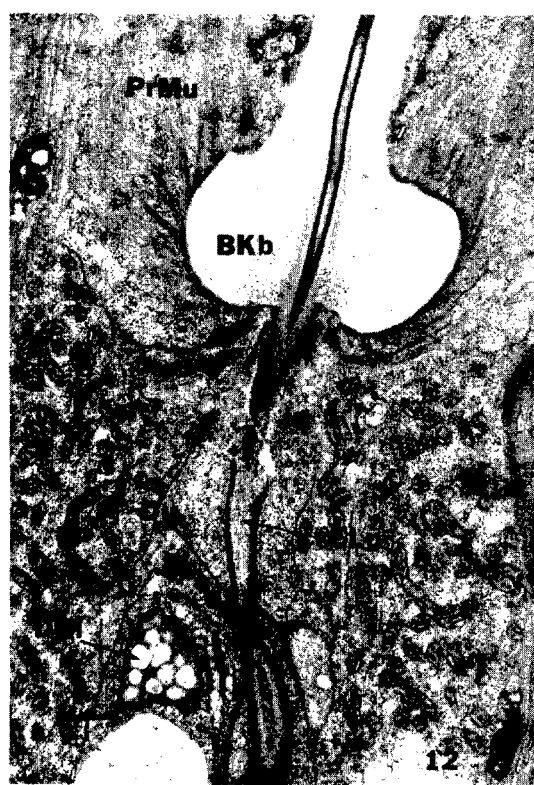
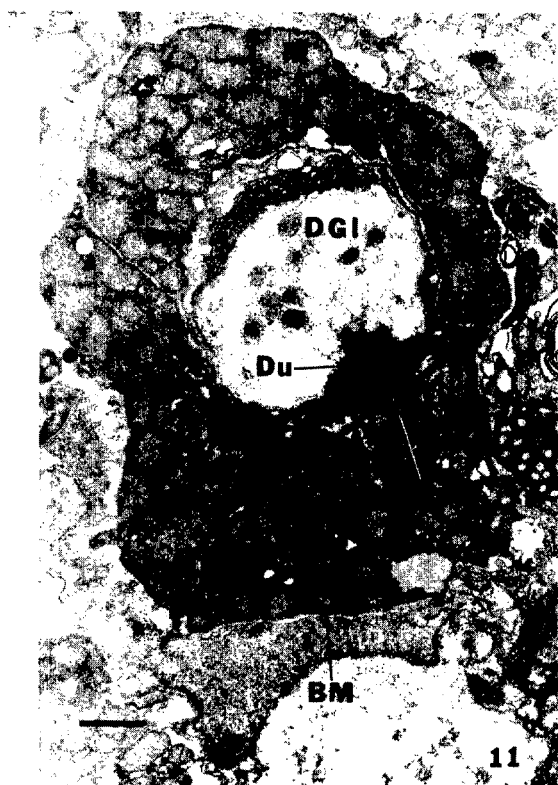
The isthmus of the esophagus is mostly enclosed by the nerve ring. The lumen is still triradiate. Membranes of the lumen complex subdivide the isthmus into many polygonal sections (Fig. 17) in which many microtubules are observed.

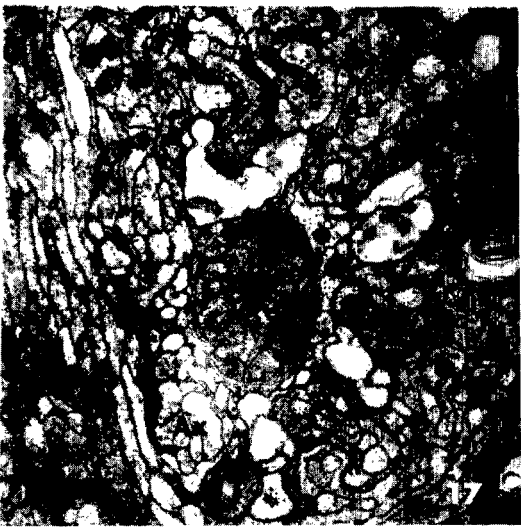
## DISCUSSION

One of the distinctive characters of the tylenchid is its massive stylet. Phylogenetic relationships of the tylenchid stylet and the stoma of Rhabditidae were interpreted by Andrassy (2). Goodey (4) hypothesized that the tylenchid stylet consists of three distinct parts: the conical end represents the fused metarhabdial teeth; the cylindrical shaft, the metarhabdions; and the basal knobs, the telorhabdions. Our observation on the

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FIG. 11-14. 11, 12. Cross and longitudinal sections through the dorsal esophageal gland ampulla (DGI) showing the duct (Du) which leads to the esophageal lumen (ELu). Six cells heavily compacted with mitochondria (M) are surrounded by a basement membrane (BM) to form the procorpus. Protractor muscles (Pr Mu) are attached to the basal knobs (BKb) of the stylet. Bar represents  $0.5\ \mu$ . 13, 14. Cross and longitudinal sections through the subventral esophageal glands (SVGI) showing the ducts leading to the esophageal lumen (ELu). Muscles in the median bulb (MeMu) are attached radially to the median bulb valve (MeVv). Bar represents  $0.5\ \mu$ .





ultrastructure of *P. penetrans* stylet reveals that the tooth portion is not a solid conical structure with a lumen through the center as generally believed. It not only covers the outer but also forms the inner lining of the anterior portion of the stylet. Therefore, the shaft and the tooth are very closely interlocked. During onset of molting, the tooth is shed while the shaft and basal knobs remain, but become invisible along with other parts of the esophagus. This process of development is similar to that of *Ditylenchus destructor* (1), *D. triformis* (6), and *Seinura* spp. (5). Later, the new tooth appears first, followed by the shaft, the knobs, and finally the median bulb valve. It seems to indicate that the shaft and knobs of the stylet are parts of the total living tissue, whereas the tooth is the product of sclerotized secretion. Recently, ultrastructure studies of *Criconemoides curvatum* (9), showing clearly cytoplasmic connections of the stylet shaft with its surrounding living cells, also support this hypothesis.

The granules found in the dorsal esophageal gland are different from those produced in the subventral glands. This difference in morphology may suggest the possible differences in their functions during feeding. Bird (3) concluded that the material exuded from the stylet of *Meloidogyne javanica* might be derived from the breakdown of granules in the ampulla of the dorsal esophageal gland. In the present study, two types of granules are found in the dorsal esophageal gland of *P. penetrans*. They may represent different organelles containing specific substances, or merely the same granules at a full or empty stage.

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FIG. 15-17. 15, 16. Cross sections through the median bulb showing the median bulb valve (MeVv) in its open and closed condition. MeMu. Median bulb muscles; M. mitochondria; Nu, Nucleus; DGI, Dorsal esophageal gland; SoMu, somatic muscles. Bar represents 0.5  $\mu$ . 17. Cross section of the isthmus showing membranes surrounding the lumen. This region of the isthmus is completely enclosed by axons (Ax) of the nerve tissue. Bar represents 0.5  $\mu$ .