Observations on the Morphology of the Pine Wood Nematode, Bursaphelenchus xylophilus

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Abstract: Scans of slash pine wood chips infested with pine wood nematodes showed coiled aggregates of 5–10 nematodes in the axial resin canals, and 1–2 nematodes folded in the radial resin canals. Observations of the pine wood nematode by SEM showed the head to be offset with six labial lobes. A smaller circle of six inner lobes surrounded the stoma. A single labial sensillum was observed on each of the two subventral and two subdorsal lobes. The two opposite lobes, without sensilla, bore a pore-like amphid. The female vulva flap was observed to be a pouch. In addition to two pairs of copulatory papillae previously described for the male, three additional papillae were observed: an additional post-anal pair, and a single papilla anterior to the cloaca. Spicules were fused ventrally. Distal spicule ends terminated in two fused half discs. Caudal alae were angular at the tail terminus, but curled inward at the corners to appear oval. Lateral fields had four lateral striations. *Key words:* vulva pouch, copulatory papillae, slash pine, scanning electron microscope.

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970 (syn. *B. lignicolus*, Mamiya and Kiyohara 1972), was found to cause widespread wilting death of red and black pines (*Pinus resinosa*, Ait. and *Pinus thunbergii*, Parl., respectively) throughout central and southwestern Japan (4,6). In Japan this nematode is vectored mainly by a long-horn beetle (Cerambycidae), *Monochamus alternatus* (3,4,5,6). The dauer larvae are carried to healthy trees and enter feeding wounds caused by these beetles. These larvae aggregate in the resin ducts, mature to adults, and lay eggs. The population increase is so rapid, it is believed they block resin flow and water transport. The decline in resin flow is the first symptom of the disease in pine trees (5). Rapid yellowing and wilting of the infested trees follow. Infected trees observed to be healthy in early summer were dead by late summer. In inoculation studies, trees were killed in 40 d.

The nematodes are devastating to the Japanese forest industry; about 8 million

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trees were killed in 1978. The Japanese Government had appropriated $35 million in 1980 to fight the disease (W. R. Nickle, personal communications). The only European country reporting the presence of the pine wood nematode is France (11).

In February 1979, Dropkin (2) reported the pine wood nematode on ornamental Austrian (*Pinus nigra,* Arnold) and Scotch (*Pinus sylvestris,* L.) pines in Missouri. Further discoveries of the nematodes followed in Arkansas, Kansas, Kentucky, Inlinois, Oklahoma, Maryland, Texas, Virginia, Louisiana, Pennsylvania, California, Florida, Mississippi, and South Carolina (13). We found these nematodes in a dead slash pine, *Pinus elliotti,* Engelm., in Baton Rouge, Louisiana, in November 1979 (1). It is now known to occur in at least 10 parishes in the state, most commonly found on slash pines and loblolly pines, *Pinus taeda,* L. The pine hosts in the other parts of the United States has been varied. The Japanese vector, *M. alternatus,* is not known to occur in the United States. A vector for the pine wood nematode in Louisiana is still unknown.

About 35 species of *Bursaphelenchus* have been described (8,9). Most are associated with insects. The pinewood nematode has certain morphological features that distinguish it from other species. Drawings from Mamiya and Kiyohara's original description of *B. lignicolus* (7) show a high offset head. Females have broadly rounded to digitate tails. The vulva has a wide overlapping anterior structure that appears as a flap in lateral view. The male spicules are large, paired, and with a rostrum. The distal ends have cuticular projections. The male tail is pointed with small terminal oval caudal alae.

Steiner and Buhrer (12) in 1934 recovered and described *Aphelenchoides xylophilus* from longleaf pine, *Pinus palustris,* from Bogalusa, Louisiana, and Orange, Texas. Illustrations of the timber nematode by Steiner and Buhrer showed an offset head, with six labial lobes. Larvae and female tails were digitate. They described six copulatory papillae for the males.

The objective of this study was to observe the external morphology of the pine wood nematode from Louisiana with the scanning electron microscope (SEM), compare these observations to those made by Mamiya and Kiyohara for *B. lignicolus* with the light microscope (LM), and to relate these comparisons with *B. xylophilus* (Steiner and Buhrer 1934) Nickle 1970.

MATERIALS AND METHODS

Wood chips of slash pine, infested with pine wood nematodes were soaked in water overnight to recover the nematodes. The nematodes were subsequently cultured and propagated on potato dextrose agar (PDA) plates colonized with *Gliocladium* spp. isolated from the same wood chips.

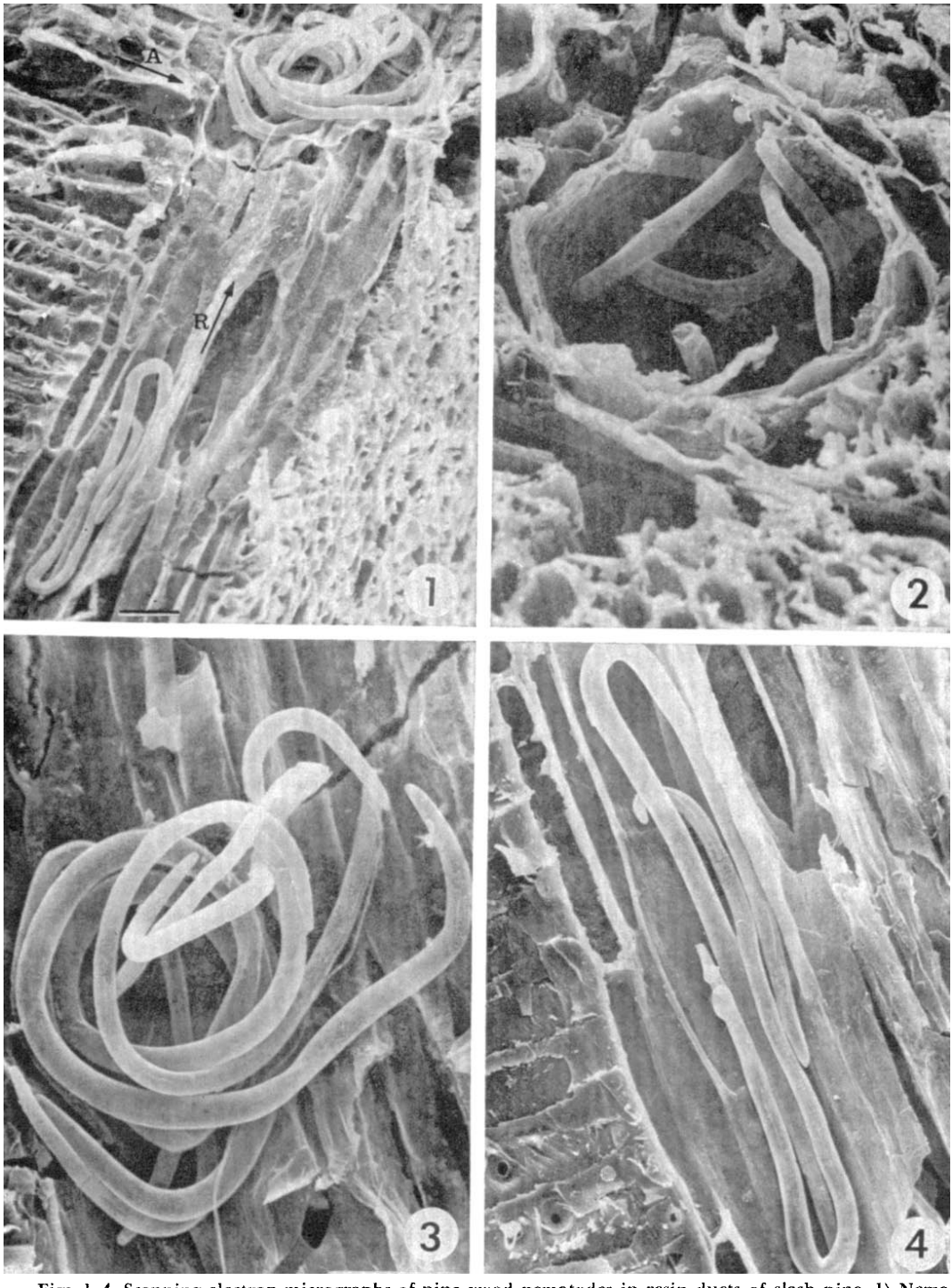
Various stages of the nematodes were flooded off the PDA plates, fixed in formalin alcohol acetic acid (FAA) overnight, and pipetted into modified beem capsules. Open ends of the capsules were covered with 13-mm-d, 5.0-μm-nucleopore membranes. The nematodes were dehydrated in an acetone series (20%, 50%, 70%, 95%), with two changes into 100% acetone for 5 minutes at each concentration, and then critical point dried. Nematodes were arranged on double adhesive tapes adhering to aluminum stubs, sputter coated with 20-nm gold palladium, observed and photographed with a Hitachi S-500 SEM at 20 kv.

We made a SEM study of the nematodes inside the naturally infested wood. Thin (1 mm) slices of infested wood chips were fixed in FAA for 2 d, washed in two changes of distilled water, dehydrated in acidified 2,2-dimethoxy propane (DMP), and critical point dried. Thin slivers of wood were removed along the grains in radial and tangential sections to expose the resin ducts containing the nematodes. These sections were mounted on stubs with television tube coat, sputter coated with 20-nm gold palladium, and viewed with the SEM at 25 kv.

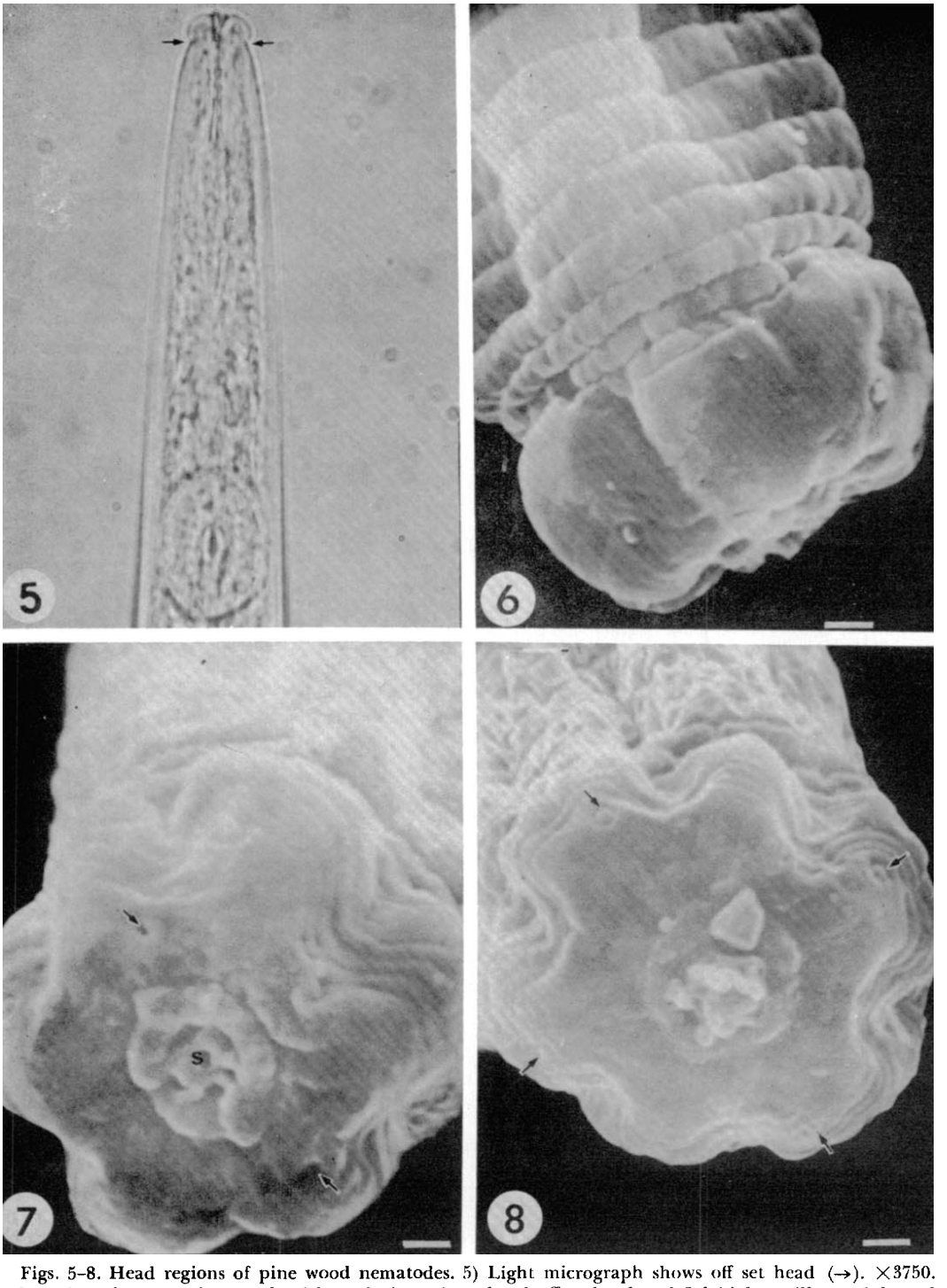
Different stages of the nematodes were mounted in water on glass slides and gently heat killed. These were then observed and photographed in bright field with a Leitz Ortholux II compound microscope.

RESULTS AND DISCUSSION

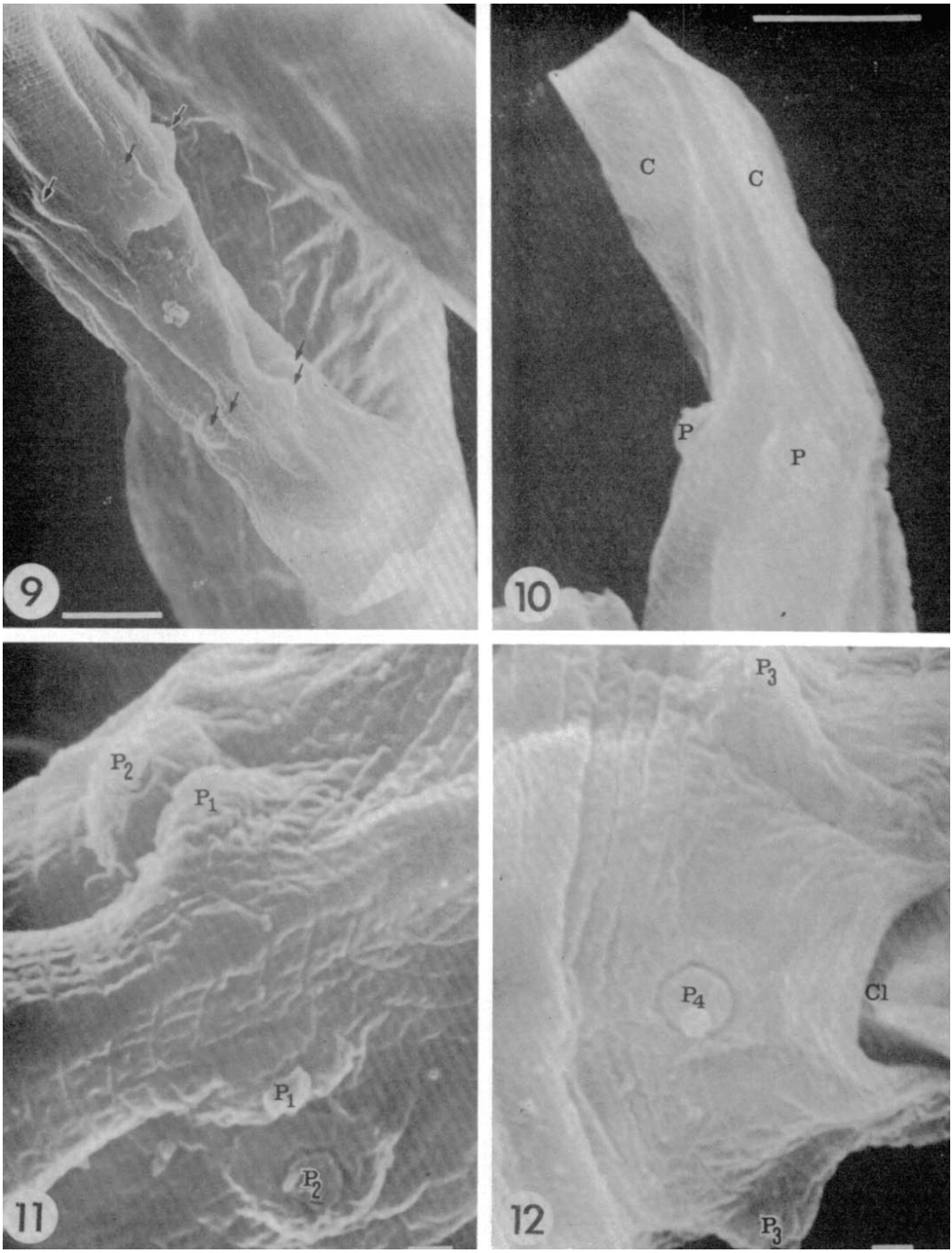
In situ scans of infested pine chips showed the pine wood nematodes infesting the resin ducts (Fig. 1). Aggregates of 5–10



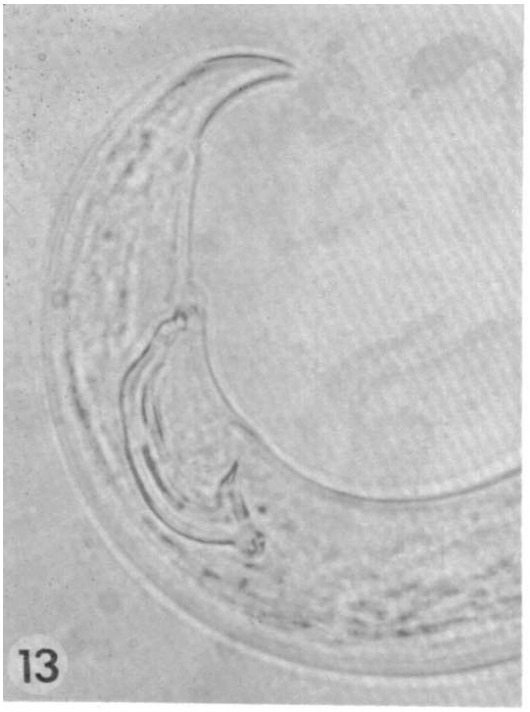
Figs. 1–4. Scanning electron micrographs of pine wood nematodes in resin ducts of slash pine. 1) Nematodes in axial (A→) and radial (R→) resin ducts. Tangential section. ×170. Bar = 53 μm. 2) Axial resin duct showing aggregates of nematodes blocking canal. Cross section. ×380. Bar = 45 μm. 3) Coil aggregate of nematodes exposed in axial resin duct. Tangential section. ×290. Bar = 43 μm. 4) Folded nematodes in the exposed radial resin duct. Tanqential section. ×250. Bar = 50 μm.

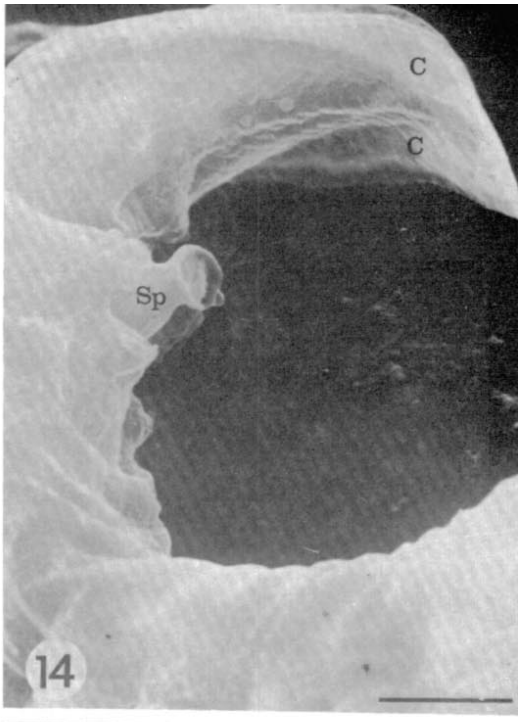


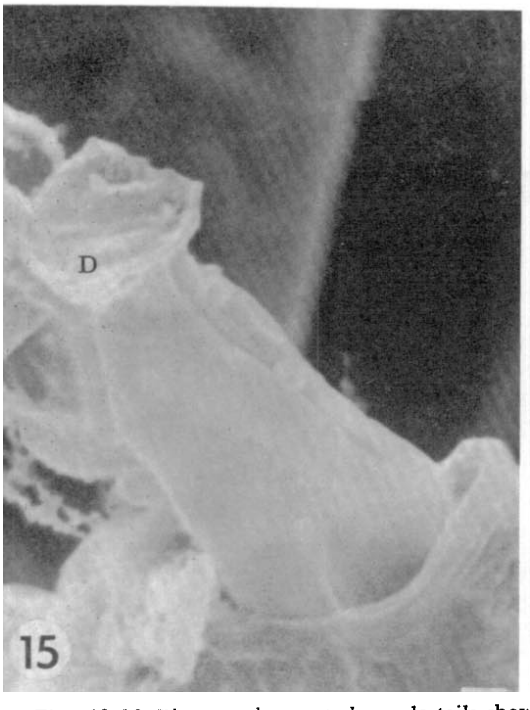
Figs. 5–8. Head regions of pine wood nematodes. 5) Light micrograph shows off set head (→). ×3750. 6) Scanning electron micrograph of lateral view of anulated offset head and 2 labial sensilla on 2 lateral lobes. ×15000. Bar = 0.5 μm. 7) Face view of head showing six labial lobes. A smaller circle of six inner labial lobes surrounds the stoma(s). Two pore like amphids on opposite lateral lobes (→). ×15000. Bar = 0.5 μm.

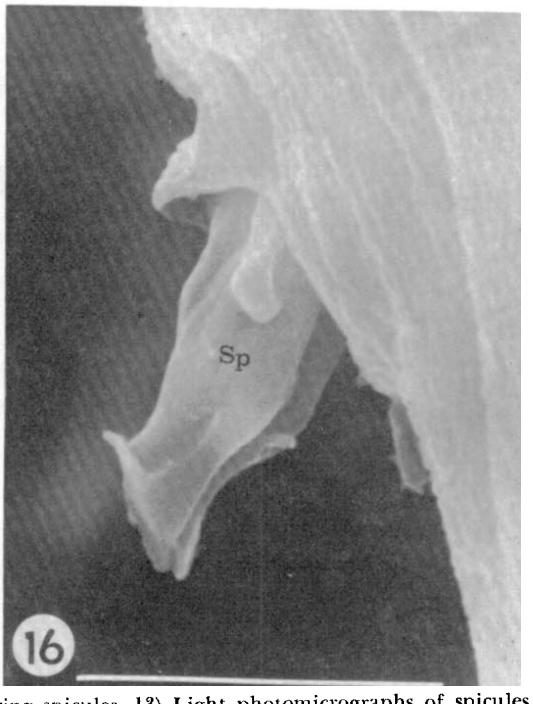


Figs. 9–12. Scanning electron micrographs of pine wood nematodes. ventral regions of male tails. 9) Male tail showing the seven copulatory papillae (→). ×2500. Bar = 5.0 μm. 10) Two adjacent pairs of post-anal copulatory papillae (P) located at origin of the caudal alae (C). ×4000. Bar = 6.0 μm. 11) Higher magnification of the two post-anal copulatory papillae in tail (P_1, P_2). ×10000. Bar = 0.5 μm. 12) A pair of pre-anal copulatory papillae (P_3) and a single papilla (P_4) anterior to cloaca (Cl). ×8500. Bar = 0.5 μm.

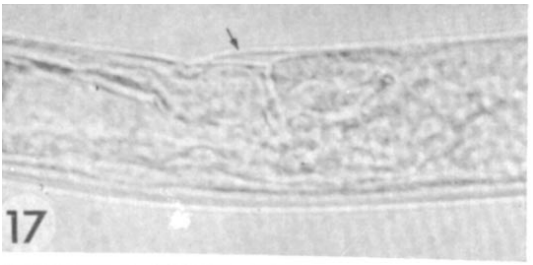


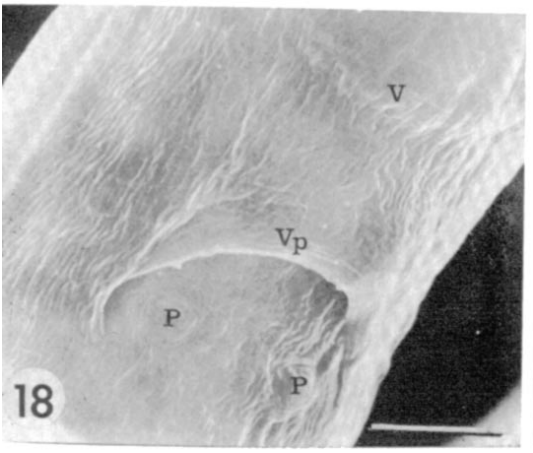


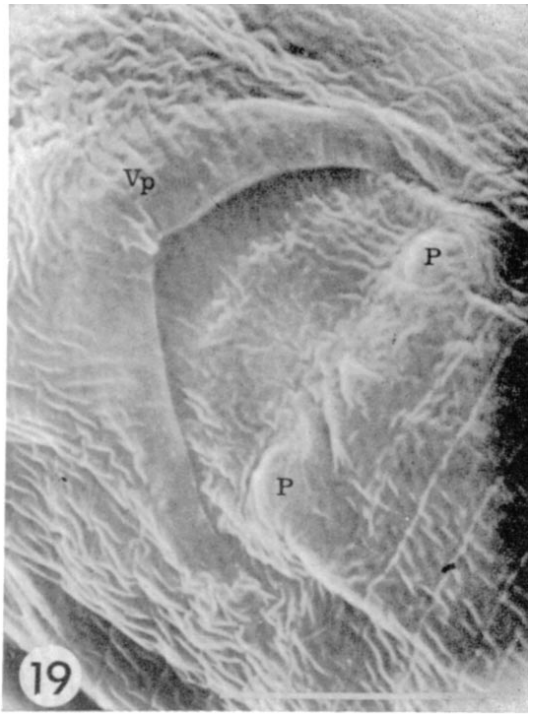




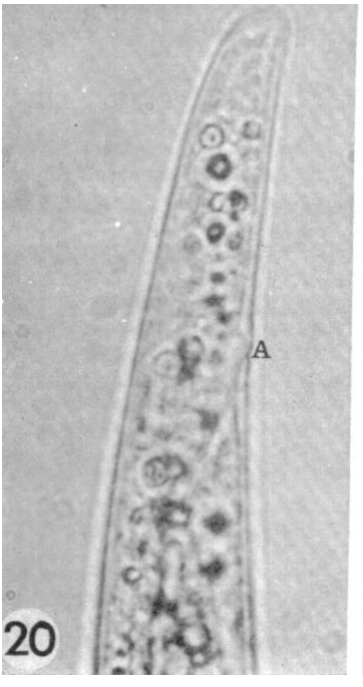
Figs. 13–16. Pine wood nematode, male tails showing spicules. 13) Light photomicrographs of spicules, extended spicule ends, and rostrum. Caudal alae not prominent. ×750. 14) Scanning electron micrographs of fused spicules (Sp) and caudal alae (C). ×3000. Bar = 6.0 μm. 15) Scanning electron micrograph of spicules' ends terminating in a disc (D). ×11000. Bar = 0.5 μm. 16) Lateral dorsal view of spicules (Sp) observed in SEM. A deep trough invaginates the dorsal of the paired spicules. ×10000. Bar = 5.0 μm.

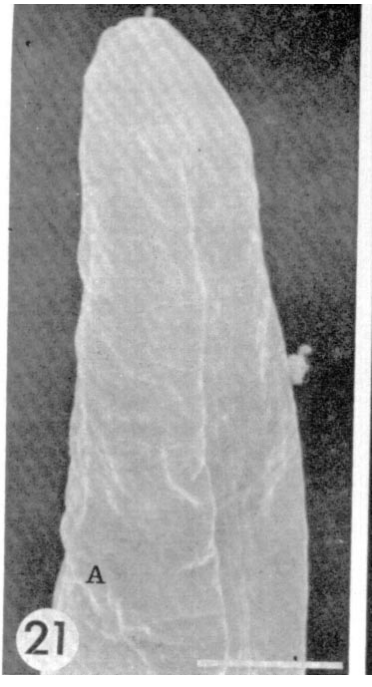


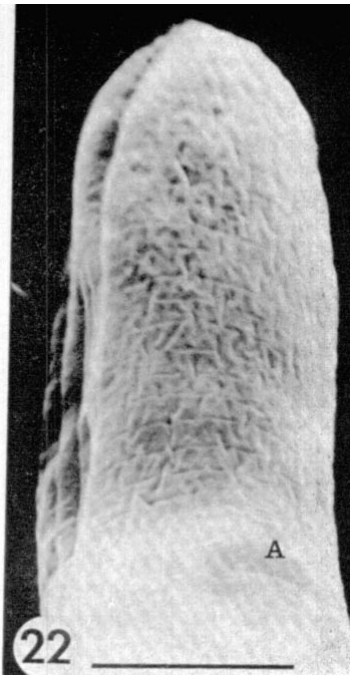




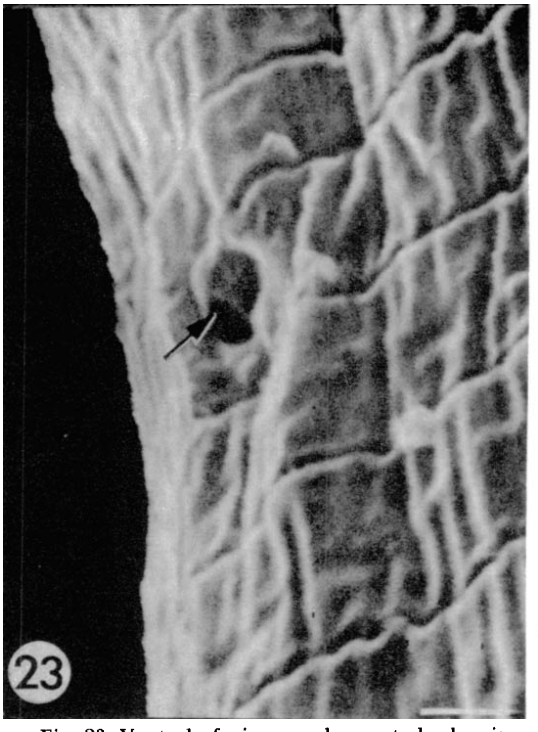
Figs. 17–19. Pine wood nematodes, vulva regions of females. 17) Light photomicrograph of vulva showing vulvar flap (→). ×2300. 18) Scanning elecron micrograph of vulva pouch (Vp). The ridge (V) shows location of vulva in the pouch. A pair of papillae (P) external to the pouch. ×3000. Bar = 4.5 μm. 19) Enlarged SEM view of pouch (Vp) and pair of mammilate papillae (P). ×5700. Bar = 5.5 μm.







Figs. 20–22. Tails of pinewood nematodes. 20) Light photomicrograph of digitate female tail. ×1650. 21) Digitate tail of larva seen with SEM. ×3000. Bar = 6.0 μm. 22) Digitate female tail seen with SEM. ×4000. A = anus. Bar = 4.0 μm.



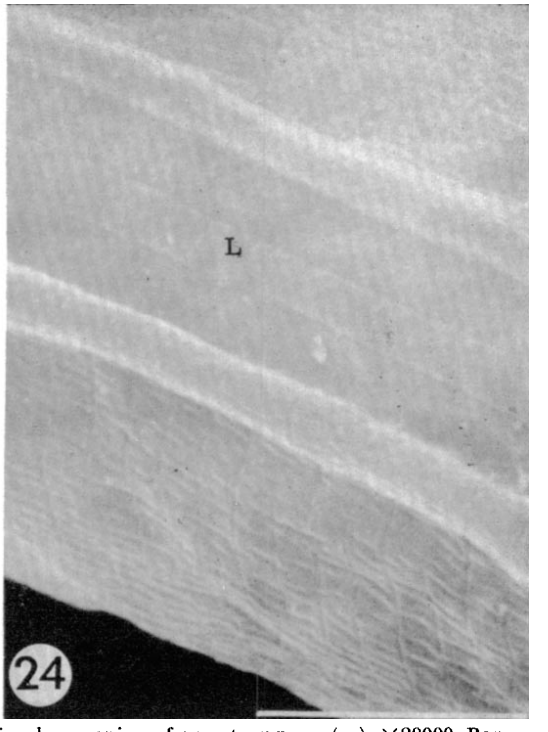


Fig. 23. Ventral of pine wood nematode showing circular opening of excretory pore (→). ×20000. Bar = 0.6 μm.

Fig. 24. Lateral field (L) of pine wood nematode with four lateral striations bordered by longitudinal ridges. ×6000. Bar = 5.5 μm.

nematodes were observed coiled in the larger axial resin canals (Figs. 2, 3), and 1–2 nematodes were folded in the narrower radial resin canals (Fig. 4). This concurs with the study of Mamiya and Kiyohara (7).

Light photomicrographs (Fig. 5) and SEM micrographs (Fig. 6) showed an offset head. SEM micrographs showed six labial lobes with 3–4 striations (Figs. 7, 8). A smaller circle of six labial lobes surrounds the stoma. A labial sensillum was observed on each of the two subventral and two subdorsal lobes (Fig. 8), and the two opposite lobes without sensilla each bear a pore-like amphidial opening (Fig. 7).

Mamiya and Kiyohara reported the presence of two pairs of copulatory papillae in the male. One pair is located pre-anal and the other at the caudal alae's origin. We observed seven copulatory papillae (Fig. 9) with the SEM, three more than previously described by Mamiya and Kiyohara. The first two, a ventrosubmedial pair, are located at the caudal alae's origin (Figs. 10–11). The second pair is adjacent to the first; these two are not visible as separate structures under LM because they fall in the same line of lateral view as the first pair. The third pair is located on the pre-anal side. The single papilla was observed directly anterior to the cloaca (Fig. 12).

The male spicules are conspicuous with small expanded ends. The caudal alae are obscure in LM (Fig. 13). Spicules observed in SEM micrographs (Figs. 14, 15) are fused. A deep trough seen in the dorsal side may account for the illusion of unfused spicules seen under LM (Fig. 16). The distal spicules' ends terminated in two fused half discs (Fig. 15). The caudal alae were cuticular extensions of the tail and spade-like at the tail terminus (Figs. 9, 10, 14), but curled inward at the corners to appear oval in dorsal view, and pointed in lateral view.

The vulvar flap seen in LM (Fig. 17) is actually a pouch with a wide anterior lip, as revealed with SEM (Fig. 18). A pair of mammilate papillae, not described before, was observed outside the pouch openings (Figs. 18, 19).

The tails of the larvae (Fig. 21) and females (Fig. 22) observed in SEM micrographs were subcyclindrical, broadly

rounded or digitate, similar to those shown in LM micrographs (Fig. 20). The excretory pore was a wide circular opening on the anterior, ventral portion of the body (Fig. 23), opposite the junction of the esophagus and the intestine. Lateral fields of all specimens of larvae, males, and females observed showed four lateral striations (Fig. 24) bordered by two longitudinal ridges.

The presence of the seven copulatory papillae in the male as revealed by our SEM study brings the resemblance of the pine wood nematode close to the timber nematode which had six copulatory papillae, as described by Steiner and Buhrer. However, no mention of the vulvar flap, a distinctive feature in the pine wood nematode was described by Steiner and Buhrer for *B. xylophilus* in 1934 (12) or by Nickle in 1970 (9).

Recent re-examination of the original material of *B. xylophilus* from the USDA Nematode Collection by Nickle and Golden (personal communication, W. R. Nickle and A. M. Golden) confirmed the presence of the vulvar flap and *B. lignicolus*-like spicules in *B. xylophilus*. Results of mating experiments of the Japanese culture and the Louisiana culture of *B. lignicolus* carried out by Nickle produced viable offsprings. Such biological and morphological research by Nickle et al. (10) conclusively place *B. lignicolus* as a synonym of *B. xylophilus*. Our observations with the SEM agree with this synonymy.

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