On the Taxonomy and Morphology of the Pine Wood Nematode, Bursaphelenchus xylophilus (Steiner & Buhrer 1934) Nickle 1970

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Abstract: During the past 3 yr, nematologists in the United States have found specimens of *Bursaphelenchus* sp. in the wood of dead and dying pine trees. This nematode-host association resembles a similar interaction reported from Japan where pine trees are being killed by the pine wood nematode. This taxonomic research was conducted to determine if the Japanese pine wood nematode and similar populations in the United States are of the same species. Based upon typical morphological characters of original specimens of *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970 that were rediscovered in the USDA Nematode Collection and genetic crosses among the Japanese and American nematode populations, it was concluded that they are all the same species, *B. xylophilus*.

During the past 3 yr, nematologists in the United States have found specimens of *Bursaphelenchus* sp. in the wood of dead and dying pine trees. This nematode-host interaction resembles similar associations found in Japan where pine trees are killed by the pine wood nematode. At present, the infestation in the United States is apparently not as severe as that in Japan where the cost of controlling this nematode disease complex exceeded $35 million in 1980. Eight million pine trees were killed by this nematode in Japan in 1978 making it the most important forest pest in Japan. Losses in the United States are mostly limited to ornamental pines (Scotch, Austrian, Japanese black), but other pines are susceptible. While it does not yet appear to be a serious problem in U.S. forests, its distribution is being monitored. A survey compiled by the U.S. Forest Service, St. Paul, Minnesota, shows that this nematode is present in 28 states in the central and eastern parts of the United States and in California. In the past, damage caused by the pine wood nematode was probably attributed to insects, fungi, drought, and other causes.

Japanese investigators suspect that the pine wood nematode entered Japan from abroad and became established in their pine species which are highly susceptible to this pest. One of the countries in which this nematode may have originated is the United States. The timber nematode, *Aphelenchoides xylophilus* (Steiner and Buhrer 1934), was isolated in 1931 from blue-stained logs of longleaf pine (*Pinus palustrus*) at a sawmill in Bogalusa, Louisiana (7). Among the more than 30 described species of *Bursaphelenchus* are *B. fraudulentus* (Rühm 1956) Goodey 1960, found in Germany in the frass of *Cerambyx scopolii* in *Prunus avium, Populus nigra* and *P. tremula* (6); *B. lignicolus* Mamiya and Kiyohara 1972 (4); and *B. mucronatus* Mamiya and Enda 1979 from declining pines in Japan (3). Only these three species have the typical spicule shape and vulval flap which are now known to occur on specimens of *B. xylophilus*. Nickle (5) transferred *Aphelenchoides xylophilus* to the genus *Bursaphelenchus* because of the typical bursa and other characters. Recently Baujard (1) placed *B. mucronatus* as a synonym of *B. lignicolus*.

This study redescribes the newly found *B. xylophilus* material from the USDA Collection and shows the results of the mating of a culture of *B. lignicolus* from Japan and the newly isolated American cultures of the pine wood nematode. Morphological information is enhanced by SEM micrographs of spicules and other diagnostic structures.

MATERIALS AND METHODS

The original material used by Steiner and Buhrer (1934) to describe *B. xylophilus* was thought to have been lost. Only six slides in extremely poor condition were found and referred to by Nickle (5). Three

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of these slides were unusable en face views and two of the other three had remains of deteriorated whole specimens (specimens on one slide had disappeared completely). Significantly however, all slides had data written in ink on slide labels that showed host, origin, collector, date of receipt, and the genus *"Pathoaphelenchus."* Beneath this generic name, in Steiner's handwriting, was the name *"xylophilus* n. sp." One slide, so labeled and dated 15 October 1929, contained specimens from *Pinus palustris* in Orange, Texas, thus providing the earliest record of the nematode in the United States. The other slides had specimens from *Pinus palustris* collected by Ross W. Davidson at a sawmill in Bogalusa, Louisiana, and received on two dates in 1931 (12 November and 24 November). Two slides with en face specimens and one with whole specimens dated 24 November probably constituted the main basis for the description. Data on the slides corresponded to the information in the files.

About 1962, in the process of sorting and salvaging some of the Steiner nematode material, one of us (AMG) found a vial containing specimens in formaldehyde solution and data showing that it contained the 24 November 1931 specimens from Louisiana. These were processed to glycerine, placed in a vial, and filed in the USDA Nematode Collection under the genus *Pathoaphelenchus* to correspond to the original records. With the recent intense interest and concern about the pine wood nematode in Japan and in the United States, a search of the Collection was initiated in early 1980. It located this vial of original specimens containing about 50 males, females, and larvae in remarkably good condition. The adults and some larvae were mounted in glycerine on slides and used in this redescription.

Photomicrographs of males and females were made with an automatic 35 mm camera attached to a compound microscope having an interference contrast system. The plate of original drawings prepared and used by Steiner and Buhrer (Fig. 1) had been kept in good condition at Beltsville.

To prepare specimens for observation with the scanning electron microscope (SEM), live nematodes, obtained in March 1980 from declining Austrian pine *(Pinus nigra)* in Finksburg, Maryland, were placed in vials containing 3% glutaraldehyde in 0.05 M phosphate buffer pH 6.8 at 22°C. Chemical fixation for 2–24 hr was followed by dehydration in an ethanol series and critical point dried in liquid carbon dioxide. The fixed specimens were placed on stubs and coated with 20–30 nm of gold-palladium in a Technics Hummer sputtering device. The coated specimens were viewed and photographed with a Hitachi S-430 SEM operating at 10 or 15 kV.

Mating studies were conducted between individuals of the Louisiana and the Pennsylvania populations and between the Japanese population and four American populations. All nematode populations were cultured on *Botryis cinerea* on PDA agar. The Japanese population was obtained from *Pinus thunbergii* collected at Tateyama, Chiba Pref. Japan in 1970 (Y. Mamiya). The American populations were isolated from *P. elliotti,* Baton Rouge, Louisiana (W. Birchfield); *P. elliotti,* Gainesville, Florida (R. P. Esser); *P. ponderosa,* Suskiyou County, California (R. W. Hackney); *P. thunbergii,* Cambridge, Maryland (R. Dekker); and *P. cembra,* Fox Chapel, Pennsylvania (J. R. Bloom). Standard mating techniques were used wherein last-stage larvae from the Japanese culture were individually placed on small petri dishes containing *B. cinerea* on PDA agar. One week later the plates were checked and those containing gravid females discarded. Ten single males from each of the four American populations and the Japanese population were added individually to the petri dishes containing nongravid females.

RESULTS

Studies of the original specimens of *B. xylophilus* revealed two significant findings indicating a close relationship of *B. xylophilus* to *B. lignicolus.* Both populations had the typically shaped spicules with expanded tips and the typical vulval flaps. The original illustrations of Steiner and Buhrer did not include the vulval flap. The spicule was not clearly shown (Fig. 1-D). However, the expanded tip of the spicule was indicated in Figure 1-C. These two important morphological characters of the

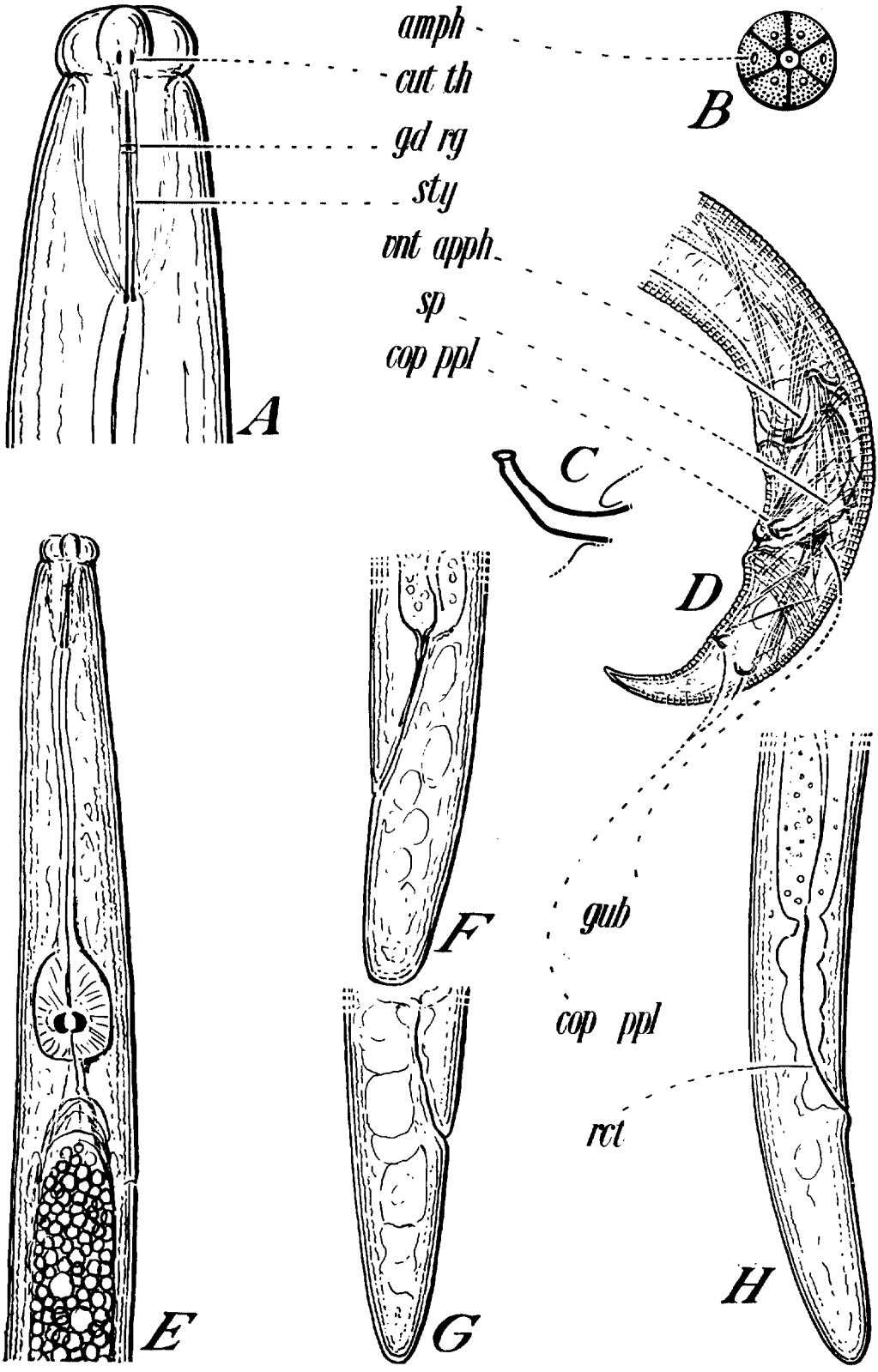


Fig. 1. Steiner and Buhrer's original drawings of *Aphelenchoides xylophilus*. A) Head of female showing stylet. B) En face view. C) Extruded spicule showing circular expansion. D) Tail of male. E) Anterior end of larva. F,G) Tails of larvae showing variation in shape. H) Tail of female.

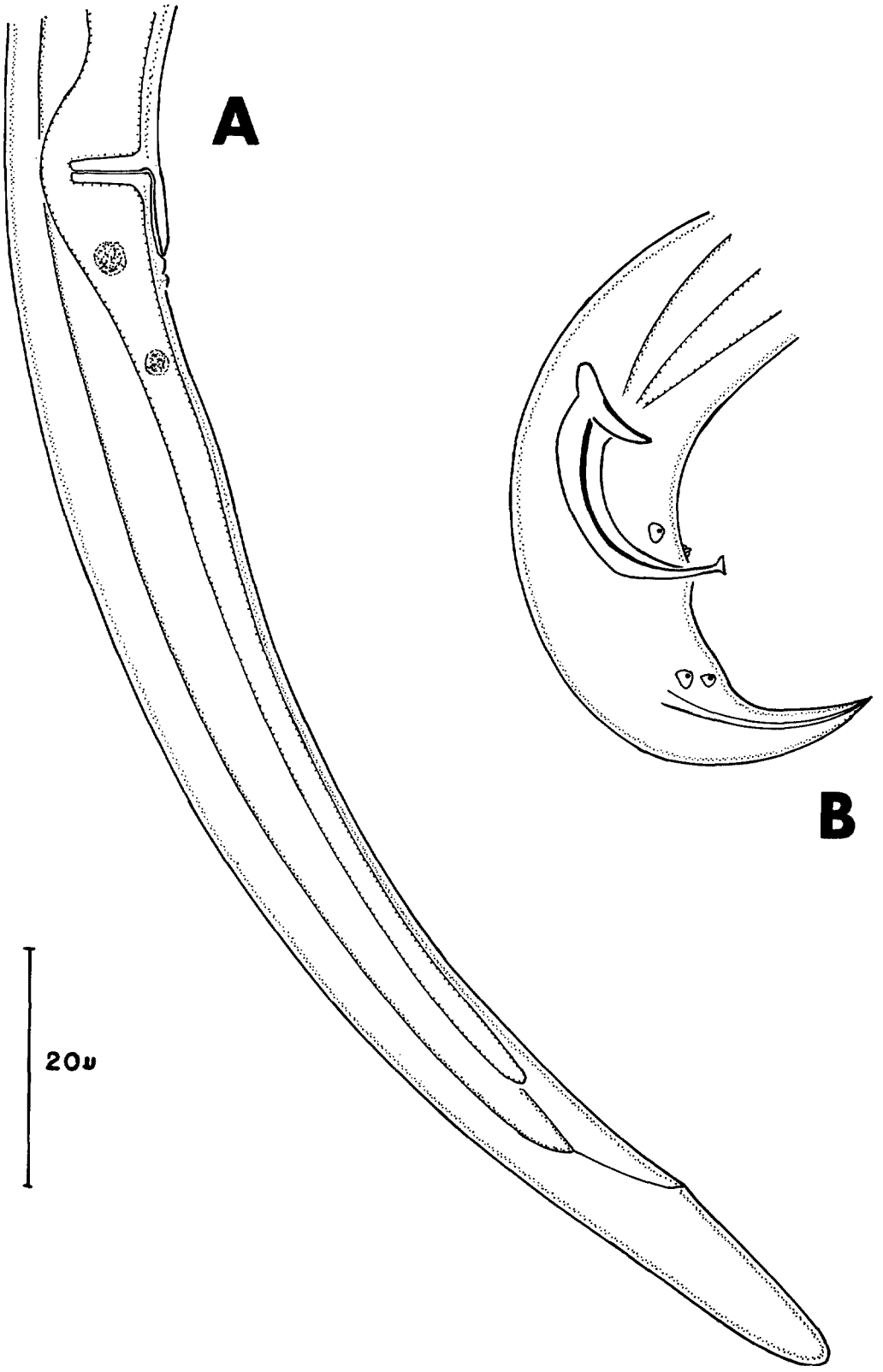
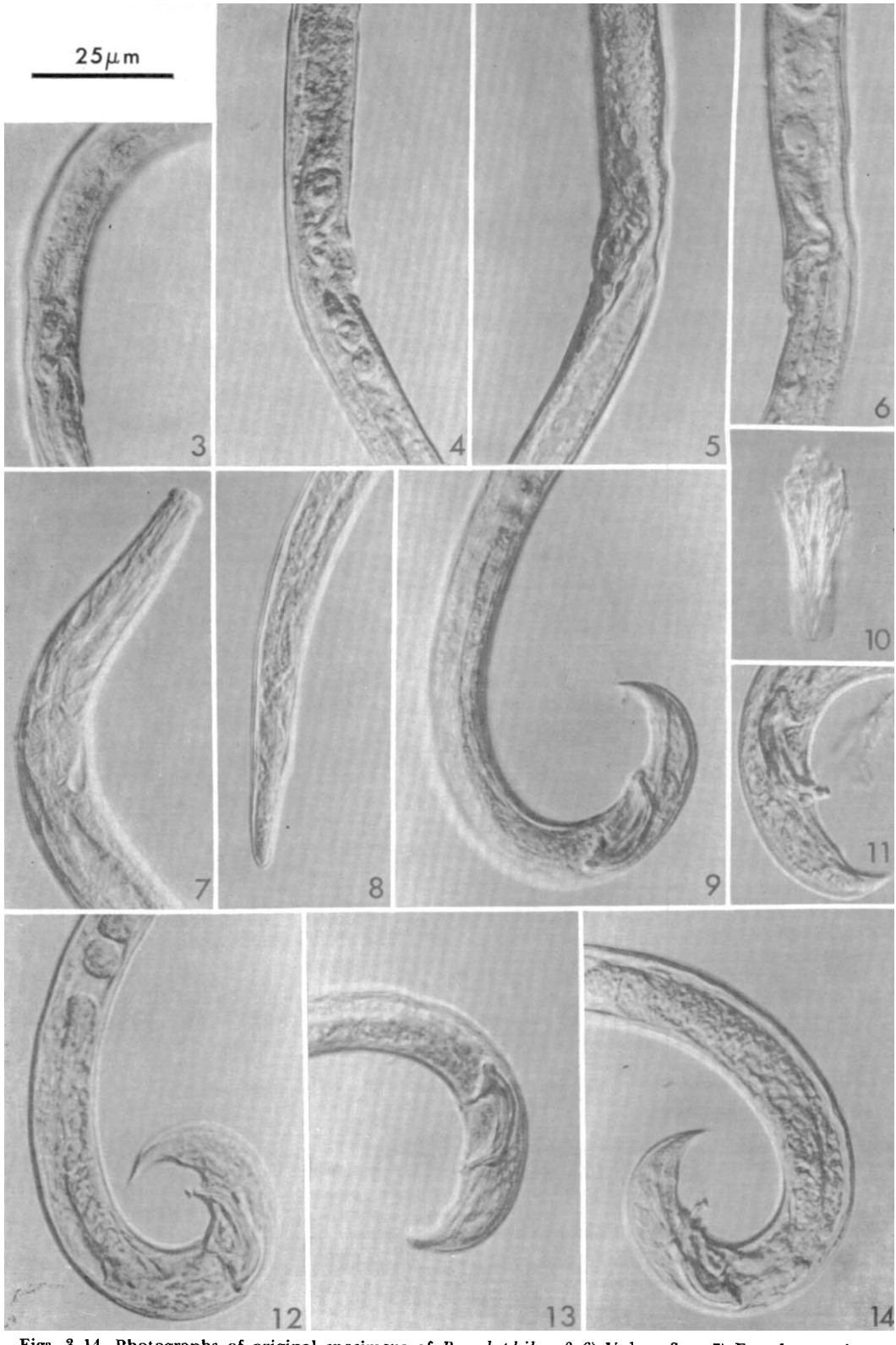
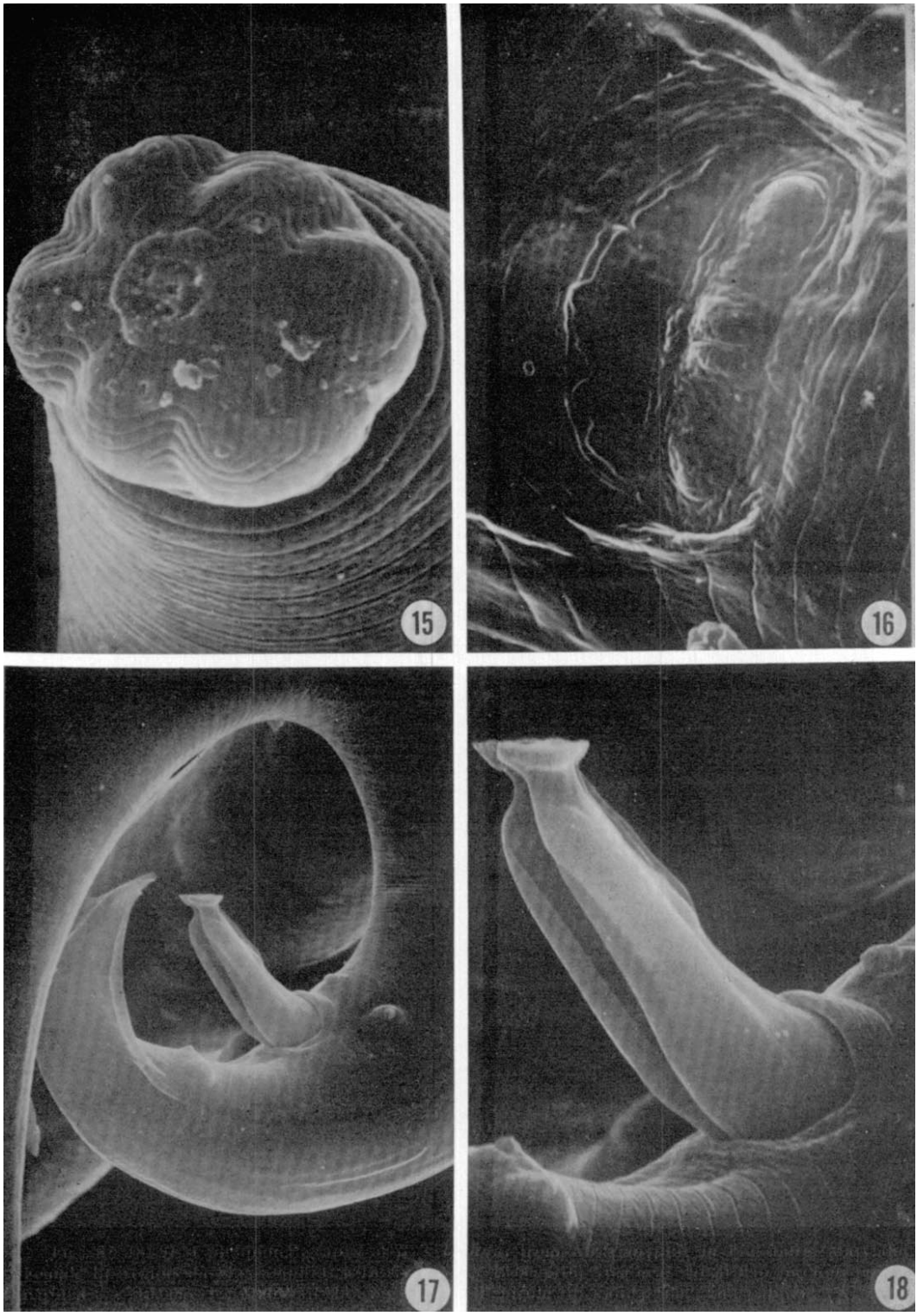


Fig. 2. Drawings of the female and male of newly found original *B. xylophilus* material. A) Female tail showing large vulvar flap and long post uterine sac. B) Male tail showing typical spicule with expanded tip, caudal alae, and tail papillae.



Figs. 3–14. Photographs of original specimens of *B. xylophilus*. 3–6) Vulvar flap. 7) Female anterior region. 8) Female posterior region. 9–14) Male tail region showing typical spicule and caudal alae.



Figs. 15–18. SEM photomicrographs of *B. xylophilus* from Austrian pine in Finksburg, Maryland. 15) Female lip region showing amphids with six lips (× 13,000). 16) Area near vulvar flap and four post-vulval papillae (× 10,000). 17) Male tail showing expanded spicule tip, caudal alae, and papillae (× 3,000). 18) Male tail showing disc-like expanded tip (× 7,500).

original specimens are more clearly illustrated in Figure 2-A, B. Photographs of the original specimens show the vulval flap in Figures 3–6 and the typical spicules in Figures 9–14. Other identifying characters and critical measurements of *B. xylophilus* were within the range determined for *B. lignicolus*.

The results from the mating experiments were positive. The Louisiana population was mated with the Pennsylvania population to try out the technique and to see if we were dealing in the United States with the same species. The Louisiana population was found to mate freely with the Pennsylvania population. The Japanese population mated freely with the Louisiana, Florida, Maryland, California, and the check Japanese populations. Two or three replicates from matings from single pairs were successful from each cross, producing many thousands of viable young in 3–4 wk. This showed that the nematodes from Japan and the United States were the same species. No morphological or biological differences that would justify forming new subspecies were found between the two populations. Among the various crosses, the one between the Louisiana and the Japanese populations was the most significant because the type location of *B. xylophilus* is Louisiana. Thus, based upon morphological characters and genetic crosses, we concluded that *B. xylophilus* and *B. lignicolus* are the same species and can be synonymized under *B. xylophilus*.

Redescription of
Bursaphelenchus xylophilus
Bursaphelenchus xylophilus
(Steiner & Buhrer 1934) Nickle 1970
Syn *Aphelenchoides xylophilus*
Steiner & Buhrer 1934
Bursaphelenchus lignicolus
Mamiya & Kiyohara 1972 n. syn.
(Figs. 1–18)

Steiner and Buhrer's measurements:

♀	1.8	7.9	8.7	74	96.2	0.9 mm
	1.0	1.5	1.5	1.6	1.0	
♂	2.2	9.3	10	M	95.9	0.77 mm
	1.2	1.6	1.6	2.0	1.5	

These measurements, especially the vulval percentages and lengths, fit well within the range given for *B. lignicolus*. Our measurements from the original material:

Male (5) lectotypes: L = 0.560 mm (0.520–0.601); a = 40.8 (35–45); b = 9.4 (8.4–10.5); c = 24.4 (21–29); stylet = 13.3 μm (12.6–13.8); spicule length = 21.2 μm (18.8–23.0).

Male hololectotype: L = 0.557 mm; a = 38; b = 8.5; c = 23; stylet = 13.8 μm; spicule length = 21.1 μm.

Females (5) lectotypes: L = 0.523 mm (0.447–0.609); a = 42.6 (37–48); b = 9.6 (8.3–10.5); c = 27.2 (23–31); V = 74.7% (72.7–77.5); stylet = 12.8 μm (12.6–13.0).

Female allolectotype: L = 0.544 mm; a = 37; b = 9.0; c = 28; V = 73.0% stylet = 12.6 μm.

These measurements indicate about a 10–15% shrinkage that is expected when specimens are kept in formalin for 30 yr and glycerine for 20 yr. Esser (2) mentions a 10% shrinkage of nematode specimens over 40 yr old.

Measurements from Mamiya and Kiyohara (1972):

Males (30): L = 0.73 mm (0.59–0.82); a = 42.3 (36–47); b = 9.4 (7.6–11.3); c = 26.4 (21–31); stylet = 14.9 μm (14–17); spicules 27.0 μm (25–30).

Females (40): L = 0.81 mm (0.71–1.01); a = 40.0 (33–46); b = 10.3 (9.4–12.8); c = 26.0 (23–32); V = 72.7 (67–78); stylet = 15.9 μm (14–18).

Description of male: Spicules large, uniquely arcuate, paired, with sharply pointed prominent rostrum; distal ends of spicules with typical disc-like expansions. Tail arcuate, terminus pointed, appearing talon-like on lateral view, surrounded by short, oval caudal alae. Seven caudal papillae, one adanal pair just pre-anal, single papilla just pre-anal centered; two post-anal pairs just before caudal alae origin.

Description of female: Lip region high, offset with six lips. Stylet with small basal swellings. Esophageal glands slender, 3–4 body widths long, overlapping dorsally. Excretory pore opposite junction of esophagus and intestine, sometimes at level of nerve ring. Hemizonid conspicuous, about two-thirds body width behind medium bulb. Ovary outstretched, oocytes usually in single

file. Post uterine sac long, extending three-fourths distance to anus. Tail subcylindrical, usually with broadly rounded terminus.

Hololectotype male: Collected on 24 November 1931 from *Pinus palustris* in Louisiana deposited T–342t, USDA Nematode Collection, Beltsville, Maryland, USA.

Allolectotype female: Same data as hololectotype. Deposited T–343t, USDA Nematode Collection, Beltsville, Maryland, USA.

Male and female paralectotypes collected on 24 November 1931 from pine in Louisiana deposited at the Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchinai, Ibaraki, 305 Japan; California Nematode Collection, University of California, Davis, California; and at USDA Nematode Collection, Beltsville, Maryland.

Diagnosis: B. xylophilus can be distinguished from other species in the genus by the shape of the spicules, the distinct vulvar flap and the lack of a digitate tail tip which is present in *B. mucronatus* which we recognize as a valid species. One of us (YM) tried unsuccessfully to mate *B. mucronatus* with *B. lignicolus,* thus indicating their separate species status. *B. fraudulentus* is similar to *B. xylophilus,* but the female has a digitate tail tip similar to *B. mucronatus.*

LITERATURE CITED

1. Baujard, P. 1980. Trois nouvelles especes de Bursaphelenchus (Nematoda, Tylenchida) et remarques sur le genre. Revue Nematol. 3:167-177.

2. Esser, R. P. 1974. Two permanent mounting methods compared after six years. Proc. Helm. Soc. Wash. 41:10-13.

3. Mamiya, Y., and N. Enda. 1979. Bursaphelenchus mucronatus n. sp. (Nematoda: Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. Nematologica 25:353-361.

4. Mamiya, Y., and T. Kiyohara. 1972. Description of Bursaphelenchus lignicolus n. sp. (Nematoda: Aphelenchoididae) from pine wood and histopathology of nematode-infested trees. Nematologica 18: 120-124.

5. Nickle, W. R. 1970. A taxonomic review of the genera of the Aphelenchoidea (Fuchs, 1937) Thorne, 1949 (Nematoda: Tylenchida). J. Nematol. 2:375-392.

6. Rühm, W. 1956. Die nematoden der Ipiden. Parasitologische Schriftenreihe 6. VEB Fischer Verlag, Jena.

7. Steiner, G., and E. M. Buhrer. 1934. Aphelenchoides xylophilus n. sp., a nematode associated with blue-stain and other fungi in timber. J. Agric. Res. 48:949-951.