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Amended Description of *Pratylenchus macrostylus* Wu, 1971 with SEM Observations[1]

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Abstract: The description of *Pratylenchus macrostylus* Wu is amended using specimens collected from Fraser fir and red spruce in the Black Mountains of North Carolina. Measurements of females in North Carolina overlap those of the type series. However, stylet length (21.8–27.8 μm, 24.7 ± 1.1) is greater in North Carolina specimens, which also have a longer body length and greater C ratio. Heads of the North Carolina specimens are divided into lateral and submedian segments which taper and fuse with oral discs. Males are rare and not important in species diagnosis. Previously described specimens in Japan differed from those in North America in key diagnostic characters of stylet and body length. This discrepancy suggests that the Japanese species may be distinct from the North American.

Key words: Abies fraseri, Fraser fir, light microscopy (LM), morphology, morphometrics, *Picea rubens, Pratylenchus macrostylus,* red spruce, scanning electron microscopy (SEM), taxonomy.

Specimens of *Pratylenchus* Filipjev, 1936, found in soil samples from Fraser fir (*Abies fraseri* (Pursh) Poir.) and red spruce (*Picea rubens* Sarg.) stands at several locations in the southern Appalachian Mountains were similar to *P. macrostylus* Wu, 1971 but differed in head shape and stylet and body lengths. *Pratylenchus macrostylus* paratypes and topotypes from the Canadian National Collection of Nematodes had head shapes and other key diagnostic characters similar to North Carolina specimens.

Previous findings of *P. macrostylus* in North America include associations with white birch (*Betula papyrifera* Marsh.) and white spruce (*Picea glauca* (Moench) Voss) in Ontario, Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in Vancouver, British Columbia (13), boreal forests in North Carolina (*Pratylenchus* spp.) (11), and balsam fir (*Abies balsamea* (L.) Mell.) in Maine (9). Outside North America, several populations in Japan have been identified as *P. macrostylus* (5,10).

A study of the morphological variation of *P. macrostylus* from North Carolina is presented here to further clarify diagnostic features and to amend the species description. The male is rare and is not described.

Materials and Methods

Females of *Pratylenchus* were collected from roots of Fraser fir and red spruce on Potato Knob and Commissary Ridge, Black Mountains, North Carolina. Specimens were extracted from washed host root tissue which had been placed in Baermann trays in a growth chamber kept at 13–16 C, similar to ambient soil temperatures of the study area. Females were fixed in hot (70–80 C) TAF (7 ml 40% formaldehyde, 2 ml Triethanolamine, 91 ml distilled water) and mounted in this same fixative for observation. Line drawings were made with a Leitz drawing tube, and photographs were taken with a bright field microscope. Females and excised stylets were prepared for scanning electron microscopy (SEM) as previously described (6). Voucher specimens were fixed in 2% glutaraldehyde, gradually dehydrated with ethanol, and mounted in dehydrated glycerin. All measurements are in micrometers (μm) unless otherwise specified.

Systematics

Pratylenchus macrostylus Wu, 1971 (Figs. 1, 2)

Female: Measurements of North Carolina specimens (Table 1) are compared with

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descriptions from Canada and Japan (Table 2). Body translucent, white, vermiform, tapering at both ends. Cuticle marked by fine annulations, four lateral lines, often faint with broken diagonal striae in middle ala. Head cap arch shaped, angular; height 3.1 ± 0.3, width 8.9 ± 0.4 (Fig. 1B–D). Head region slightly set off from body, marked with two annuli in lateral view, rarely one. In SEM (Fig. 2A, B), stoma slit-like, labial sensilla pore-like, often obscure; prestoma ovoid. Amphidial openings small, slit-like. Medial lips angular, narrowing near amphidial openings; similar to those of Group 2 (2). Cephalic framework strongly developed, hexaradiate; vestibule and vestibule extension prominent (Fig. 2D–F). Stylet (Fig. 1E–J) strongly developed; cone equal to length of shaft and knobs; shaft cylindrical, fluted (Fig. 2C) and slightly constricted near junction with knobs; knobs prominent, flattened, often cupped anteriorly with anterior projections tapered, rounded, smooth posteriorly (Fig. 1G, H). Procorpus wide, cylindrical, narrowing posteriorly near junction with metacarpus (Fig. 1A). Distance of dorsal esophageal gland orifice (DEGO) to stylet base 4.1–8.4; DEGO ampulla distinct. Metacorpus muscular; cuticularized valve plates prominent (Fig. 1A). Nerve ring distinct, hemizonid usually conspicuous, immediately anterior to excretory pore. Basal gland lobe overlaps intestine ventrally, length of overlap 28.3–78.3 (50.0 ± 12.1); slight dorso-lateral overlap near esophagointestinal valve. Dorsal esophageal gland nucleus conspicuous, anterior to subventral gland nuclei. Subventral esophageal gland nuclei near excretory pore, parallel or perpendicular to body axis. Esophagointestinal valve faint. Excretory pore in vicinity of esophagointestinal valve. Intestine filled with globules; rectum cuticularized. Anus small, pore-like. Ovary one, outstretched, rarely flexed; spermatheca round to ovoid, 16.5–25.0 (20.5 ± 2.2) long, 11.0–18.8 (14.5 ± 2.2) wide, distance from spermatheca to vulva variable, 40.5–98.4 (57.9 ± 12.4) (Fig. 1K). Uterus with tricolumella of 15 cells, occasionally 12. Postvulval uterine sac short, rounded, occasionally conical posteriorly. Phasmids pore-like. Tail typically conoid (Fig. 2G, H); terminus shape variable, usually bluntly rounded with no striations, rarely crenate (Figs. 1L–O, 2I); number of tail annuli 17–26.

Biology: Fraser fir and red spruce are believed to be the primary hosts of *P. macrostylus* in the spruce–fir ecosystem of North Carolina. Infected root tissues included mycorrhizae, short-feeder and long-feeder roots, and supporting roots. Lesions in host tissue were not always visible externally, but necrotic regions containing numerous females, juveniles, and eggs were detected when roots were teased apart. More than 500 nematodes per gram of feeder root were extracted in Baermann trays. These nematodes appear to damage their hosts, but many confounding factors are present (1,3) making this assessment uncertain.

In the Black Mountains, *P. macrostylus* occurs throughout the elevational range of the spruce–fir ecosystem (1,700–2,036 m). These mountains receive more than 175 cm of precipitation annually, and mean monthly temperature ranged from −3 to 15 C (8).

Discussion

Primary distinguishing features of *Pratylenchus macrostylus* are a long stylet with anteriorly flattened knob and a high lip region with rounded to convex, angular shape. Additional characteristics include number of head annules (usually two), position of vulva, structure of lateral field, round-to-oval spermatheca, absence of sperm in spermatheca, length of postvulval uterine branch, and conical tail. In the original line drawings, the head annules appear to be continuous with those of the body and the outline lacks angularity. However, the head cap is slightly set off and angular in paratypes and topotypes.

In the original description, measurements for only one male (allotype) and females with empty spermathecae were reported (12). The frequency of males in Canada was not indicated. Parthenogenetically reproducing species of *Pratylenchus*

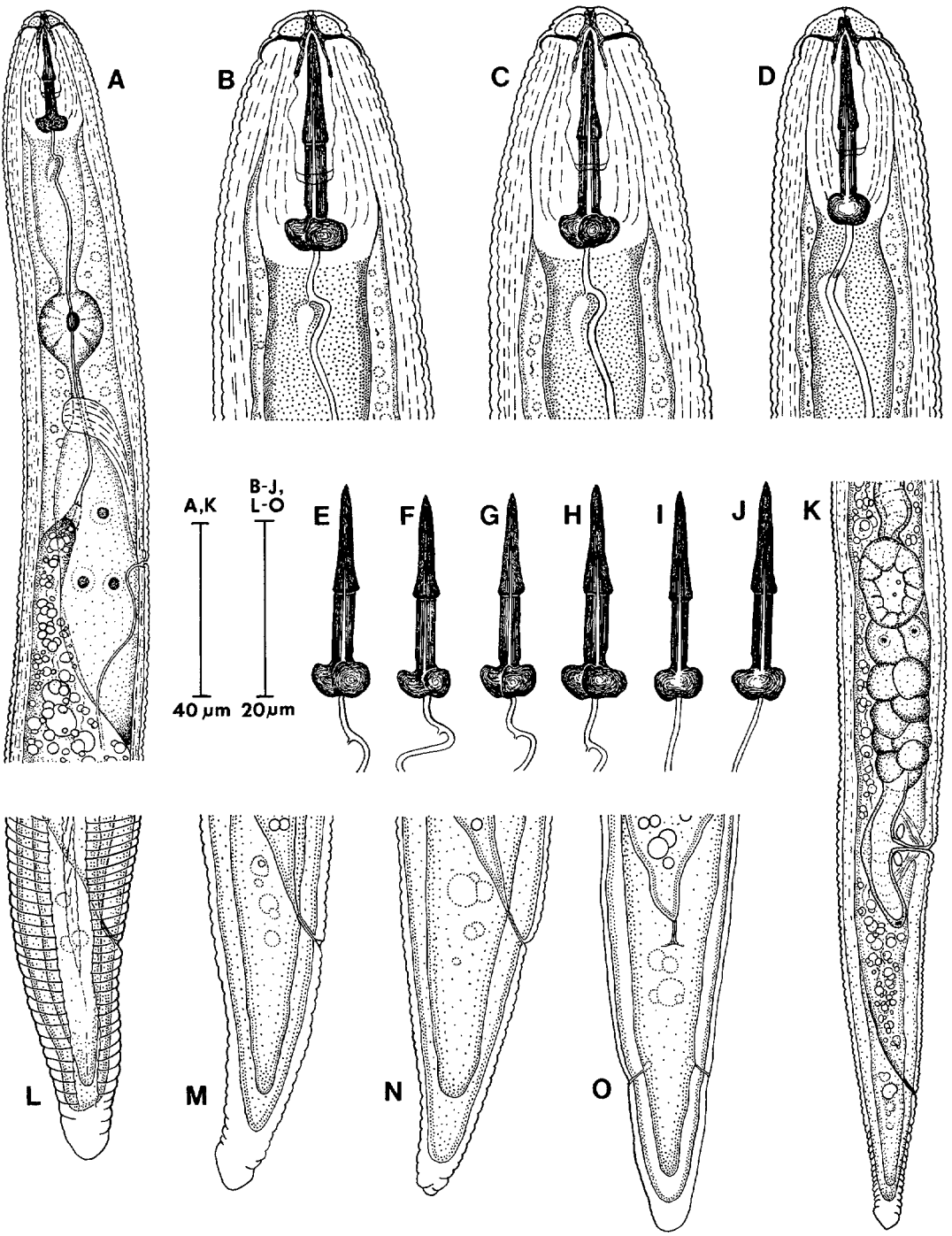

FIG. 1. Line drawings of females of *Pratylenchus macrostylus* from North Carolina. A) Esophageal region, lateral. B, C) Cephalic regions, lateral. D) Cephalic region, ventral. E–H) Stylets, lateral. I, J) Stylets, ventral. K) Posterior region. L–N) Variations in tail morphology, lateral. O) Tail, ventral.

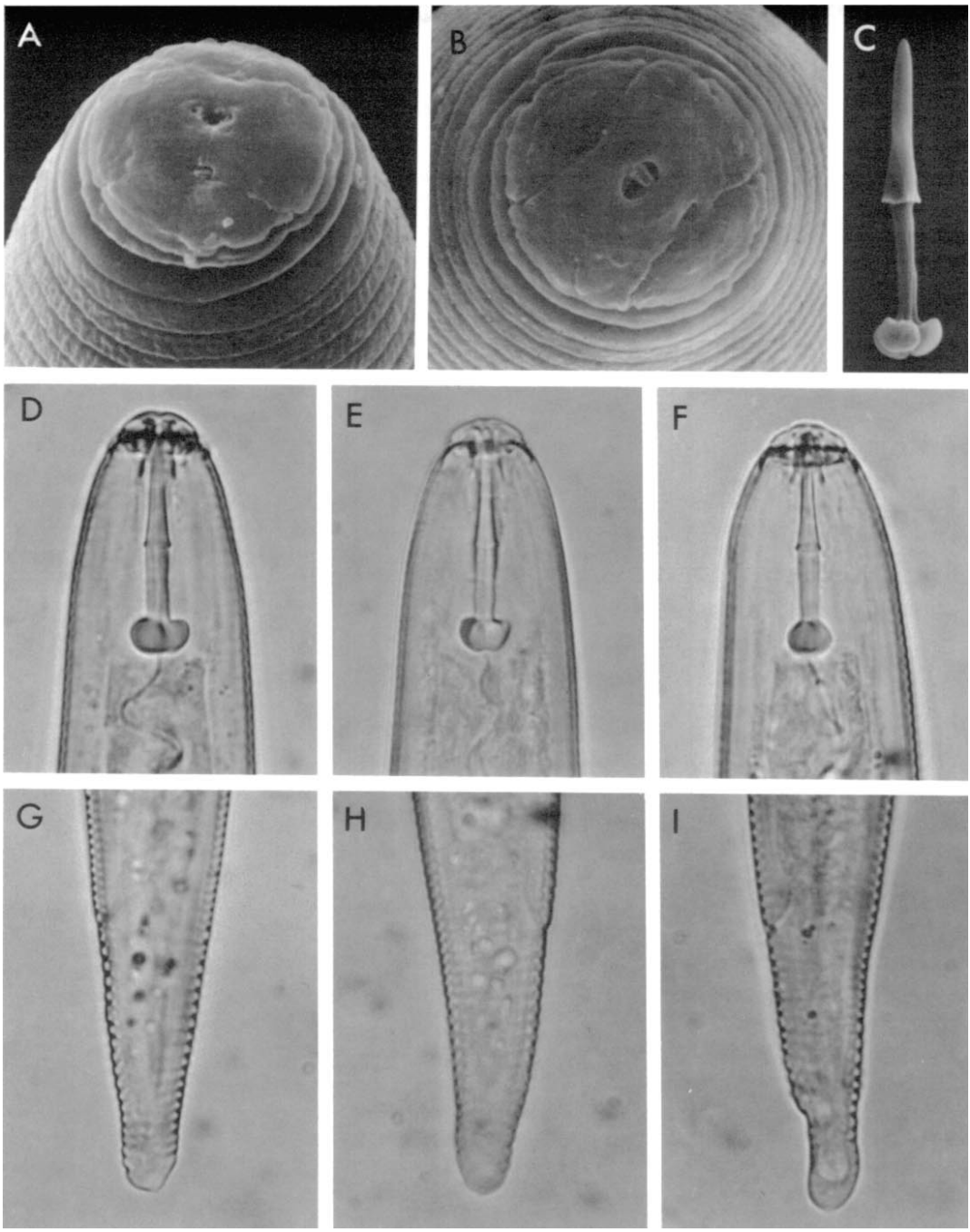

FIG. 2. SEM (A–C) and LM (D–E) photographs of *Pratylenchus macrostylus* from North Carolina. A) Cephalic region, lateral. B) Cephalic region, face view. C) Stylet. D, E) Cephalic region, lateral. F) Cephalic region, medial. G–I) Tails, lateral.

commonly have empty spermathecae and few males are present. In a key for the genus *Pratylenchus* (7), males were indicated as rare in *P. macrostylus* but were placed under the dichotomy of "males common, spermatheca filled with sperm" which is misleading. In addition, males are rarely encountered in collections in North Carolina, with only three found among several thousand individuals.

TABLE 1. Measurements of 25 females of *Pratylenchus macrostylus* Wu from North Carolina.

Character	Range	Mean	Standard error mean	Standard deviation	Coefficient of variation (%)
Linear (μm)					
Body length	584.5–828.4	684.4	11.9	59.6	8.6
Body width	23.4–37.8	28.4	0.8	3.9	13.7
Tail length	26.2–36.6	29.9	0.5	2.6	8.6
Excretory pore to head end	93.0–129.0	109.8	1.8	8.8	8.1
Stylet length	21.8–27.8	24.7	0.2	1.1	4.3
Stylet knob width	5.2–7.6	6.4	0.1	0.6	9.6
DEGO to knob base	4.1–8.4	5.9	0.2	0.9	14.9
Esophagus length	91.4–126.9	105.2	1.8	9.0	8.6
Postvulval uterine sac	10.2–32.4	20.5	1.3	6.3	30.6
Anus to vulva distance	47.32–88.7	63.1	2.7	13.6	21.6
Ratios					
a	17.5–34.2	24.5	0.8	4.1	16.9
b	5.6–7.9	6.5	0.1	0.6	9.7
b′	8.6–25.3	14.5	0.1	4.0	9.3
c	18.4–29.1	23.0	0.6	2.8	12.2
G_1	40.6–58.5	50.9	0.9	4.6	9.1
V	83.5–88.4	86.4	0.3	1.5	1.7

The tricolumella of the uterus typically is comprised of three rows of five cells each in specimens from North Carolina and Canada. However, variance specimens with four cells per row are common. In these specimens, the three posterior cells of the columella are reduced in size and slightly set off from the uterus proper. Columella cells are globular in most females but are flattened and undifferentiated in others. They may not develop until oocytes in the ovary ripen as in *Rotylenchus robustus* (de Man) Filipjev (4). Structure of the tricolumella in *Pratylenchus* spp. may have diagnostic value.

Although *P. macrostylus* from North Car-

TABLE 2. Comparison of selected measurements of *Pratylenchus macrostylus* Wu from Canada, North Carolina, and Japan.

Character	Locality		
	Canada† (n = 22)	North Carolina (n = 25)	Japan‡ (n = 27)
Linear (μm)			
Body length	510–680	584.5–828.4	429–698§
Stylet length	21–24.7‖	21.8–27.8	17.5–20.8§
Stylet core length	10.5–12.1	11.1–14.5	7.4–11.4
Stylet knob width	4–5.8	5.2–7.6	2.5–5.1
Excretory pore to head end	86–107	93.0–129.0	70.7–97.3
Tail length	25–40	26.2–36.6	24.0–37.9
Postvulval uterine sac	18–32	10.2–32.4	9.5–30.3
Ratios			
a	22–33	17.5–34.2	22.8–30.2§
b	5.0–7.4	5.6–7.9	6.5–9.9
c	16–24	18.4–29.1	14.1–20.7§
V	85.0–88.8	83.5–88.4	82.3–89.0§

† Measurements after Wu (13).
‡ Measurements after Minagawa (11).
§ n = 81.
‖ n = 50.

olina has a longer stylet than the Canadian specimens, their ranges in lengths overlap. North Carolina specimens also are similar in head shape, number of head annuli, stylet knob shape, lateral field structure, position of vulva, spermatheca shape and content (empty), and tail shape. Other linear measurements and ratios overlap as well, but their means were higher for North Carolina specimens, which had generally larger, more robust females. These morphological differences may represent diverging characteristics of two geographically separated populations.

Pratylenchus macrostylus from *Quercus acutissima* Carruth., *Q. dentata* Thunb., *Q. senata* Murray, and *Prunus janaskura* Sieb. ex Koidzd. was redescribed in Japan (10). Other populations of *Pratylenchus* were reported from loquat, *Eriobotrya japonica* Lindley, and apple, *Malus domestica* Borkh., in Japan (5) which were later identified as *P. macrostylus* (10). Japanese populations have smaller, less robust females with a more slender, cylindrical tail shape than those in Canada, but several other morphological characteristics overlap (Table 2). However, stylet length, the primary distinguishing feature, is much shorter than in the types. These differences suggest that the Japanese nematodes may be closely related to, but a separate species from, the North American *P. macrostylus*.

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