Description and SEM Observations of Meloidogyne chitwoodi n. sp. (Meloidogynidae), a Root-knot Nematode on Potato in the Pacific Northwest

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Abstract: Meloidogyne chitwoodi n. sp. is described and illustrated from potato (*Solanum tuberosum*) originally collected from Quincy, Washington, USA. This new species resembles *M. hapla,* but its perineal pattern is basically round to oval with distinctive and broken, curled, or twisted striae around and above the anal area. The vulva is in a sunken area devoid of striae. Vesicles or vesicle-like structures are present in the median bulb of females. The larva tail, being short and blunt with a hyaline tail terminal having little or no taper to its rounded terminus, is distinctively different from *M. hapla.* SEM observations revealed the nature of the perineal pattern and details of the head of larvae and males, and showed the spicules to have dentate tips ventrally. Hosts for *M. chitwoodi* n. sp. include potato, tomato, corn, and wheat but not strawberry, pepper, or peanut. The latter three crops are excellent hosts for *M. hapla.* The known distribution of this new root-knot species presently involves certain areas of Idaho, Washington, and Oregon. The common name "Columbia root-knot nematode" is proposed for *M. chitwoodi* n. sp. *Key Words:* taxonomy, morphology, *Meloidogyne,* root-knot, new species, SEM ultrastructure, potato, *Solanum tuberosum,* hosts.

In December 1974 an infected 'Russet Burbank' potato and dissected root-knot specimens which had been tentatively identified as *Meloidogyne hapla* Chitwood, 1949 were received from Aberdeen, Idaho, for deposit in the USDA Nematode Collection, Beltsville, Maryland. The specimens were filed for later study as part of the "*M. hapla* complex" (2). When examined critically in 1977, they were found to represent an undescribed species of *Meloidogyne.* Concurrently in 1977, and subsequently, this new *Meloidogyne* species has been found and identified in several areas of the Pacific Northwest, specifically in Idaho, Washington, and Oregon (4). The present taxonomic report on the description of this new root-knot species represents only a part of the extensive research being conducted in the Pacific Northwest on this destructive pest of potato (4).

Specimens used in this description were obtained from cultures originating at Quincy, Washington, and grown on potato in a growth chamber and in an isolated area of a greenhouse.

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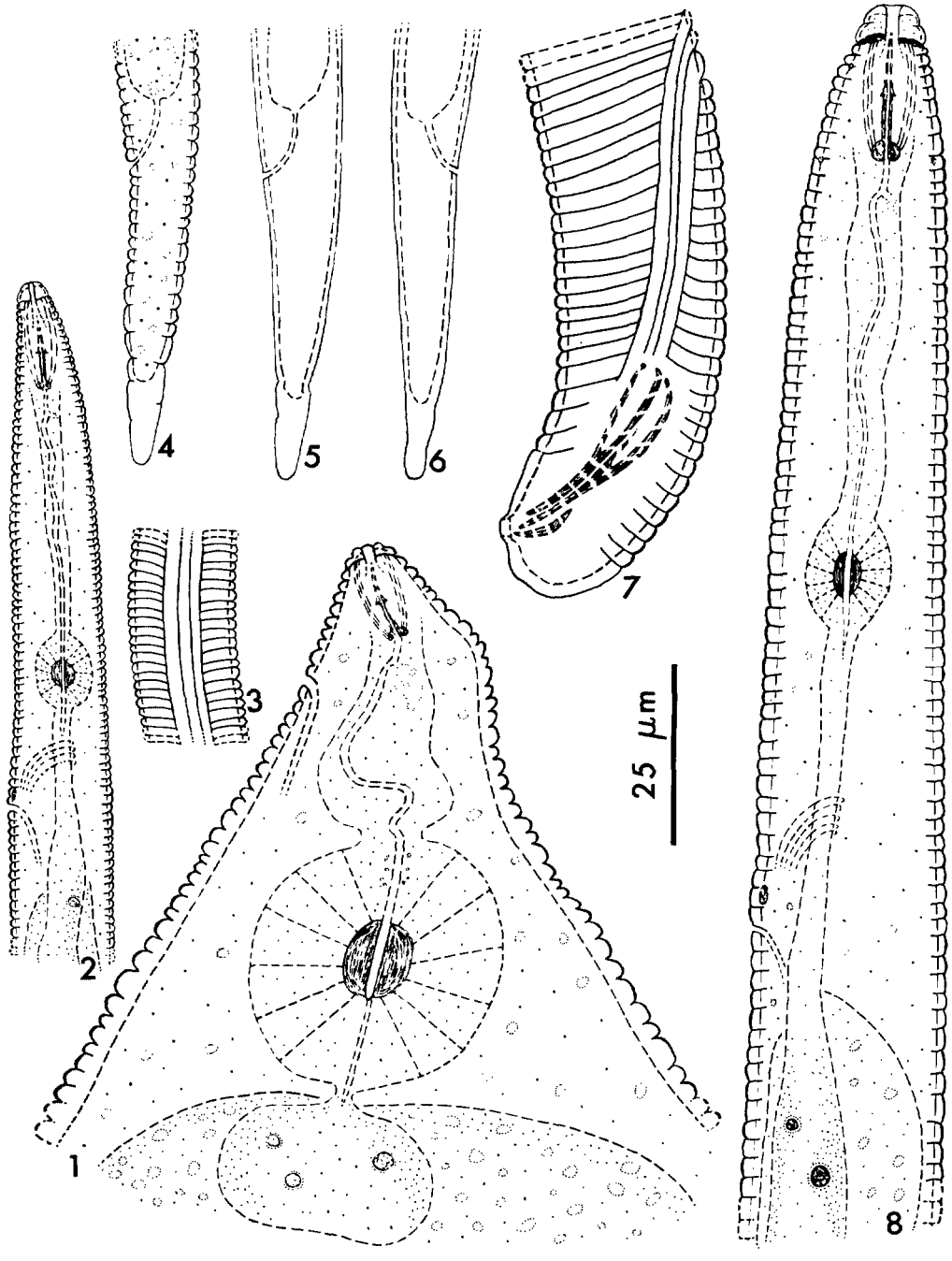
Larvae for morphological examination were generally recovered from fresh infected roots, or egg sacs, that were kept in petri dishes with a small amount of water. Females were later dissected from the roots after fixation overnight in 3% formaldehyde solution. Males were obtained in this same manner or by soil sieving followed by Baermann funnel extraction. The procedures used for measuring, drawing, and preparing specimens were essentially the same as those used by Golden and Birchfield (3), except that some fixed females were cut and mounted in lactophenol solution. Photomicrographs of perineal patterns and eggs were made with an automatic 35-mm camera attached to a compound microscope having an interference contrast system, and those of infected roots and whole females were made with a 8.3 × 10.8-cm sheet-film camera attached to a dissecting microscope. In making the scanning electron micrographs, living specimens were fixed in 3% glutaraldehyde solution with 0.05 M phosphate buffer (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO_2, sputter-coated with 20–30 nm layer of gold-palladium, and examined with a scanning electron microscope (SEM).

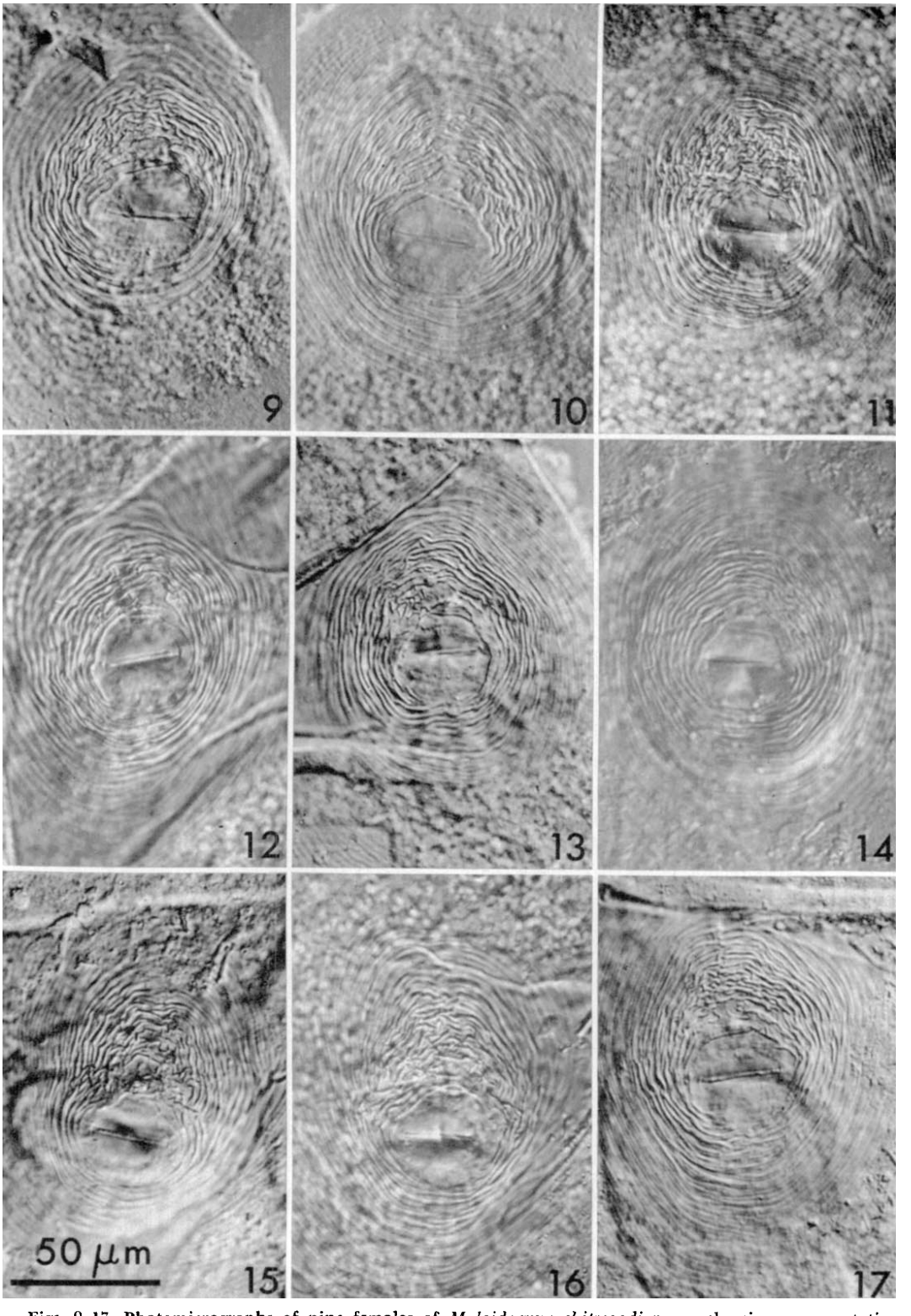
Meloidogyne chitwoodi n. sp.

Females (60): Length 430–740 μm (mean 591 μm, standard deviation [SD] 60 μm); width 344–518 μm (422 μm, SD 42); a = 1.1–1.8 (1.4, SD 0.2); b = 3.8–5.0 (4.4, SD

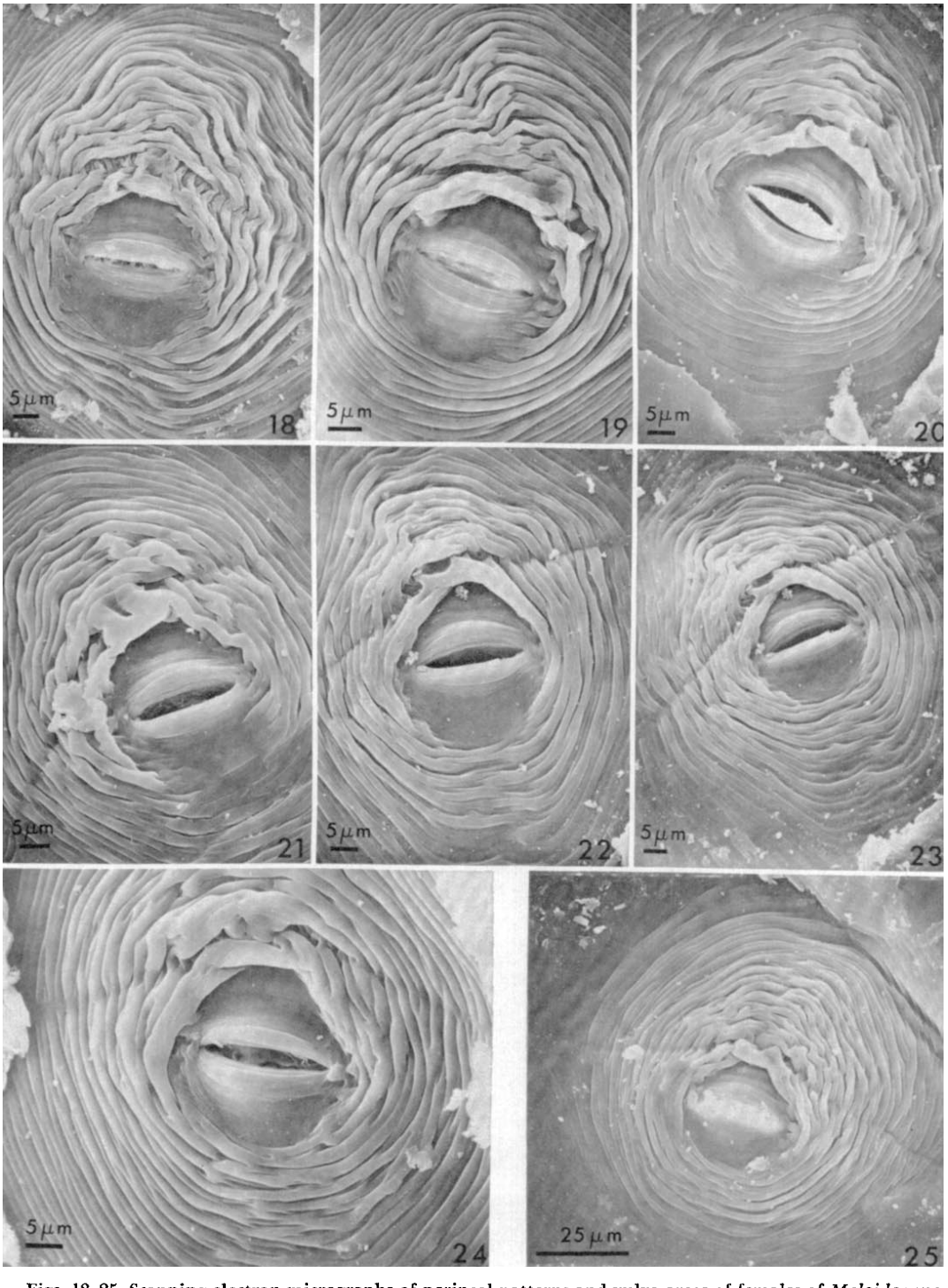


Figs. 1–8. Drawings of *Meloidogyne chitwoodi* n. sp. 1) Anterior region of female (note vesicles or vesicle-like structures in the median bulb, clustered around the lumen anterior to the center valve plates). 2–6) Larvae: Consecutively, anterior region, cuticular annulation and lateral field at midbody, tails showing shapes with blunt hyaline tail terminals. 7–8) Male.

0.4); stylet 11.2–12.5 µm (11.9 µm, SD 0.3); width of stylet knobs 3.4–4.3 µm (3.8 µm, SD 0.3); dorsal esophageal gland orifice (DGO) 3.4–5.5 µm (4.2 µm, SD 0.6) from base of stylet; center of median bulb 52–80 µm (63 µm, SD 7) from anterior end; excretory pore 10–27 µm (18 µm, SD 5) from anterior end; vulva slit length 19–32 µm (27 µm, SD 3); distance from vulva slit to anus 13–22 µm (18 µm, SD 2).



Figs. 9–17. Photomicrographs of nine females of *Meloidogyne chitwoodi* n. sp. showing representative perineal patterns.



Figs. 18–25. Scanning electron micrographs of perineal patterns and vulva areas of females of *Meloidogyne chitwoodi* n. sp.

Holotype (female): Length 598 μm; width 474 μm; a = 1.3; b = 4.3; stylet 12 μm; DGO 4.3 μm from base of stylet; width of stylet knobs 3.9 μm; vulva slit length 29.2 μm; distance from vulva slit to anus 18.1 μm.

Description. Females: Body pearly white, globular to pear-shaped, with slight pos-

terior protuberance visible occasionally, and with distinct neck situated anteriorly on a median plane with terminal vulva (Fig. 36). Esophageal and anterior region often appearing as illustrated (Fig. 1). Several small vesicles or vesicle-like structures usually present within the median bulb and are clustered around the lumen anterior to the valve plates of the median bulb (Fig. 1). Head with distinct but weak cephalic framework, offset from neck but variable in exact shape, and bearing a labial cap and usually one cephalic annule. Excretory pore clearly visible and commonly located at a distance equal to about 1 1/2 stylet lengths from anterior end. Stylet small but strong, having a dorsal curvature and rounded knobs that slope posteriorly. Perineal pattern (Figs. 9–17, 18–25) quite distinctive with striae around and above anal area being broken, curved, twisted, or curled as illustrated; overall shape of pattern appearing round to oval, sometimes with inner striae forming an arch of varying degrees. Punctation not observed. Vulva sunken in an area variable in shape and devoid of striae.

Males (30): Length 887–1268 μm (1068 μm, SD 100); a = 28–46 (36, SD 4); b = 6–9 (7.2, SD 1); c = 140–226 (162, SD 20); stylet 18.1–18.5 μm (18.3 μm, SD 0.2); DGO 2.2–3.4 μm (3 μm, SD 0.4) from base of stylet; center of median bulb 61–77 μm (71 μm, SD 5) from anterior end; spicules 26–29 μm (27 μm, SD 1.2); gubernaculum 6.5–8.2 μm (7.7 μm, SD 0.6); tail 4.7–9.0 μm (6.8 μm, SD 0.9).

Allotype (male): Length 1046 μm; a = 39; b = 5.6; c = 174; stylet 18.1 μm; DGO 2.6 μm; spicules 26 μm; gubernaculum 7.3 μm; tail 6 μm.

Description. Males: Body slender, vermiform, tapering slightly at both extremities. Head slightly offset, with large labial disc (head cap) and a large post labial annule not divided into annules (Figs. 8, 30–32). Cuticular annules distinct, becoming more prominent a short distance from either end. Midbody width 22–37 μm (30 μm, SD 3.9). Lateral field (Figs. 7, 34) with four lines, center band smaller than outer two, and with some areolation evident with SEM but difficult to resolve with optical microscopy. Stylet, knobs, cephalids, hemizonid, excretory pore, and anterior portion commonly appear as illustrated (Fig. 8). Testis one or two. Spicules arcuate, and under SEM can be seen to have dentate tips ventrally (Fig. 33). Phasmids located at or anterior to cloaca. Tail short, rounded (Fig. 7).

Second-stage larvae (60); Length 336–417 μm (390 μm, SD 16); a = 24.5–29.8 (27.5, SD 1.2); b = 3.3–3.8 (3.6, SD 0.2); c = 7.9–9.6 (8.9, SD 0.4); stylet 9.0–10.3 μm (9.9 μm, SD 0.3); DGO 2.6–3.9 μm (3.2 μm, SD 0.2) from base of stylet; center of median bulb 43–56 μm (51 μm, SD 3) from anterior end; head width 4.7–5.2 μm (5 μm, SD 0.2); head height 1.7–2.6 μm (2.3 μm, SD 0.2); hw/hh ratio 1.8–2.7 (2.3, SD 0.2); tail length 39–47 μm (43 μm, SD 1.8); hyaline tail terminal 8.6–13.8 μm (11 μm, SD 1); caudal ratio A 1.9–3.9 (2.5, SD 0.3); caudal ratio B 3.0–5.7 (4.2, SD 0.7).

Description. Larvae: Body small, vermiform, 12.5–15.5 μm (14.2 μm, SD 0.6) in width at midbody, tapering at both extremities but more so posteriorly. Head not offset, with weak cephalic framework, and bearing a labial disc and a large post-labial annule without striations (Figs. 2, 26–29). Cuticular annulation on most of body very fine, measuring about 1 μm. Lateral field with four lines, not areolated (Fig. 3). Stylet, knobs, hemizonid, excretory pore, and anterior portion generally appearing as illustrated (Fig. 2). Cephalids indistinct and not shown. Phasmids small, difficult to see, and located in anterior 1/3 of tail. Rectum not inflated. Tail commonly appearing as illustrated (Figs. 4–6), having a short, blunt hyaline portion which remains almost the same diameter for its length and with little or no taper, thus giving relatively high caudal ratios, especially caudal ratio B. Tail terminus rounded.

Eggs (25): Length 79–92 μm (85 μm, SD 3.6); width 40–46 μm (42 μm, SD 1.8); L-W ratio 1.8–2.3 (2, SD 0.1). Egg shell hyaline, without visible markings, when observed with optical microscopy.

Holotype (female): Isolated by J. H. O'Bannon and G. S. Santo from potato tubers originally from Quincy, Washington, in October 1978 and subsequently grown on potato ('Russet Burbank') in an isolated greenhouse area. Slide T-323t, USDA Nematode Collection (USDANC), Beltsville,

Maryland, USA.

Allotype (male): Slide T-324t. Same data as holotype. USDANC, Beltsville, Maryland, USA.

Paratypes (males, females, larvae, and eggs): USDANC, Beltsville, Maryland, USA; University of California Nematode Survey Collection (UCNSC), Davis, California, USA; Nematology Department, Rothamsted Experimental Station, Harpenden, Hertfordshire, England; Canadian National Collection of Nematodes, Ottawa, Canada; Laboratoire des Vers, Museum National d'Histoire Naturelle, Paris, France; Institute voor Dierkunde, Laboratorium voor Morfologie en Systematiek der Dieren, Ledeganckst, 35, B-9000, Gent, Belgium; and Laboratory voor Nematologie, Binnehave 15, Wageningen, Netherlands.

Type host and locality: Roots and tubers of potato, *Solanum tuberosum,* in a field near Quincy, Washington, USA.

Diagnosis: Meloidogyne chitwoodi n. sp. is related to *M. hapla* Chitwood, 1949 in some respects but differs markedly in (i) the perineal pattern, including its overall round to oval shape and especially by the presence of broken, curved, twisted, or curled striae around and above the anal area, and by the sunken vulva in an area without striae; (ii) the presence of vesicles or vesicle-like structures in the median bulb of the females as described above; and (iii) the nature of the larval tail, being short and blunt with the hyaline tail terminal showing little or no taper to its rounded terminus. Although *M. querciana* Golden, 1979 has a sunken vulva in an area devoid of striae, other characteristics of this species, including the nature of the perineal striae and length of the female stylet (average 17.5 μm), readily distinguish it from *M. chitwoodi* n. sp.

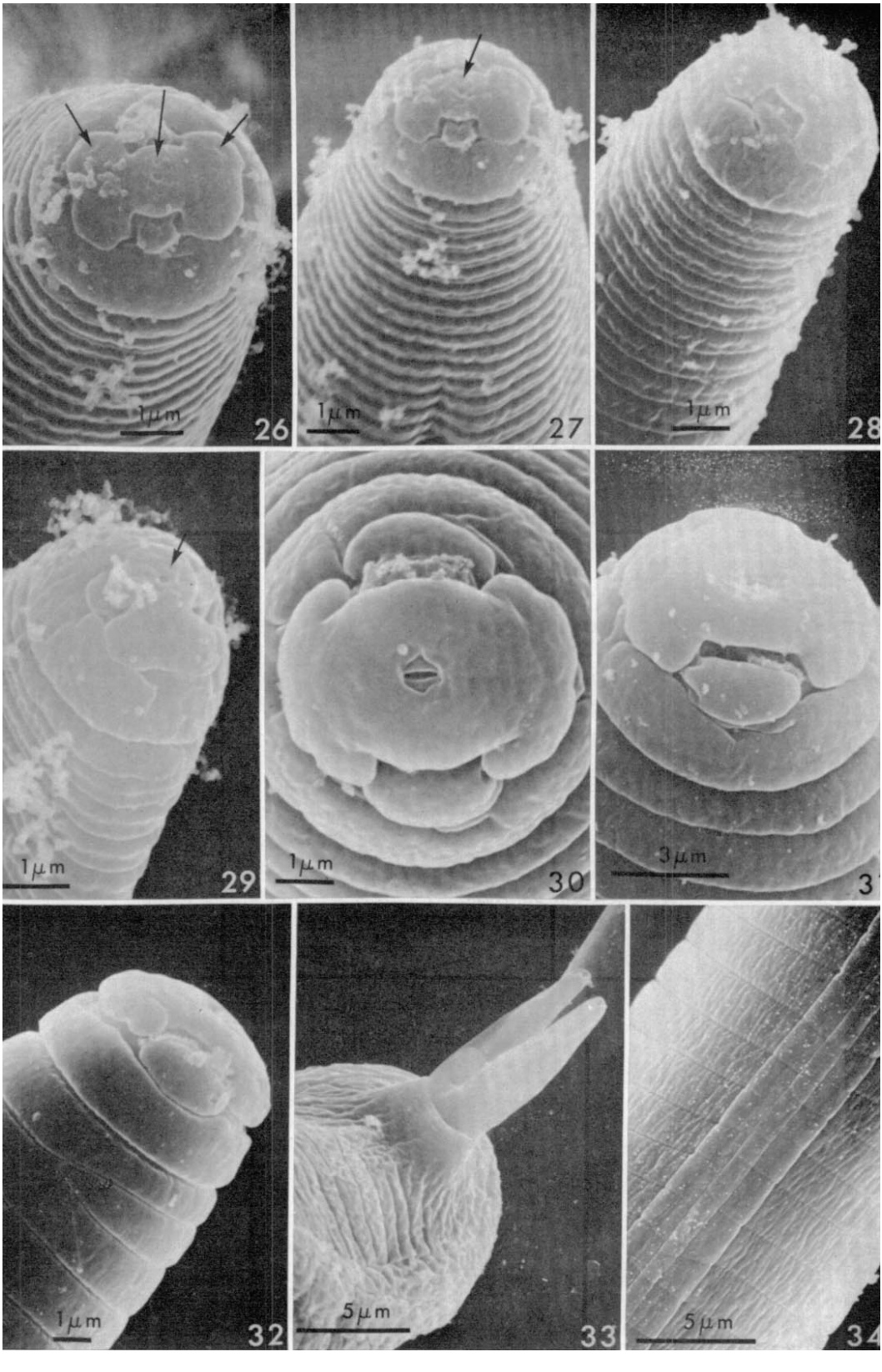
The presence of vesicles or vesicle-like structures in the median bulb of females of *M. chitwoodi* n. sp. is unique in *Meloidogyne* species, as far as is known. In describing *M. naasi,* however, Franklin (1) first reported such structures in this root-knot species, but they were present in the median bulb of males and larvae only, not in the females. In the present case these structures have been consistently seen in the females but not observed in males and larvae.

SEM examination of specimens confirmed observations made with optical microscopy and showed much more detail. On the perineal patterns (Figs. 18–25), the sunken vulva in an area devoid of striae and the nature of the striae on the surrounding patterns is evident. Anterior views of larvae (Figs. 26–29) and males (Figs. 30–32) clearly revealed the presence of only one large post-labial (head) annule without striations and showed the amphidial openings and nature of the labial disc. Further, on the male, the dentate tips of the spicules on the ventral surface (Fig. 23) and details of the lateral field (Fig. 34) are evident.

In repeated greenhouse tests, *M. chitwoodi* n. sp., unlike *M. hapla,* did not develop on an unknown variety of strawberry but did develop on 'Belrus' potato. As reported recently by Santo et al. (4), this new species not only heavily attacked potato ('Russet Burbank') and tomato ('Rutgers'), both being hosts for *M. hapla,* but also readily developed on wheat and corn, non-hosts for *M. hapla.* In their tests, *M. chitwoodi* n. sp. also failed to develop on peanut and pepper, which are good hosts for *M. hapla.* Morphological examination of specimens from tomato and 'Belrus' potato showed no distinct differences from those described and illustrated above for this new species.

As pointed out by Santo et al. (4), this nematode causes little or no galling on roots of potato and 'Rutgers' tomato. The small roots of these plants can become heavily infected (Figs. 35, 37, 39), but the infection, especially on rootlets, might be easily overlooked without the most careful examination. The females commonly protrude from the root surface and become surrounded posteriorly by a large, filled egg sac which

Figs. 26–34. Scanning electron micrographs of larvae and males of *Meloidogyne chitwoodi* n. sp. 26–29) Larvae, head region and heads. (In Figs. 26, 27, and 29 note the six inner labial sensilla surrounding the oral aperture; in Fig. 26 note two of the four cephalic sensilla.) 30–34) Males. 30–32) Heads. 33) Spicules (note dentate tips). 34) Lateral field about midbody (note narrow middle band and areolation by very fine striae).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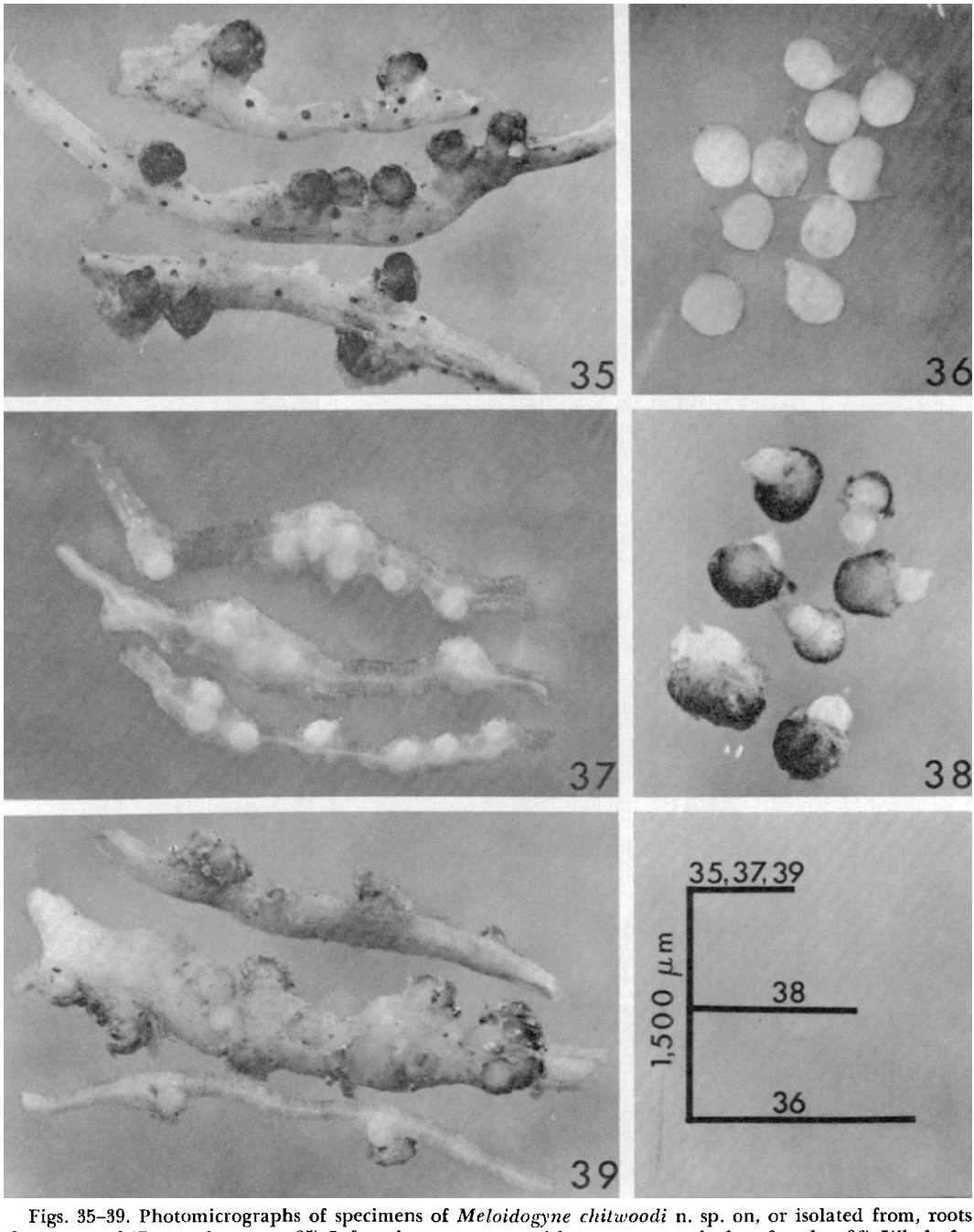

Figs. 35–39. Photomicrographs of specimens of *Meloidogyne chitwoodi* n. sp. on, or isolated from, roots of potato and 'Rutgers' tomato. 35) Infected potato roots with egg sacs attached to females. 36) Whole females. 37) Infected potato roots showing females with egg sacs removed. 38) Females (from tomato) with egg sacs attached (note the two females with fused egg sacs, lower left). 39) Infected tomato roots with most egg sacs still attached to females.

becomes brown to dark brown in older females (Figs. 35, 38, 39).

Though *M. hapla* was originally described from potato in New York and occurs on this crop in the Pacific Northwest and many other areas, it is commonly known as the "northern root-knot nematode." We propose for *M. chitwoodi* n. sp. the common name "Columbia root-knot nematode," a reference to the general area of its present occurrence.

The species name is given in honor of

the late eminent zoologist and nematologist, Benjamin G. Chitwood.

LITERATURE CITED

1. Franklin, M. T. 1965. A root-knot nematode, Meloidogyne naasi n. sp., on field crops in England and Wales. Nematologica 11:79-86.

2. Golden, A. M. 1976. Meloidogyne taxonomy—current status, some problems, and needs. Pp. 13–21 *in* Proceedings of the Research Planning Conference on Root-knot Nematodes, Meloidogyne spp. North Carolina State University, Raleigh.

3. Golden, A. M., and W. Birchfield. 1972. Heterodera graminophila n. sp. (Nematoda: Heteroderidae) from grass with a key to closely related species. J. Nematol. 4:147-154.

4. Santo, G. S., J. H. O'Bannon, A. M. Finley, and A. M. Golden. 1980. Occurrence of a new root-knot nematode (Meloidogyne sp.) in the Pacific Northwest. Plant Disease 64:951-952.