

# Meloidogyne incognita wartellei n. subsp. (Meloidogynidae), a Root-knot Nematode on Resistant Soybeans in Louisiana<sup>1</sup>

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**Abstract:** *Meloidogyne incognita wartellei* n. subsp. is described and illustrated from roots of soybean (*Glycine max* L.) near Washington, Louisiana. It is rather limited in distribution in that state, being known at five locations comprising about 60,000 acres. It not only attacks commonly susceptible soybeans but is a destructive pest on other commercial soybean varieties that are resistant to other forms of the *M. incognita* group in the area. This new subspecies is related most closely to *M. i. incognita* and *M. i. acrita*, but differs especially in the females having a delicate stylet with small, rounded knobs sloping posteriorly; dorsal esophageal gland orific further back ( $5\text{ }\mu\text{m}$ ) from base of stylet; and excretory pore often two to three stylet lengths (sometimes more) from the anterior end. Also, males are often without detectable head annules and with an average stylet length of  $22.4\text{ }\mu\text{m}$ . Comments and morphometric data are given on *M. i. incognita* and *M. i. acrita*. **Key Words:** taxonomy, morphology, resistance-breaking, *Glycine max*.

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In 1968 a root-knot nematode was found heavily attacking soybean (*Glycine max* L. cv. Bragg) on the Wartelle Farm in St. Landry Parish, Louisiana (14). This variety has resistance to the known populations of the *Meloidogyne incognita* group occurring in the range of cultivation of this particular soybean. Subsequent host-range tests in Louisiana (7) and morphological examination in Beltsville, Maryland, suggested that

the nematode was a new race of *M. incognita*, which became known as the "Wartelle race." During the past three years or so, however, this nematode has been examined more critically. It appears to have certain morphological characters which can be used to distinguish it from closely related forms (4) and is described herein as a new subspecies of the *M. incognita* group.

This nematode is a highly destructive pest on soybean, not only on the usual root-knot-susceptible varieties but also on varieties having resistance to other known forms of the *M. incognita* group. In the presence of "regular" root-knot in the area, the resistant Bragg variety yields 30–40 bushels of soybeans per acre; in a field with this nematode, however, the yield from Bragg is from 2 to 12 bushels per acre. The yield of the Forrest variety is similarly reduced in the presence of this nematode. As of now, no commercial varieties available to Louisiana growers have resistance to this parasite, although certain breeding lines and varieties show some resistance (14). Also, more recently, a modified root-gall index technique was developed for screening large numbers of soybean lines for resistance to this nematode (9), and the nature of inheritance of resistance to this pest has been determined in a soybean line (1). Intensive cooperative research to develop suitable resistant varieties is under way by James M. Epps (USDA, ARS, Nematology Investigations, 605 Airways Blvd., Jackson, Tennessee 38301), E. E. Hartwig (Delta Branch Experiment Station, ARS/USDA, Stoneville, Mississippi 38776), and D. R. Gilman (Louisiana State University, Baton Rouge, Louisiana 70803). So far, the nematode appears to be limited to about five locations in Louisiana amounting to about 60,000 acres, although it might well be more widespread. It is of interest, although of little consolation to the soybean grower, that this pest does not attack the Centennial sweet potato, which is highly susceptible to the common forms of *M. incognita* in sweet-potato-growing areas (7).

Specimens used herein were obtained from cultures on Lee and Bragg soybeans in a growth chamber at Beltsville, Maryland, and from several additional field collections in St. Landry Parish, Louisiana, at

different times during the past three or four years, the latest being in October, 1977. Larvae and males were generally recovered from infected fresh roots kept in petri dishes with a small amount of water, and females were later dissected from the roots after overnight fixation in 3% formaldehyde solution to harden the specimens. The procedures used, including measuring, drawing, photomicrographing, and preparing specimens, were essentially as used by Golden and Birchfield (5) except that some fixed females were cut and mounted in lactophenol solution.

*Meloidogyne incognita wartellei* n. subsp.

FEMALES (25): Length 456–1135  $\mu\text{m}$  (mean 748  $\mu\text{m}$ , standard deviation (SD) 148  $\mu\text{m}$ ); width 361–585  $\mu\text{m}$  (464  $\mu\text{m}$ , SD 75);  $a = 1.1\text{--}2.2$  (1.6, SD 0.3);  $b = 4.3\text{--}6.7$  (5.4, SD 0.7); stylet 13.6–15.3  $\mu\text{m}$  (15.0  $\mu\text{m}$ , SD 0.6); width of stylet knobs 3.0–4.3  $\mu\text{m}$  (3.7  $\mu\text{m}$ , SD 0.4); dorsal esophageal gland orifice 4.06–6.0  $\mu\text{m}$  (5.0  $\mu\text{m}$ , SD 0.6) from base of stylet; vulva slit length 21–30  $\mu\text{m}$  (25.2  $\mu\text{m}$ , SD 2.1); distance from vulva slit to anus 16–20  $\mu\text{m}$  (18  $\mu\text{m}$ , SD 1.1).

HOLOTYPE (female): Length 774  $\mu\text{m}$ ; width 473  $\mu\text{m}$ ;  $a = 1.6$ ;  $b = 4.9$ ; stylet 15.1  $\mu\text{m}$ ; width of stylet knobs 3.0  $\mu\text{m}$ ; dorsal esophageal gland orifice 4.6  $\mu\text{m}$  from base of stylet.

Body pearly white, globular to pear-shaped, without posterior protuberance, and with distinct neck situated anteriorly on a median plane with vulva. Esophageal and anterior region appearing about as illustrated (Fig. 6). Head not offset from neck, bearing a labial cap and generally only one prominent cephalic annule. Cephalic framework present but weak. Stylet quite delicate, with small rounded knobs commonly sloping posteriorly (Figs. 16–23). Center of median bulb 59–90  $\mu\text{m}$  (73  $\mu\text{m}$ , SD 9.3) from anterior end. Excretory pore distinct, and though variable in exact position, generally located well posterior to base of unprotruded stylet, being 13–98  $\mu\text{m}$  (45  $\mu\text{m}$ , SD 19) from anterior end. Vulva and anus terminal. Perineal pattern with arch, and generally with rather fine lines which may be wavy, discontinuous, or broken (Figs. 7–15).

MALES (25): Length 1203–2450  $\mu\text{m}$  (1797  $\mu\text{m}$ , SD 332);  $a = 32\text{--}52$  (43, SD 5.7);  $b = 6\text{--}11$  (8, SD 1.4);  $c = 122\text{--}372$  (184,

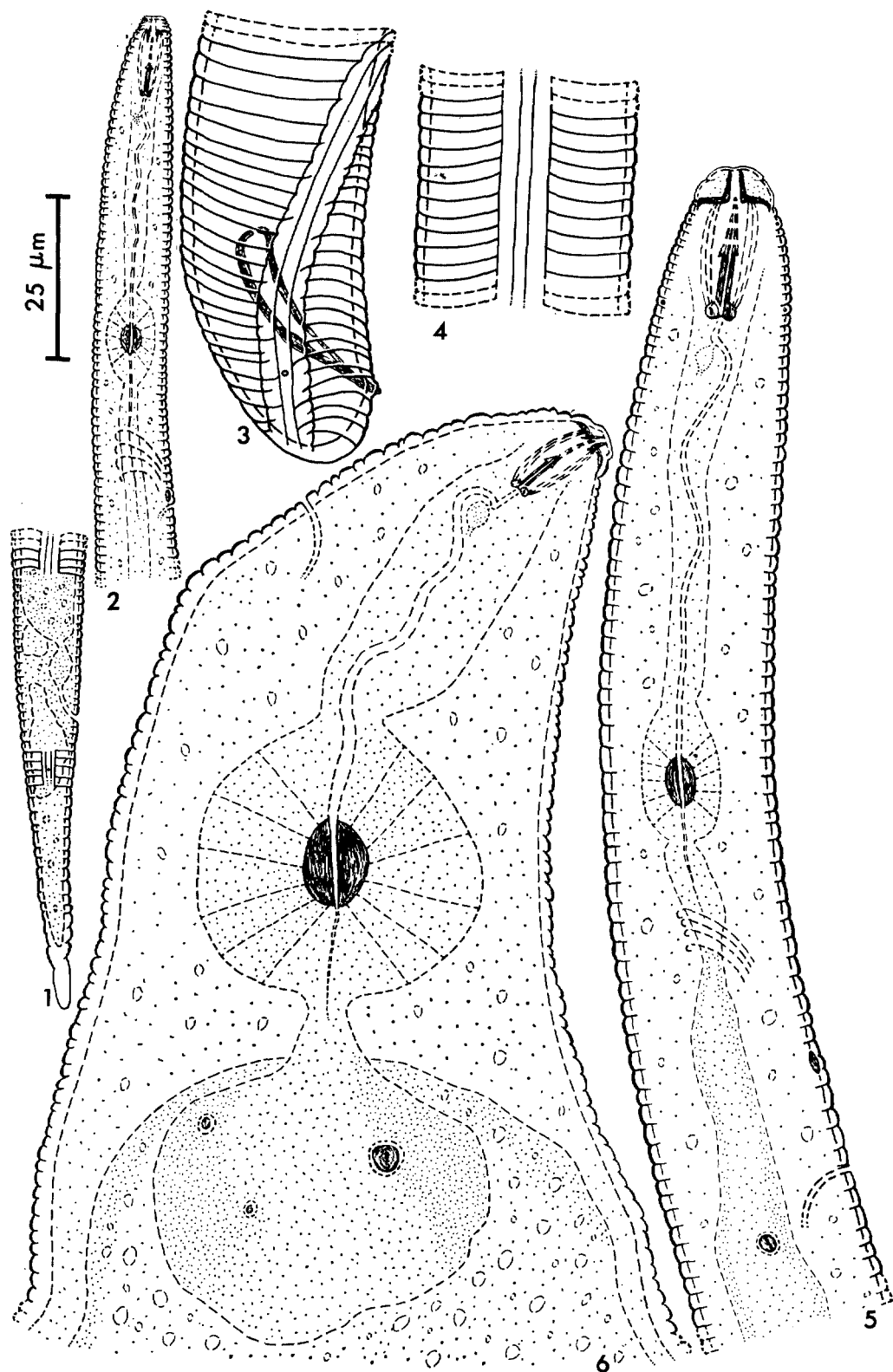


FIG. 1-6. Drawings of *Meloidogyne incognita wartellei* n. subsp. 1-2) Larva. 3-5) Male. 6) Anterior region of female.

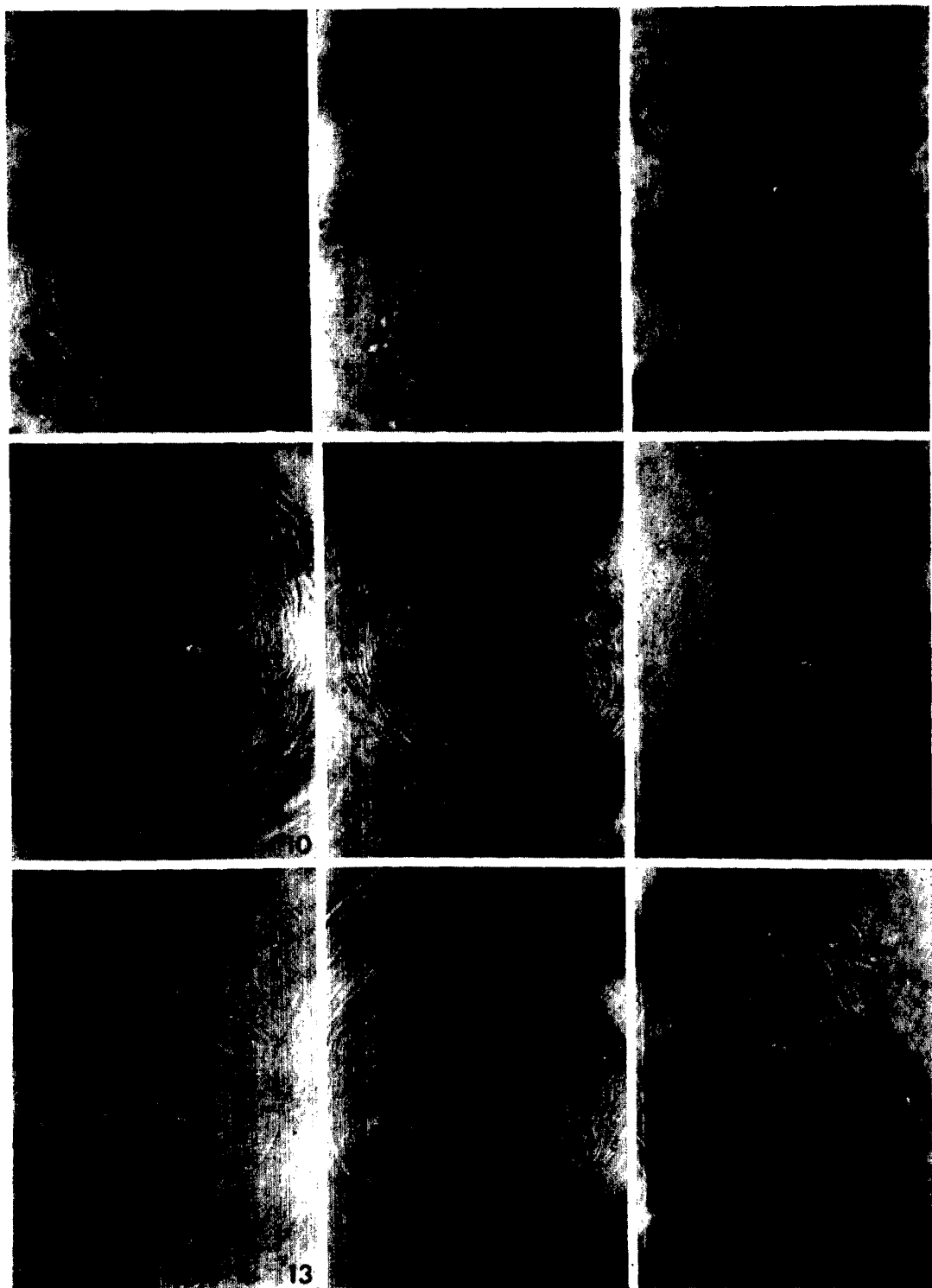


FIG. 7-15. Photomicrographs of representative perineal patterns of nine different females of *Meloidogyne incognita wartellei* n. subsp.

SD 57); stylet 21.3–23.8  $\mu\text{m}$  (22.4  $\mu\text{m}$ , SD 0.7); width of stylet knobs 5–5.6  $\mu\text{m}$  (5.2  $\mu\text{m}$ , SD 0.23); dorsal esophageal gland orifice 2.6–3.4  $\mu\text{m}$  (3  $\mu\text{m}$ , SD 0.4) from base of stylet; spicules 28–36  $\mu\text{m}$  (33  $\mu\text{m}$ , SD 2.4); gubernaculum 9.4–10.2  $\mu\text{m}$  (9.6  $\mu\text{m}$ , SD 0.4); tail 6–12  $\mu\text{m}$  (10  $\mu\text{m}$ , SD 1.5).

**ALLOTYPE** (male): Length 1815  $\mu\text{m}$ ; a = 43; b = 7.9; c = 165; stylet 22.8  $\mu\text{m}$ ; orifice of dorsal esophageal gland 2.9  $\mu\text{m}$  from base of stylet; spicules 34  $\mu\text{m}$ ; gubernaculum 9  $\mu\text{m}$ ; tail 11  $\mu\text{m}$ .

**Description:** Body slender, vermiform tapering slightly at both extremities. Mid-body width 32–46  $\mu\text{m}$  (43  $\mu\text{m}$ , SD 5.7). Head only slightly offset, with massive labial annule (head cap), and commonly without visible postlabial annules although occasional specimens might show 1–2 faint striations. Cuticular annulation prominent, with annules at midbody measuring about 3.5  $\mu\text{m}$ . Lateral field (Figs. 3, 4) with 4 lines, generally not areolated but outer 2 lines often crenate, especially in posterior portion, and about 1/4 of body width, the latter at widest part being 32–46  $\mu\text{m}$  (40  $\mu\text{m}$ , SD 3.5). Stylet, knobs, cephalids, hemizonid, excretory pore, and anterior portion appear about as illustrated (Fig. 5). Median bulb center 91–111  $\mu\text{m}$  (102  $\mu\text{m}$ , SD 7) from anterior end. Testis generally 1, although sometimes 2. Spicules arcuate, tips rounded. Phasmids distinct, anterior to cloaca (Fig. 3). Tail short, rounded (Fig. 3).

**LARVAE** (50): Length 352–404  $\mu\text{m}$  (385  $\mu\text{m}$ , SD 12.5); a = 26–31 (28, SD 1); b = 2.0–2.6 (2.3, SD 0.11); c = 7.4–9.0 (8.1, SD 0.33); stylet 9.5–10.6  $\mu\text{m}$  (10.3  $\mu\text{m}$ , SD 0.31); dorsal esophageal gland orifice 2.2–3.0  $\mu\text{m}$  (2.4  $\mu\text{m}$ , SD 0.23) from base of stylet; center of median bulb 49–56  $\mu\text{m}$  (53  $\mu\text{m}$ , SD 2) from anterior end; head width 5.04  $\mu\text{m}$ ; head height 2.2–2.8  $\mu\text{m}$  (2.6  $\mu\text{m}$ , SD 0.28); hw/hh ratio = 1.8–2.3 (2.1, SD 0.22); tail length 43–51  $\mu\text{m}$  (47.4  $\mu\text{m}$ , SD 2.3); hyaline tail terminal 9.6–13  $\mu\text{m}$  (11.2  $\mu\text{m}$ , SD 0.8); caudal ratio A = 2.4–3.3 (2.9, SD 0.19); caudal ratio B = 3.8–5.8 (4.9, SD 0.42).

**Description:** Body vermiform, tapering at both extremities but much more so posteriorly. Head not offset, with weak cephalic framework, and bearing 3 postlabial annules. Body cuticular annulation fine but readily visible. Lateral field (Fig. 1) with 4 lines, not areolated, and 1/4 body

width, the latter being 13–17  $\mu\text{m}$  (15  $\mu\text{m}$ , SD 1.3) at its widest part. Stylet, knobs, hemizonid, excretory pore, and anterior portion appearing about as illustrated (Fig. 2). Cephalids indistinct and not shown. Center of median bulb 48–58  $\mu\text{m}$  (53  $\mu\text{m}$ , SD 2.6) from anterior end. Phasmids small but distinct, located about one anal body width posterior to level of anus (Fig. 1). Rectum generally inflated (Fig. 1) at least to some extent. Tail somewhat variable in exact shape but commonly appearing as illustrated (Fig. 1).

**EGGS** (25): Length 77–94  $\mu\text{m}$  (86  $\mu\text{m}$ , SD 4.1); width 35–48  $\mu\text{m}$  (41  $\mu\text{m}$ , SD 3.3); L/W ratio 1.8–2.4 (2.1, SD 0.15). Egg shell hyaline, without visible markings.

**HOLOTYPE** (female): Originally collected by Wray Birchfield 30 May 1975 (and subsequently grown on soybean in an isolated growth chamber). Slide T-275t, USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

**ALLOTYPE** (male): Slide T-276t. Same data as holotype. USDANC, Beltsville, Maryland, USA.

**PARATYPES:** Males, females, larvae, and eggs: USDANC, Beltsville, Maryland; University of California Nematode Survey Collection (UCNSC), Davis, California, USA; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada.

**TYPE HOST AND LOCALITY:** Roots of soybean (*Glycine max* L.) on the Wartelle Farm, St. Landry Parish near Washington, Louisiana.

**DIAGNOSIS:** *Meloidogyne incognita wartellei* n. subsp. differs from the most closely related forms, *M. incognita incognita* (Kofoid & White, 1919) Chitwood, 1949, and *M. incognita acrita* Chitwood, 1949, in the following characteristics:

*Females:*

1. The stylet of *wartellei* is more delicate than in *incognita* or *acrita*, and its smaller knobs show greater slope to the rear.

2. The average distance of the dorsal esophageal gland orifice behind the stylet is 5.0  $\mu\text{m}$  for *wartellei*, 3.8  $\mu\text{m}$  for *incognita*, and 4.0  $\mu\text{m}$  for *acrita*.

3. The excretory pore is usually about two stylet lengths posterior to the base of the unprotruded stylet in *wartellei*, one

stylet length or less in *incognita*. In *acrita* the excretory pore is commonly very near the base of the stylet.

#### Males:

1. Lip annulation is generally lacking in *wartellei*. There are three prominent annules in the lip region of *incognita*, and three faint ones in *acrita*.

2. The stylet length averages 22–23  $\mu\text{m}$  in *wartellei* and *acrita* and 25.5  $\mu\text{m}$  in *incognita*.

We fully recognize that a subspecies cannot always be identifiable apart from its nominate subspecies or other subspecies in the same species group. In accordance with the "75-percent rule" relative to subspecies, however, as commonly used in zoological systematics and as discussed by Mayr (8), we feel that the data and information presented above justify subspecific status for this former "Wartelle race." Furthermore, it is believed that this action can in time result in better information on the occurrence and distribution of this destructive soybean pest and contribute to its control, especially in the development and use of resistant soybean varieties.

#### Comments and Morphometrics on *Meloidogyne incognita incognita* and *M. incognita acrita*

These two subspecies were among the species described by Chitwood (2), in 1949, when he reestablished the genus *Meloidogyne* Goeldi, 1887. His observations and measurements on these two forms at that time were accurate and are still valid. The perineal pattern of the female was used as an important diagnostic character, and his photomicrographs (in particular Fig. 6E and F) illustrate the pattern characteristics of these two forms. In general, *incognita* patterns have rather fine, distinct and continuous lines which may be wavy (or zig-zag) on one or both sides, especially in the vicinity of the anal area. On the other hand, *acrita* patterns commonly have heavy or coarse lines that are irregularly broken and discontinuous and tend to be straight, typically without the wavy nature. Some other workers, particularly Taylor *et al.* (11) and Sasser (10), reported similar pattern characteristics in these two forms.

Eventually, however, various workers began considering these two forms to be the

same taxon, referring to it as the species *incognita*. The trend was perhaps spurred on by the formal synonymy of *acrita* under *incognita* by Goodey (6), in 1963, and given further impetus by Whitehead (13) in his extensive *Meloidogyne* report in 1968. Nevertheless, a good many workers, including Terent'eva (12) and some other Russian scientists, continued to recognize these forms as two separate subspecies. Recently, *acrita* was raised to full specific rank by Esser *et al.* (3), indicating a need and justification for recognizing *acrita* as a separate taxon from *incognita*. Because of the obvious close relationship of these two forms, however, we prefer at this time to consider *acrita* as a subspecies in the *incognita* group rather than as a full separate species.

#### Measurements of *M. incognita incognita* (Kofoid & White, 1919) Chitwood, 1949

After Chitwood (2). FEMALES: Length 510–690  $\mu\text{m}$ ; width 300–430  $\mu\text{m}$ ; stylet 15–16  $\mu\text{m}$ ; width of stylet knobs 4–5  $\mu\text{m}$ ; dorsal esophageal gland orifice 2–4  $\mu\text{m}$  "(mean 3  $\mu$ )" from base of stylet.

MALES. Length 1.2–2.0 mm; a = 39–48; b = 8–17; stylet 23–26  $\mu\text{m}$  "(usually 25–26  $\mu$ )"; width of stylet knobs 5.5–6.5  $\mu\text{m}$ ; dorsal esophageal gland orifice 1.7–3.5  $\mu\text{m}$  from base of stylet "(mean 2.5  $\mu$ )"; spicules 34–36  $\mu\text{m}$  (along chord of their arc).

LARVAE: Length 360–393  $\mu\text{m}$ ; a = 29–33; b = 5.6–6.4; c = 8–9.4; stylet 10  $\mu\text{m}$ ; dorsal esophageal gland orifice 2–2.5  $\mu\text{m}$  from base of stylet.

EGGS: Length 80–98  $\mu\text{m}$ ; width 30–38  $\mu\text{m}$ .

Our measurements were essentially identical to those above, and data on only certain additional characters are included here, and similarly for *acrita*.

FEMALES (25): Excretory pore from anterior end 17–43  $\mu\text{m}$  (28  $\mu\text{m}$ , SD 7); center of median bulb from anterior end 64–89  $\mu\text{m}$  (77  $\mu\text{m}$ , SD 8); length of vulva slit 20–27  $\mu\text{m}$  (24  $\mu\text{m}$ , SD 2.1); distance from vulva slit to anus 15–20  $\mu\text{m}$  (17  $\mu\text{m}$ , SD 1.8).

LARVAE (25): Center of median bulb from anterior end 48–54  $\mu\text{m}$  (52  $\mu\text{m}$ , SD 2.1); head width 4.5–5.0  $\mu\text{m}$  (4.9  $\mu\text{m}$ , SD 0.2); head height 2.8  $\mu\text{m}$  head width/head height ratio = 1.8 tail length 44–52  $\mu\text{m}$

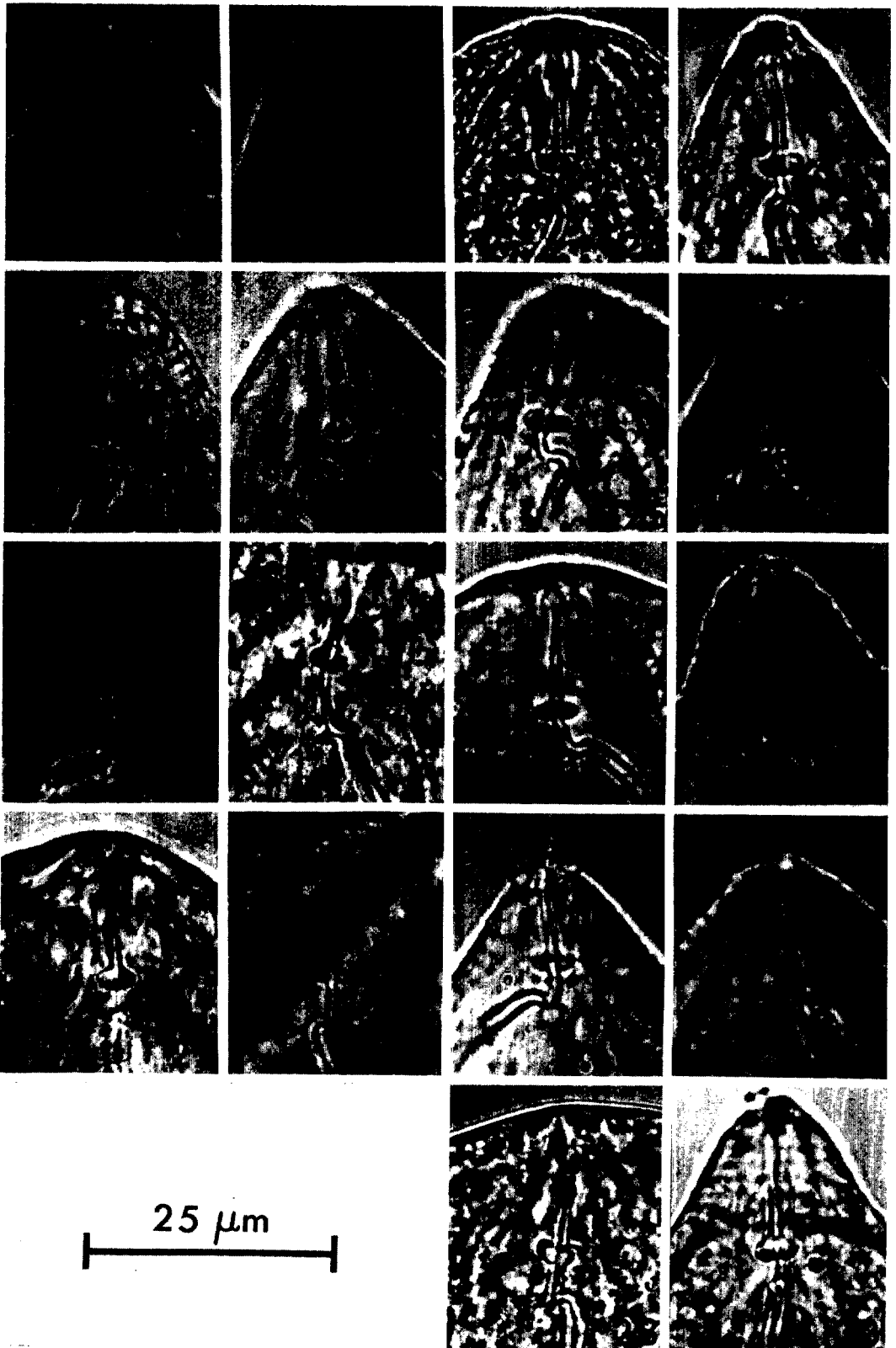


FIG. 16-33. Photomicrographs of anterior portion of certain *Meloidogyne* females showing especially stylet knobs and dorsal esophageal gland orifice. 16-23) *M. incognita wartellei* n. subsp. 24-28) *M. incognita acrita*. 29-33) *M. incognita incognita*. (Arrows indicate location of DGO.)

(48  $\mu\text{m}$ , SD 2) ; hyaline tail terminal 9.5–14  $\mu\text{m}$  (11  $\mu\text{m}$ , SD 1.6); caudal ratio A = 2.4–3.6 (3, SD 0.4); caudal ratio B = 3.6–6.3 (5.1, SD 0.9); phasmids located in center of lateral field about one anal body diameter posterior to level of anus.

**MALES:** No additional characters of significance were measured. It should be mentioned, however, that the upper range of 32.7  $\mu\text{m}$  for the male stylet length given by Whitehead (13) is far greater than any we have ever observed. That measurement might represent an error, although his average stylet length of 25.0  $\mu\text{m}$  seems reasonable.

Measurements of *M. incognita acrita*

Chitwood, 1949

After Chitwood (2). **FEMALES:** Length 440–670  $\mu\text{m}$ ; width 364–545  $\mu\text{m}$ ; stylet 16  $\mu\text{m}$ ; width of stylet knobs 5  $\mu\text{m}$ ; dorsal esophageal gland orifice 3–5  $\mu\text{m}$  from base of stylet.

**MALES:** Length 1.0–1.6 mm; a = 39–46; b = 11–13; stylet 20–24  $\mu\text{m}$  “(usually 23–24  $\mu$ )”; width of stylet knobs 5–6  $\mu\text{m}$ ; dorsal esophageal gland orifice 2–4  $\mu\text{m}$  posterior to base of stylet “(usually 2–3  $\mu$ )”; spicules 29–34  $\mu\text{m}$  (chord of arc).

**LARVAE:** Length 345–396  $\mu\text{m}$ ; a = 22–28; b = 6.1–7.1; c = 7.0–7.5; stylet 10–11  $\mu\text{m}$ ; dorsal esophageal gland orifice 2–2.5  $\mu\text{m}$  posterior to base of stylet.

**EGGS:** Length 82–93  $\mu\text{m}$ ; width 36–39  $\mu\text{m}$ .

Authors' measurements on additional characters:

**FEMALES (25):** Excretory pore from anterior end 9–23  $\mu\text{m}$  (15  $\mu\text{m}$ , SD 4.2); center of median bulb from anterior end 69–94  $\mu\text{m}$  (80  $\mu\text{m}$ , SD 7.4); length of vulva slit 20–26  $\mu\text{m}$  (24  $\mu\text{m}$ , SD 1.5); distance from vulva slit to anus 15–23  $\mu\text{m}$  (18  $\mu\text{m}$ , SD 1.7).

**LARVAE (30):** Center of median bulb from anterior end 46–58  $\mu\text{m}$  (51  $\mu\text{m}$ , SD 2.4); head width 5.0  $\mu\text{m}$ ; head height 2.8  $\mu\text{m}$ ; head width/head height ratio = 1.8; tail length 41–53  $\mu\text{m}$  (47.6  $\mu\text{m}$ , SD 2.9); hyaline tail terminal length 8–14  $\mu\text{m}$  (10.7  $\mu\text{m}$ , SD 1.3); caudal ratio A = 2–3.6 (2.6, SD 0.32); caudal ratio B = 3–8 (4.5, SD 1); phasmids located in center of lateral field about one anal diameter posterior to level of anus.

**MALES:** No additional characters of significance were measured.

The perineal patterns as discussed above and as illustrated especially by Chitwood (2), Taylor *et al.* (11), and Sasser (10) have been the main basis for identifying *M. i. incognita* and *M. i. acrita*. It appears now, however, that the location of the excretory pore in females and particularly the stylet length in males can serve as helpful supplemental characters in separating these forms. In any case, it seems obvious that identification of *Meloidogyne* forms is quite complex, and that not only must a good many females (anterior and posterior portions of 12–20 or more specimens) be examined but other developmental stages should also be studied whenever possible.

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