Additional notes on the morphology of Meloidogyne brevicauda

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Summary — Females, males, and second-stage juveniles of *Meloidogyne brevicauda* Loos, 1953 were examined by light and scanning electron microscopy. The labial disc of females and males is distinctly separated from the lips by a deep groove. In the second-stage juvenile, the labial disc and lips are divided by a groove but are joined by a small band of cuticle. Head annulations are absent in all three life stages and the lips extend posteriorly to the first body annule. The tail of the male is marked by a crescent-shaped fold around the posterior portion of the cuticle surrounding the cloacal opening. The tail of the second-stage juvenile is marked by transverse grooves that radiate from the posterior end of the lateral field. Also, the phasmids open on the ventral edge, rather than in the middle of the lateral field. The morphology of *M. brevicauda* is quite different from the four most common species and most others examined closely, but is similar in many respects to that of *M. coffeicola* Lordello & Zamith, 1960, *M. indica* Whitehead, 1968, *M. propora* Spaull, 1977, and *M. nataliei* Golden, Rose & Bird, 1981.

Résumé — Notes supplémentaires sur la morphologie de Meloidogyne brevicauda — Des femelles, des mâles et des juvéniles de deuxième stade de Meloidogyne brevicauda Loos, 1953 ont été examinés en microscopies optique et électronique à balayage. Le disque labial des femelles et des mâles est distinctement séparé des lèvres par une rainure profonde. Chez les juvéniles de deuxième stade (J2), le disque labial et les lèvres sont également séparés par une rainure, mais demeurent reliés entre eux par une petite bande de cuticule. L'annélation de la tête est absente chez les adultes et les J2, et les lèvres s'étendent postérieurement au premier anneau du corps. La queue du mâle se distingue par un repli en forme de croissant soulignant la partie postérieure de la cuticule entourant l'ouverture cloacale. La queue des J2 se distingue par des rainures transversales qui divergent à partir de l'extrémité postérieure du champ latéral. Les phasmides s'ouvrent ainsi sur le côté ventral plutôt qu'au milieu du champ latéral. La morphologie de *M. brevicauda* est assez différente de celle des quatre espèces les plus communes et de celle de la plupart des autres espèces examinées en détail, mais elle est semblable, par plusieurs aspects, à celle observée chez *M. coffeicola* Lordello & Zamith, 1960, *M. indica* Whitehead, 1968, *M. propora* Spaull, 1977, et *M. nataliei* Golden, Rose & Bird, 1981.

Key-words : Meloidogyne, morphology, nematodes.

Meloidogyne brevicauda Loos, 1953 was described nearly 40 years ago as a root-knot species parasitizing mature tea in Sri Lanka (Loos, 1953). It was the first description of a new species of root-knot nematode after Chitwood (1949) described or delineated five species and placed them into the genus Meloidogyne Goeldi, 1887. Since 1953, M. brevicauda has been reported only in Sri Lanka and India (Rao, 1970; Silvapalan, 1972, 1978; Lamberti et al., 1987). Very few additional morphological studies have been completed since it was described (Whitehead, 1968). The purpose of this paper was to reexamine the morphology of M. brevicauda. Some of the morphological details that were in the original description were quite different from that described for most root-knot nematodes. We wanted to confirm and clarify these morphological characters using light and scanning electron microscopy. Our hope was to delineate new characters that may help reveal the phylogenetic relationships of *M. brevicauda* with other species within the genus and subfamily. We have previously reported results from preliminary investigations (Eisenback & Gnanapragrasam, 1986).

Materials and methods

Specimens of *Meloidogyne brevicauda* were recovered from infected roots of tea collected from a plantation located 80 km from the Tea Research Institute of Sri Lanka, at an altitude of 1050 m, where the climate is much cooler than that of the Tea Research Institute. Second-stage juveniles, males, and females were prepared for light and scanning electron microscopy (SEM) as previously described (Eisenback & Hirschmann, 1979; Eisenback *et al.*, 1980). At least 30 specimens of each life stage were examined.

Results

Females

The anterior end of the female (Fig. 1 A) contains the esophagus which includes a narrow procorpus, a large

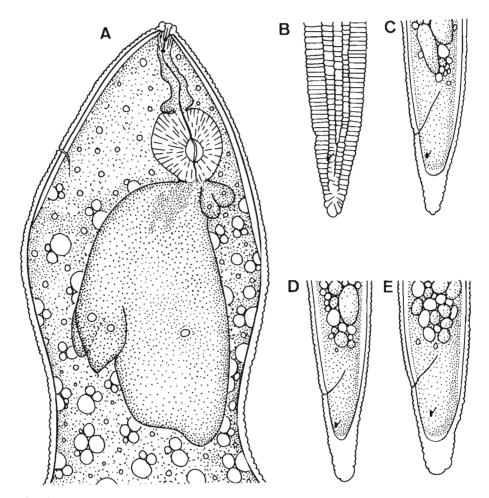


Fig. 1. *Meloidogyne brevicauda* Loos, 1953. A : Female head including esophagus; B : Surface morphology of juvenile tail; C-E : Optical section of medial plane of juvenile tail.

metacorpus, a narrow isthmus, and a large glandular region that has one dorsal and two subventral esophageal gland lobes, each with a large nucleolus and nucleus. Two large nucleated esophago-intestinal cells lie at the dorsal base of the metacorpus at the junction with the intestine. The labial disc and lips are prominent and protrude from the regular body contour (Figs 1 A; 2 A, C). The slit-like stoma is located in the oval-shaped prestoma, which is situated centrally on the labial disc and surrounded by six small pore-like openings of the inner labial sensilla (Fig. 2 C). The labial disc is raised above the surrounding lips. The subventral and subdorsal lip pairs are separate and do not fuse to form one dorsal and one ventral lip. These lips are often termed medial lips because specimen orientation is difficult to discern in the SEM. The lateral lips are large and fused with the lateral edges of the medial lips. Large elongate oval-shaped openings of the amphids separate the labial disc and lateral lips. A distinct head annule is apparently absent. The stylet is slender, but the knobs are large and rounded, and often slope posteriorly (Fig. 2 A-B). The cone gradually increases in width posteriorly and overlaps the shaft, which also widens gradually posteriorly.

The perineal pattern of *M. brevicauda* is quite distinctive (Fig. 3). The striae are smooth, coarse, and continuous; they completely encircle the perineum and extend anteriorly for some distance. The dorsal arch is high and squarish to rounded. The lateral fields are usually indistinct, but they may be marked by irregularities (Fig. 3 A), or small, wing-like projections (Fig. 3 D). The striae may completely encircle the large, rounded tail terminus.

Males

The slit-like stoma of the male of *M. brevicauda* is located in the ovate prestoma which is surrounded by six small, pore-like openings of the inner labial sensilla (Fig. 4 A-C). Some of the sensilla may open into the

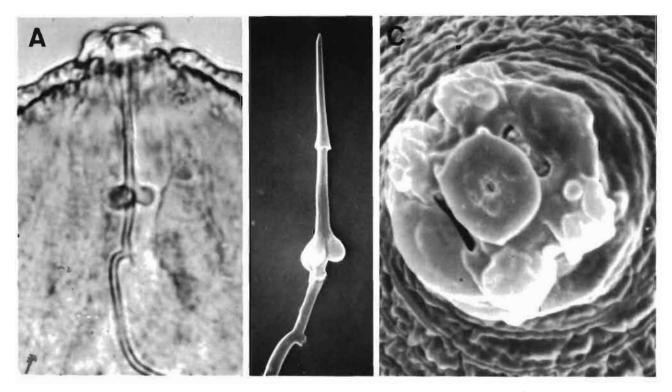


Fig. 2. *Meloidogyne brevicauda* Loos, 1953 females; light (LM) and scanning electron micrographs (SEM) A : LM of stylet and head. B : SEM of excised stylet. C : SEM of face view of head.

prestoma, rather than on the labial disc (Fig. 4 B). The large, round labial disc is separated from the lips by a deep indentation which is obvious in both LM and SEM (Fig. 4 A, D). The large subdorsal and subventral lip pairs are fused and form one dorsal and one ventral lip. They extend posteriorly and lie next to the first body annule. The lateral lips are also large and adjacent to the first body annule. Head annulations are absent.

The lips are set off from the body annulations because the diameter of the lips is larger than that of the first body annule. The first body annule is also narrower than the remaining body annules (Fig. 4 A, C). The tail is marked by a crescent-shaped fold in the cuticle surrounding the posterior portion of the cloacal opening (Fig. 4 E-F).

SECOND-STAGE JUVENILES

The slit-like prestoma is located in the ovate prestoma in second-stage juveniles of *M. brevicauda* (Fig. 5 A-C). Six pore-like openings of the inner labial sensilla surround the prestoma. The labial disc is squarish to rectangular and connected to the medial lips by a narrow piece of cuticle. The subdorsal and subventral lips are fused and form one dorsal and ventral lip. They extend posteriorly, and may be indented medially (Fig. 5 C). These large medial lips are apparent in both LM and SEM (Fig. 5 A, D). The lateral lips are also large and adjacent to the first body annule. The amphidial openings appear as small elongate oval openings between the labial disc and medial lips. Head annulations are absent.

The tail of second-stage juveniles of *M. brevicauda* is broad and short (Figs 1 B-E; 5 F-G). It is marked by transverse grooves that radiate from the posterior end of the lateral field. The tail has a clear hyaline portion that is nearly one-third of its length. The phasmids open on the ventral edge of the lateral fields, nearly midway between the anus and tail tip (Fig. 1 B-E).

Discussion

The original description of M. brevicauda is very detailed. The drawings are similar to the morphology revealed by SEM. Only a few features were clarified by additional examinations of females, males, and second-stage juveniles.

Our observations of females are very similar to those in the original (Loos, 1953). The description of the medial and lateral lips agrees with our observations in almost every detail. The morphology of the stoma, prestoma, and inner labial sensilla are additional features revealed by SEM. Likewise, the shape of the lips and the labial disc are clarified. Observations of perineal patterns

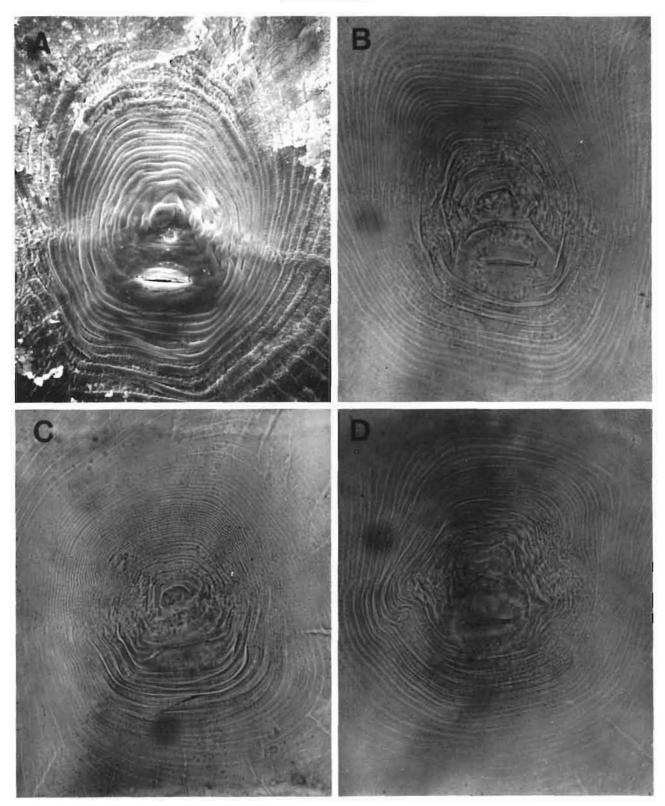


Fig. 3. Meloidogyne brevicauda Loos, 1953; perineal patterns. A : SEM; B-D : light micrographs.

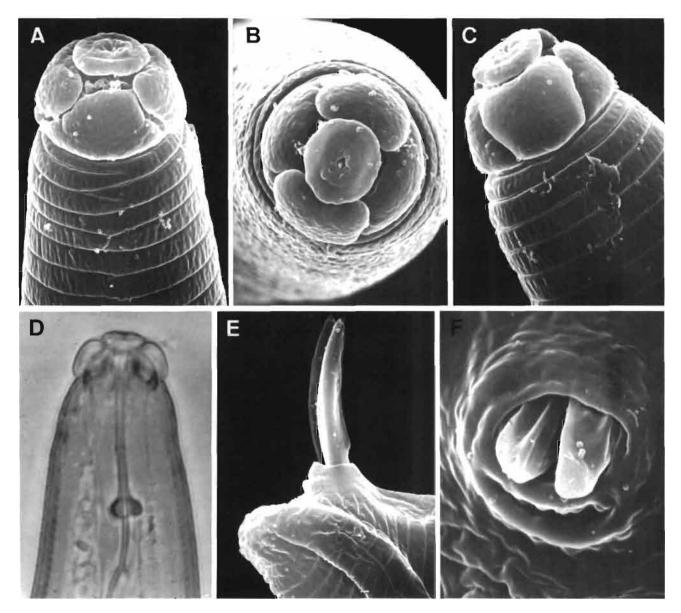


Fig. 4 Meloidogyne brevicauda Loos, 1953 males. Light (LM) and scanning electron micrographs (SEM). A-C : SEM of head in lateral, face, and medial views, respectively; D : LM of head in lateral view; E-F : SEM of protruded spicules in lateral view and retracted in ventral view, respectively; note the two pore-like openings on the tips of the spicules and the fold in the cuticle posterior to the cloacal opening.

by SEM and LM show additional morphological features and some of the variability among specimens.

The external morphology of the anterior end of females of *M. brevicauda* is quite different from that of the four most common species of root-knot nematodes, *M. arenaria, M. hapla, M. incognita,* and *M. javanica* (Eisenback *et al.,* 1983). In most species the labial disc is fused with the medial lips, but in *M. brevicauda,* the labial disc is distinctly separated from the lips by a deep groove in the cuticle. Usually, the medial lips are fused into one dorsal and one ventral lip; in *M. brevicauda*, however, they are distinctly separate. Likewise, a distinct head annule that may be further divided by irregular or incomplete annulations is commonly present on the anterior end of root-knot females, but it is absent in *M. brevicauda*. The internal morphology is quite similar to that of most *Meloidogyne* species (Eisenback, 1984).

The original description and drawings of males of

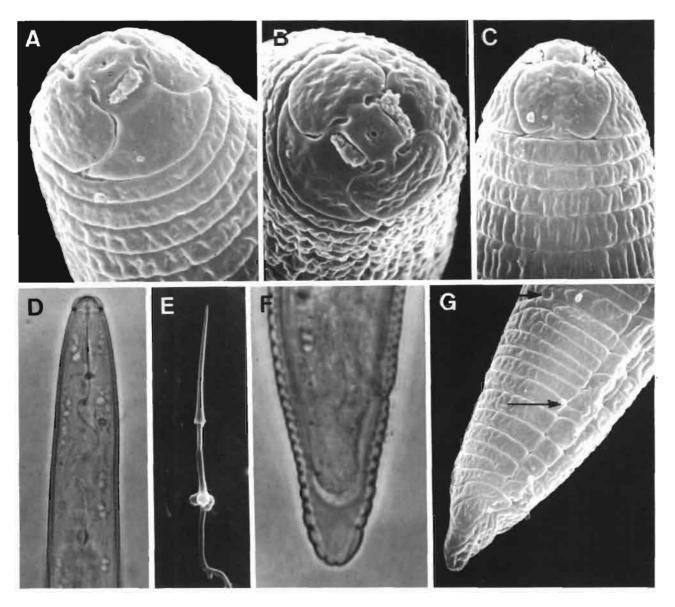


Fig. 5. *Meloidogyne brevicauda* Loos, 1953, second-stage juveniles; light (LM) and scanning electron micrographs (SEM). A-C : SEM of head in nearly lateral, face, and medial views, respectively; D : LM of head in lateral view; E : SEM of excised stylet; F : LM of tail; G : SEM of tail showing lateral field, and anal (upper arrow) and phasmidial opening (lower arrow).

M. brevicauda were very similar to our observations. The labial disc and lips appeared more set-off and the stylet was thinner and more delicate than was illustrated previously. Likewise, the lateral lips were thought to fuse with the medial lips in Figure 1 F of the original description, but SEM revealed that they are separate structures (Fig. 4 C). Also, the crescent-shaped fold posterior to the cloacal opening was not reported by Loos (1953).

The shape of the head of males of *M. brevicauda* is very different from that of most root-knot males (Eisenback & Hirschmann, 1980; Jepson, 1984). The labial disc of most males of *Meloidogyne* is fused with the medial lips, but in *M. brevicauda*, the labial disc is distinctly separated from them by a deep groove. Furthermore, most root-knot males have a large head annule that may be further divided by irregular or incomplete annulations. Males of *M. brevicauda* do not have a head region; the lips are immediately adjacent to the first body annule, similar to that of *Meloinema kerongense* Choi & Geraert, 1974 as observed by Coomans and De Grisse (1981). The lips of males of *Meloidogyne propora* Spaull, 1977 also appear to be adjacent to the first body annule (Spaull, 1977).

The crescent-shaped fold surrounding the cloacal opening posteriorly does not occur in males of the most common species (Eisenback & Hirschmann, 1981; Jepson, 1987). Other species of rootknot known to possess a similar cuticular structure include only *M. nataliei* Golden, Rose, & Bird, 1981 (Golden *et al.*, 1981) and *M. propora* (Spaull, 1977). This fold may be present in other species, but it is only clearly visible by SEM.

Drawings of second-stage juveniles of *M. brevicauda* in the original publication are similar to our LM and SEM observations. The head labial disc is distinctly set-off from the lips, and the lips are clearly illustrated (Loos, 1953). As revealed by SEM, the medial lip pairs are fused to form one dorsal and one ventral lip, and they are distinctly separated from the lateral lips and the labial disc. All of the lips extend posteriorly to the first body annule; whereas in most other second-stage juveniles of root-knot nematodes, a large head annule lies posterior to the lips. This head annule may be smooth, or interrupted by irregular or incomplete annulations (Eisenback & Hirschmann, 1979).

The tail of second-stage juveniles of M. brevicauda is very different from the four common species named above. The very short tail is the character from which the species name was derived. The location of the phasmidial opening on the ventral edge of the lateral field is most unusual because in other root-knot species it generally occurs in the middle of the field.

The morphology of *M. brevicauda* is quite different from four common species and most other species that have been examined closely. Its morphology is similar in many respects to *M. coffeicola* Lordello & Zamith, 1960, *M. indica* Whitehead, 1968, *M. propora*, and *M. nataliei*. Additional studies on the cytology, biology, and hostparasite relationship of *M. brevicauda* are necessary for the proper placement of this species in the genus *Meloidogyne*.

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