

Differentiation with SEM of six species of *Meloidogyne* (Nemata: Heteroderidae) found in Costa Rica

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Abstract: The morphology of one isolate each of *Meloidogyne incognita*, *M. javanica*, *M. hapla*, *M. exigua*, *M. salasi*, and *M. arabicida*, from Costa Rica was studied with the scanning electron microscope. Good characteristics for the differentiation of the six species were found in the labial and cephalic regions of females, males, and second-stage juveniles, but the single most reliable morphological character was the female perineal pattern.

Key words: root-knot nematodes, *Meloidogyne*, SEM, systematics, Costa Rica, morphology.

The wide geographical distribution, wide host range, severe pathogenic effects, and synergistic interactions with other disease-causing agents make *Meloidogyne* Goldi, 1887 (Nemata: Heteroderidae) the most important plant-parasitic nematode genus in Costa Rica (López & Salazar 1989). Currently *M. incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. javanica* (Treub, 1885) Chitwood, 1949; *M. hapla* Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949; *M. exigua* Goldi, 1887; *M. salasi* López, 1984, and *M. arabicida* López & Salazar, 1989 have been identified, but several undescribed forms are known to occur (R. López, unpublished data).

Efforts have been made to gather as much information as possible on the species present in Costa Rica because of their economical importance. Management strategies aimed at reducing the severity of the damage caused by root-knot nematodes include the use of chemicals, crop rotation, resistant cultivars, biological antagonists, and other cultural practices. Most of these tactics require correct species identification for successful implementation. The differentiation of root-knot nematode spe-

cies is facilitated by the scanning electron microscope (SEM), as many of their important morphological characteristics are near the limit of resolution of the light microscope.

Research was initiated to study with the SEM the morphology of all of the described species present in Costa Rica, to find useful differentiating characteristics. Unfortunately, *M. arenaria* could not be included since efforts made to recuperate it from the only location it was found previously, were unsuccessful. The results of this study are presented herein.

MATERIAL AND METHODS

One isolate each of *M. incognita*, *M. javanica*, *M. hapla*, *M. exigua*, and *M. salasi* were selected among several greenhouse cultures; the first three were maintained on tomato (*Lycopersicon esculentum* Mill) and *M. exigua* and *M. salasi* were reproduced on coffee (*Coffea arabica* L.) and rice (*Oryza sativa* L.), respectively. Topotypes of *M. arabicida*, from coffee roots, were included for comparison in this study also. Females, males, and second stage juveniles (J2) were processed for observa-

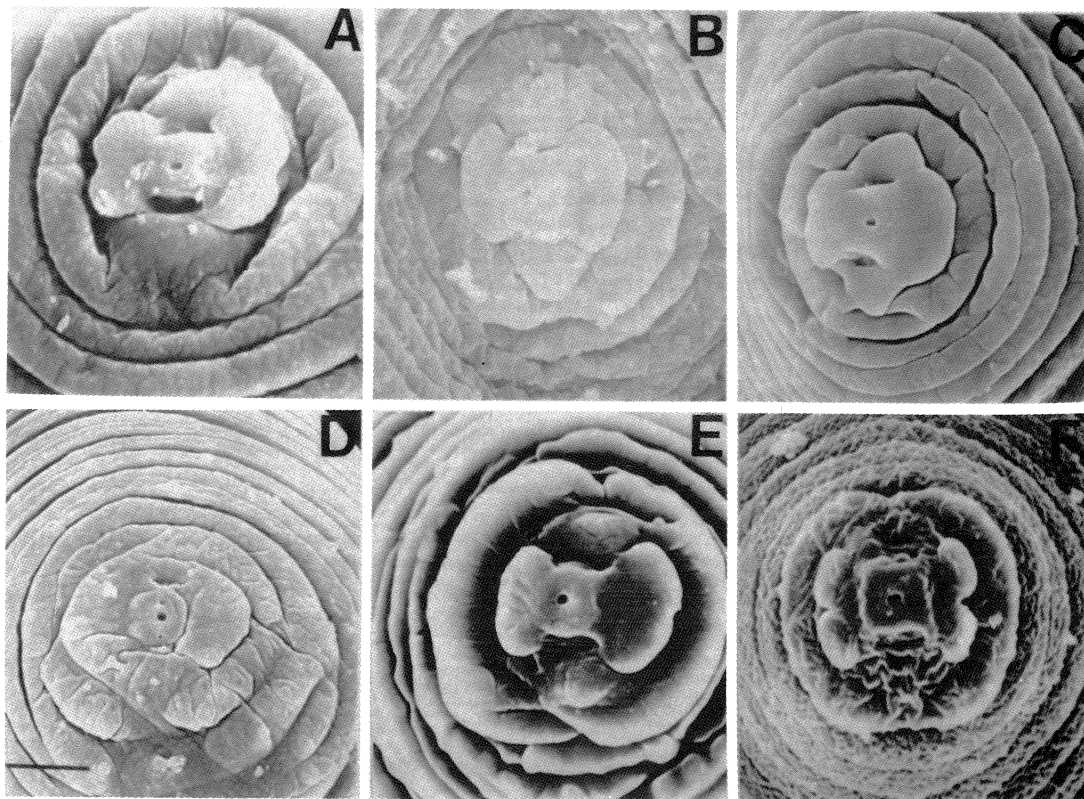


Fig. 1. Photomicrographs of the en face view of females of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E), and *M. arabicida* (F). Scale: 2 μ m.

tion on the SEM by a previously described method (López & Salazar 1990). At least 25 specimens of each life stage for each species were observed. The terminology of Eisenback & Hirschmann (1979, 1980) and Eisenback *et al.* (1990) was used to describe the morphological features.

RESULTS

The female *en face* patterns have common morphological features which include an oval or rounded prestoma located on the center of the labial disc, surrounded by six inner labial sensilla which appear as small, rounded pores, two slit-like amphidial apertures posterior to the lateral edges of the labial disc, and one complete or incomplete annulation. In *M. incognita* (Fig. 1A) the rectangular labial disc has two small protuberances on the ventral side, is slightly raised centrally, and fused with the medial lips which are wider than the labial disc;

together they form a dumbbell-shaped head cap. The medial lips often have a small indentation which divides them in two. The lateral lips are prominent and fused to the head region laterally. In *M. javanica* (Fig. 1B) the labial disc is rounded, slightly raised centrally and fused with the medial lips; two small protuberances occur ventrally. The medial lips are wider than the labial disc, and together they form a dumbbell-shaped head cap. The lateral lips are large and trapezoidal. *M. hapla* (Fig. 1C) is characterized by a rectangular labial disc that is fused with the wider medial lips; the ventral lip is larger than the dorsal lip. Generally they are fused with the triangular lateral lips. *M. exigua* (Fig. 1D) has a small, rounded labial disc that either is fused with the wider medial lips or is separated from them by a short striae. The medial lips often are fused with the lateral lips. In *M. salasi* (Fig. 1E) the rounded labial disc fuses with the lower and wider medial lips. The ventral lip is larger than the labial lip. The

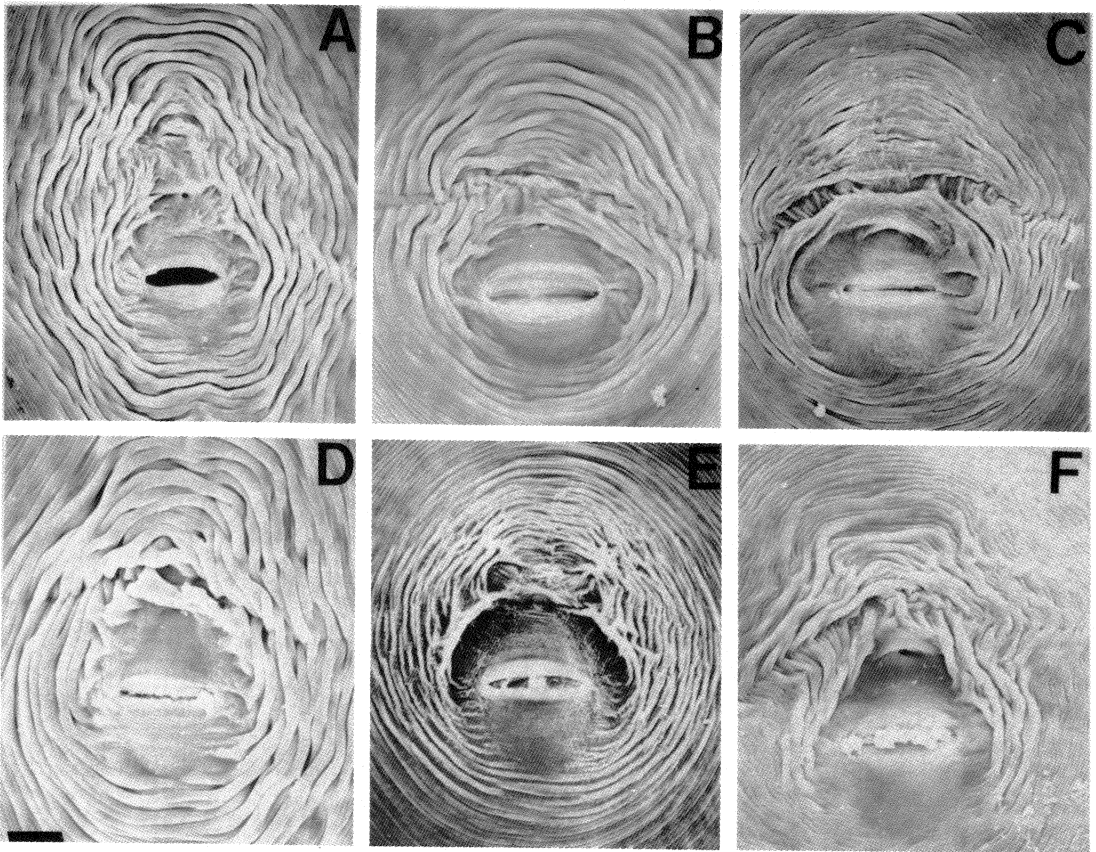


Fig. 2. Female perineal patterns of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E), and *M. arabicida* (F). Scale: 10 μ m.

lateral lips are small and rounded. *M. arabicida* (Fig. 1F) has a square labial disc that is fused with the larger medial lips and forms a dumbbell-shaped head cap. The medial lips have a deep indentation that divides them in two. The lateral lips are fused almost completely with the head region.

Perineal patterns of females are illustrated in Fig. 2. In *M. incognita* (Fig. 2A) the dorsal arch is high and trapezoidal; the striae are relatively coarse, smooth and slightly wavy to zigzag. In *M. javanica* (Fig. 2B) the dorsal arch is rounded and distinct lateral lines divide the dorsal and ventral striae. The striae are coarse, continuous, and often smooth. In *M. hapla* (Fig. 2C) the perineal patterns are oval-shaped, and the striae are closely spaced, fine, and continuous. The dorsal arch is trapezoidal and low. Dorsal and ventral striae are often divided by a deep furrow. In *M. exigua* (Fig. 2D) the perineal pattern is oval-shaped, and the striae

are coarse, continuous, and slightly wavy. In *M. salasi* (Fig. 2E) the patterns are rounded or slightly oval-shaped. The striae are mostly fine, continuous, and smooth. The dorsal arch is wide and squarish. In *M. arabicida* the dorsal arch is high and rectangular, and the striae are coarse on the central and lateral sides, but fine on the proximal portions of the dorsal arch and the vulva. The dorsal and ventral striae extend laterally to form wings in one or both sides.

The *en face* and lateral views of the anterior region of males are illustrated in Figs. 3 and 4, respectively. Features shared by all species include an oval or hexagonally-shaped prestoma that is encircled by six inner labial sensilla located in the center of the labial disc, a slit-like stomatal opening, two medial lips fused with the labial disc and forming a head cap, and two slit-like amphidial apertures posterior to the lateral edges of the labial disc. In most males of *M. incognita* (Fig. 3A,4A) the large

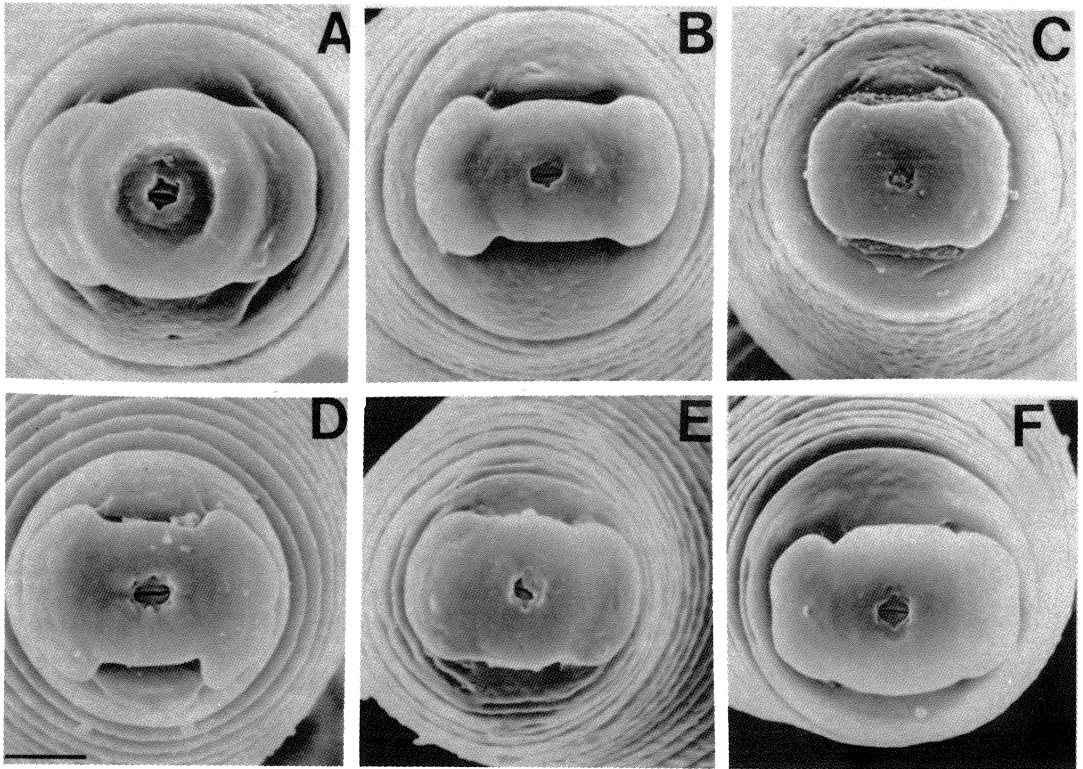


Fig. 3. En face view of males of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E), and *M. arabicida* (F). Scale: 2 μ m.

and round labial disc is concave centrally and is raised above the slightly smaller medial lips. The trapezoidal lateral lips are delimited by short striae that extend from the medial lips to the head region. The head region has a single annule. In *M. javanica* males (Fig. 3B,4B) the labial disc is large but the medial lips are slightly wider. The amphidial apertures are located laterally between the labial disc and the cephalic region. In *M. hapla* males (Fig. 3C,4C) the labial disc is rounded. The medial lips are nearly as wide as the labial disc. The cephalic cap is smaller than the cephalic region. Males of *M. exigua* (Fig. 3D,4D) have an elongate labial disc, with slightly curved lateral edges. The medial lips are wider than the labial disc. The lateral lips are trapezoidal, prominent, and delimited by short striae extending from the medial lips to the cephalic region. *M. salasi* males (Fig. 3E,4E) have a rounded and large labial disc. The medial lips are nearly as wide as the labial disc. The lateral lips are inconspicuous and delimited by very short striae that

extend from the medial lips to the cephalic region. There are one to three rows of short, incomplete annulations at different levels of the cephalic region. In *M. arabicida* males (Fig. 3F,4F) the labial disc is rounded and wider than the medial lips. The lateral lips are not discernible and the cephalic region has a single annule.

The en face and lateral views of the J2 are illustrated in Figs. 5 and 6, respectively. Common morphological characters include an oval-shaped prestoma centrally located on the labial disc and encircled by six inner labial sensilla that appear as small, rounded openings. The labial disc is fused with the wider medial lips and four cephalic sensilla, appearing as two small, rounded depressions on each medial lip, are present. The slit-like amphidial apertures are between the lateral edges of the labial disc and the small lateral lips. The medial lips and the labial disc fuse and form a dumbbell-shaped head cap. The *M. incognita* J2 (Fig. 5A,6A) have a small, rounded labial disc, triangular lateral lips, and one to three irregular and

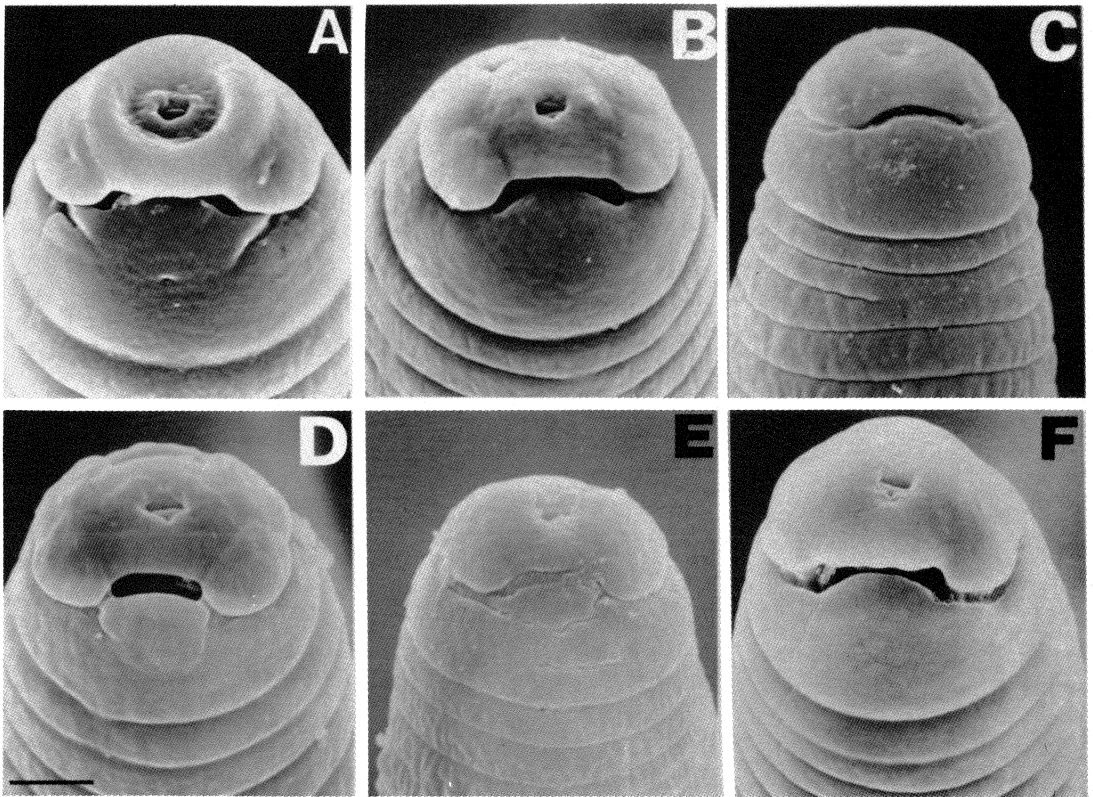


Fig. 4. Lateral view of the cephalic region of males of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E) and *M. arabicida* (F). Scale: 2 μ m.

incomplete striations on the head region. In *M. javanica* (Fig. 5B,6B) the labial disc is oval; the lateral lips are triangular or trapezoidal and the cephalic region is smooth. The J2 of *M. hapla* (Fig. 5C,6C) have a rounded labial disc, slightly arched lateral lips, and a smooth cephalic region. The *M. exigua* J2 (Fig. 5D,6D) have a rounded labial disc, rounded lateral lips, and a cephalic region which is smooth. In J2 of *M. salasi* (Fig. 5E,6E) the labial disc is oval-shaped, the lateral lips are narrow, and the cephalic region is smooth. *M. arabicida* J2 (Fig. 5F,6F) have a rounded labial disc, narrow and slightly rounded lateral lips, and one short, incomplete striation on each lateral sector of the cephalic region.

DISCUSSION

The results of this study confirm previous findings (Eisenback & Hirschmann 1979, Eisenback *et al.* 1980) regarding the usefulness

of the SEM to reveal good morphological characters for differentiating species of root-knot nematodes. Females, males, and J2 of these parasites have the same basic cephalic and labial characters, but their expression is different for each species (Eisenback *et al.* 1980). Accurate species identification is essential for the implementation of an effective nematode management program (Rammah & Hirschmann 1990). Characters that are useful for identification of species must be stable and present in all isolates of a given species, and should not be altered by fixation and preparation techniques. In this study only one isolate of each species was used, so some differences may be attributed to the particular isolate used, but it may not be characteristic for the species.

In females, several differences were evident in the *en face* view, and especially the perineal patterns. Both *M. incognita* and *M. javanica* have two small protuberances on the ventral side of the labial disc; they are absent in the

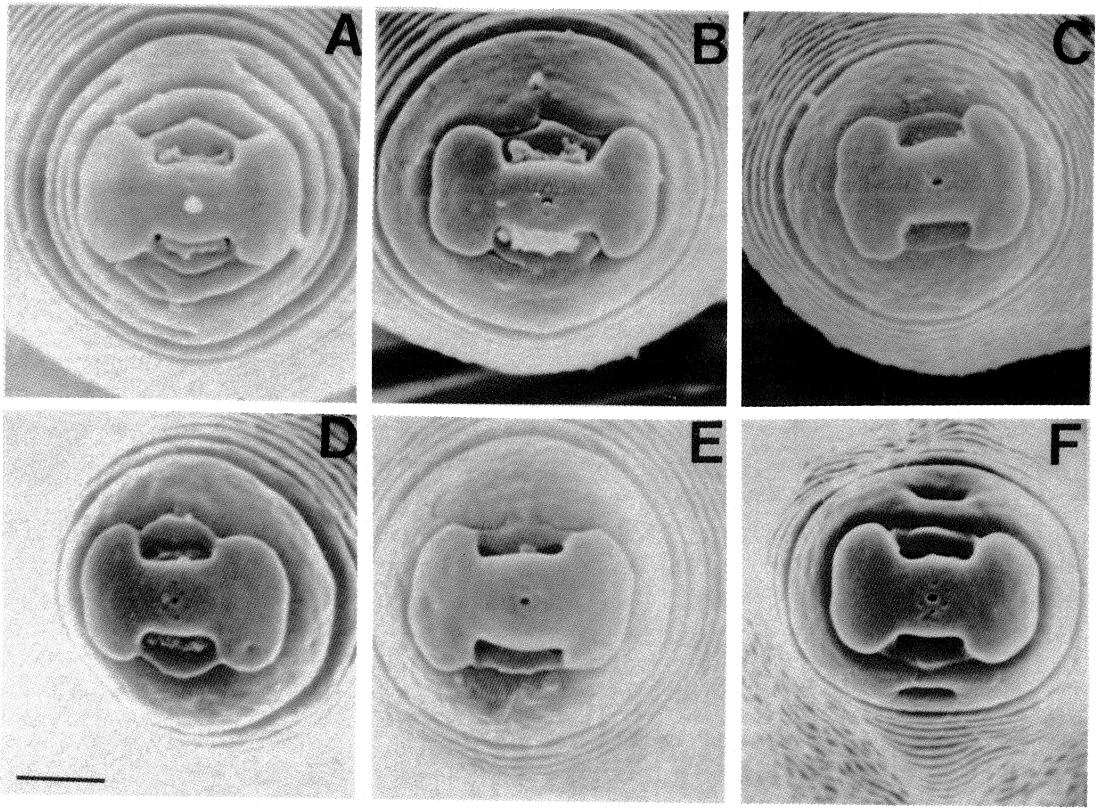


Fig. 5. En face view of second-stage juveniles of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E), and *M. arabicida* (F). Scale: 1 μ m.

other species examined. The labial disc is rectangular (*M. incognita*, *M. hapla*), square (*M. arabicida*) or rounded (*M. javanica*, *M. exigua*, *M. salasi*). The shape, size, and degree of fusion of the lateral lips to the cephalic region differs among the species, except that in *M. arabicida* they are not discernible. The presence of a deep indentation on the medial lips of *M. arabicida* is useful to differentiate it from the other species, except for *M. incognita*. The perineal pattern was the most reliable character for distinguishing females of the species studied. The overall shape of pattern, the shape of the dorsal arch, the morphology of the striae, manifestation of the lateral lines, and the presence of "wings" were all useful morphological features (Fig. 2) for differentiating species. Similar information is obtained with the light microscope (LM) and some characters not seen with the SEM are evident, i.e., the punctations of *M. hapla* (Eisenback *et al.* 1980). These aut-

hors consider information gather with the LM as quite adequate.

This study has demonstrated that cephalic morphology of males provides useful characters for differentiating species (Figs. 3 and 4). The shape of the labial disc, i.e., rounded (*M. incognita*, *M. hapla*, *M. salasi*, *M. arabicida*) or elongate (*M. javanica*, *M. exigua*); its size in relation to that of the medial lips, i.e., about the same (*M. hapla*, *M. salasi*); wider than (*M. incognita*, *M. arabicida*); or smaller than (*M. javanica*, *M. exigua*); and the presence of lateral lips (*M. incognita*, *M. exigua*, *M. salasi*); or absence (*M. javanica*, *M. hapla*, *M. arabicida*) are useful characters. Characteristics such as absence or presence of different numbers of head annulations, and occurrence of a concave labial disc of *M. incognita* males is considered to be variable (Jayaprakash *et al.* 1982). Another useful character is the size of the head cap. It can be as wide as the head region (*M. exigua*);

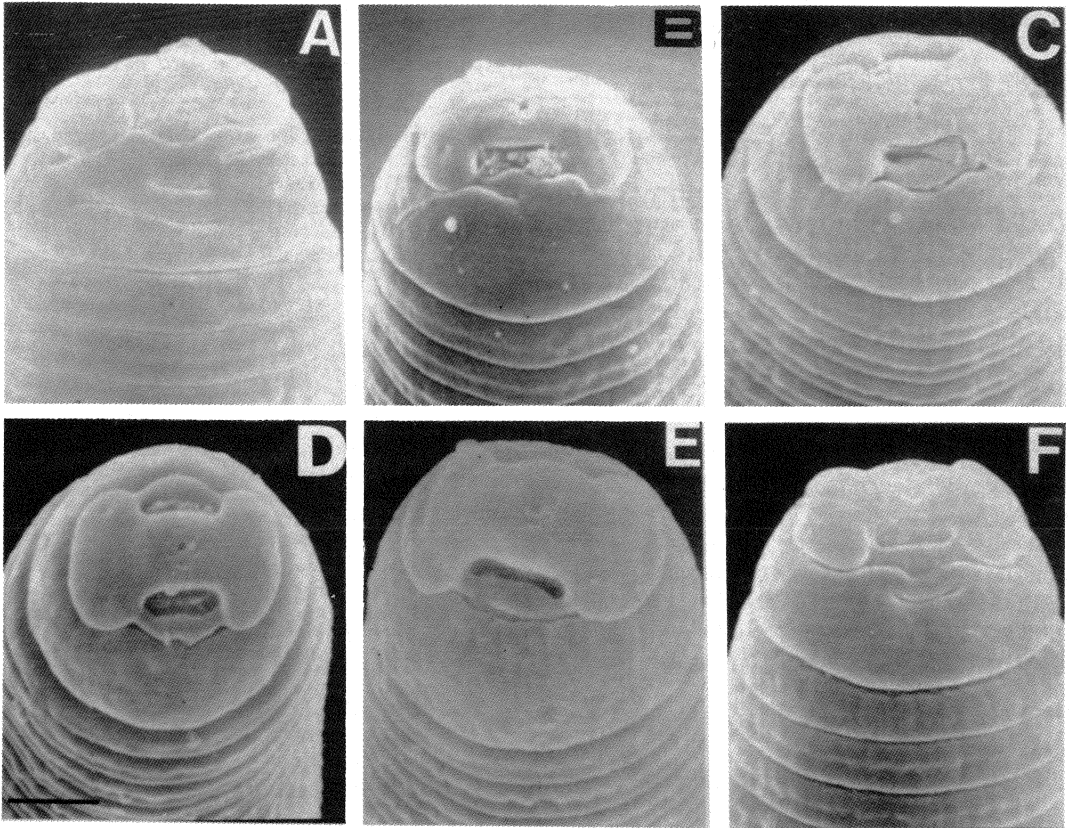


Fig. 6. Photomicrographs of the cephalic region (lateral view) of second-stage juveniles of *Meloidogyne incognita* (A), *M. javanica* (B), *M. hapla* (C), *M. exigua* (D), *M. salasi* (E), and *M. arabicida* (F). Scale: 1 μ m.

almost as wide (*M. incognita*, *M. javanica*, *M. salasi*, *M. arabicida*); or narrower (*M. hapla*). The way the labial disc fuses to the medial lips, as seen in the frontal view, is helpful also. The lateral junctions may have deep indentations (*M. exigua*); they may be shallow (*M. incognita*, *M. javanica*, *M. arabicida*); or they may be absent (*M. hapla*, *M. salasi*).

In J2, as in the other life stages studied, there are some important morphological differences among the species. The labial disc may be rounded (*M. incognita*, *M. hapla*, *M. exigua*, *M. arabicida*) or oval (*M. javanica*, *M. salasi*). The cephalic region may be smooth, with a single annule (*M. javanica*, *M. hapla*, *M. exigua*, *M. salasi*); have one short, incomplete striation on each lateral sector (*M. arabicida*); or from one to three incomplete striations located at different levels (*M. incognita*). The occurrence of striations may vary in some species as has be-

en reported for *M. incognita* (Jayaprakash *et al.* 1982, Okamotoi & Yaegashi 1981) and *M. javanica* (Rammah & Hirschmann 1990).

The shape of the lateral lips, and the expression of the four cephalic sensilla are also variable and are not considered as reliable characters for differentiating these species. Also the shape of the head cap is similar among species and therefore is not a useful character for identification.

In conclusion, differentiation of root-knot nematodes could be accomplished with the SEM alone, particularly when females, males and J2 of a given isolate are available and utilized. However, as pointed out by other authors (Hirschmann 1985, Jepson 1987), it is likely that as many characters as possible from all life stages are necessary for an accurate identification. A combined approach, including LM observations, measurements, cytological and

biochemical characteristics, and host response, may be the best way for the identification of species of *Meloidogyne*.

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RESUMEN

La morfología de un aislamiento de *Meloidogyne incognita*, *M. javanica*, *M. hapla*, *M. exigua*, *M. salasi* y *M. arabicida*, provenientes de varias localidades de Costa Rica, fue estudiada con el microscopio electrónico de rastreo. En las regiones labial y cefálica de hembras, machos y segundos estados juveniles fueron encontradas buenas características para la diferenciación de las especies, pero la más confiable fue el diseño perineal de las hembras.

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