Chapter 6
Taxonomy of Coffee-Parasitic Root-Knot Nematodes, *Meloidogyne* spp.

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Abstract *Meloidogyne* species are characterized primarily on morphological features of females, males and second-stage juveniles. Based on these characters, identifying the 17 coffee-parasitic *Meloidogyne* species is a difficult task even for well-qualified taxonomists. This chapter outlines the most diagnostic morphological and morphometric features for *Meloidogyne* taxonomy, and presents the useful characters for identification of those 17 species. In recent years, esterase phenotyping has become a practical and reliable taxonomic tool for this genus. Unfortunately, only 12 out of the 17 coffee-parasitic species have had their phenotypes characterized; *M. africana, M. decalineata, M. kikuiensis, M. megadora* and *M. oteifae* can only be identified by morphological features. Recently, a new identification tool has been developed: the multiplex PCR (SCAR primers) allows unambiguous differentiation of *M. exigua, M. incognita* and *M. paranaensis* from Brazil, with prospects for extending this method to other species. This chapter concludes by outlining studies and initiatives that should be undertaken to facilitate and improve the reliability of coffee-related *Meloidogyne* taxonomy.

Keywords Morphology · esterase phenotyping · SCAR markers · races · intraspecific variability · distribution

6.1 Introduction

Root-knot nematodes (RKNs) are classified in the genus *Meloidogyne*, which was established by Göldi (1887) and includes 17 coffee-parasitic valid species. *Meloidogyne* species are characterized primarily on morphological features of females, particularly the perineal pattern. Features of males and second-stage juveniles (J2) are complementary. Nonetheless, reliable identification of *Meloidogyne* species based on morphology is a formidable task, even for well qualified taxonomists with expertise in the genus.

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In most RKN-surveys conducted in coffee (*Coffea* sp.) plantations and nurseries worldwide (summarized by Campos and Villain, 2005), the perineal pattern was the main taxonomic feature used for species identification. Nonetheless, species identification based exclusively on this feature is difficult and uncertain for some coffee-parasitic populations, since it requires observation and subjective judgment of morphological aspects and comparison with figures presented in the species' original descriptions. Furthermore, different species may have similar perineal patterns; such is the case for *M. paranaensis* Carneiro, Carneiro, Abrantes, Santos and Almeida, *M. konaensis* Eisenback, Bernard and Schmitt, *M. izalcoensis* Carneiro, Almeida, Gomes and Hernandez, *M. inornata* Lordello and *M. mayaguensis* Rammah and Hirschmann, whose perineal patterns are similar to *M. incognita* (Kofoid and White) Chitwood.

Therefore, cases of misidentification are probably numerous. For example, reports of coffee-parasitic *M. incognita* populations in Guatemala and El Salvador, which had been based on perineal patterning, should be regarded with caution because recent surveys conducted in those countries, with the aid of enzyme phenotyping, have not detected *M. incognita*; instead, *M. paranaensis* and *M. izalcoensis* have been found (Carneiro et al., 2004; 2005b).

Conversely, perineal patterning can be a complementary tool for taxonomy based on enzyme phenotyping and other biochemical or molecular methods. Indeed, species-specific esterase phenotypes have been characterized for 12 of the 17 coffee-parasitic *Meloidogyne* species. Furthermore, Randig et al. (2002) have developed a polymerase chain reaction (PCR)-based assay to identify RKNs associated with coffee in Brazil. Three RAPD markers have been transformed into sequence-characterized amplified region (SCAR) markers, which are specific for *M. exigua* Göldi, *M. incognita* and *M. paranaensis*. Currently, only five coffee-parasitic *Meloidogyne* species from Africa have not had their enzymatic phenotypes characterized; for these species, identification remains based on morphological features only.

This chapter aims to assist nematologists, plant pathologists and other scientists in identifying the 17 coffee-parasitic *Meloidogyne* species. Initially, the basic RKN-morphology is presented, and the taxonomic reliability of several morphological and morphometric features is discussed. The diagnostic features for each of the 17 species are presented, as well as drawings from their original descriptions (some of them have been published without scale bars). Advances in biochemical and molecular taxonomy are outlined as well.

6.2 Morphology and Morphometry in *Meloidogyne* Taxonomy

Because of the morphological and morphometric similarities between *Meloidogyne* species, the most appropriate approach is to ponder a combination of differential characters of nematode females, males and J2.

Females (L = 380 − 1348 μm) are pear-shaped to spheroid, with a short (see *M. kikuyensis* de Grisse) to elongated (see *M. coffeicola* Lordello and Zamith) neck.

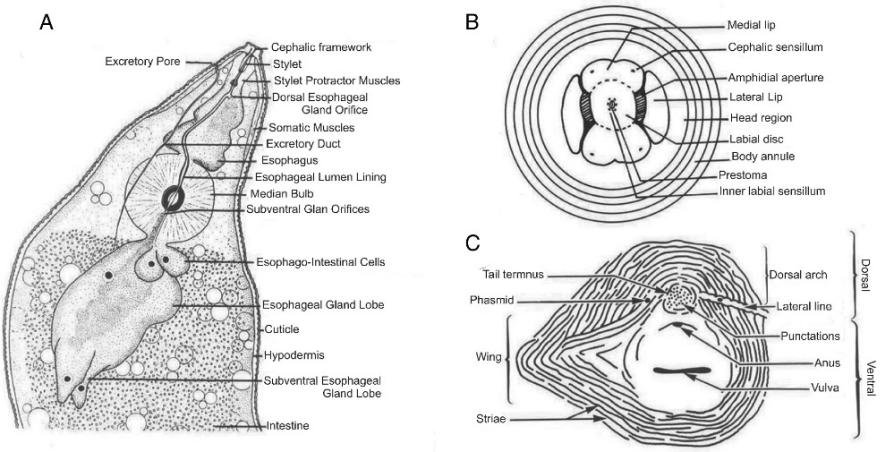

Fig. 6.1 Female morphology of root-knot nematodes (*Meloidogyne* sp.). (**A**) Anterior region. (**B**) Head morphology as revealed by SEM, in face view. (**C**) Perineal pattern (from Eisenback and Triantaphyllou, 1991, with permission)

Their body is white and not transformed into a cyst-like structure upon death. The cephalic region ('head') presents a cuticular framework (Fig. 6.1A), a labial region with six lips, median lips fused into two pairs, and one asymmetrical or symmetrical postlabial annule. The amphid apertures are slit-like (Fig. 6.1B). The stylet is robust (10–25 μm long), with three basal knobs. The positioning of the dorsal oesophageal gland orifice in relation to the base of the stylet knobs (DEGO position) is about 2–10 μm, but this character is variable within populations and species. The excretory pore is located anterior to the median bulb, usually 15–25 annules posterior to the lip region; nonetheless, this positioning varies a lot within and among *Meloidogyne* species, which makes it a poor diagnostic character. The oesophageal glands are usually five-lobed, and they overlap the intestine. The body cuticle presents simple cross annulations, which form a variable, somewhat circular pattern around the vulva and anus, which is called the perineal pattern (Fig. 6.1C). The phasmids are situated on either side of and dorsal to the anus. The eggs are not retained in the body; instead, they are deposited in a gelatinous matrix which is extruded through the anus. The females are usually endoparasitic, inducing the formation of galls ('knots') on the roots of most hosts. A more detailed morphological description of RKNs can be read in Jepson (1987) and Eisenback and Triantaphyllou (1991).

Males are vermiform, with their length (700–2,000 μm) varying according to the environmental conditions during their development. Therefore, the character body length and morphometric ratios relating it to oesophagus and tail lengths or body width are nearly useless for taxonomy. The head (Fig. 6.2A,C) presents a labial cap with six lips, and the median lips are more or less fused into two pairs, assuming a dumb-bell shape; these features provide several good diagnostic features. The amphid apertures are slit-like, conspicuous, leading to broad pouches in the lateral lips. Usually there is only a single postlabial annule, although additional, incomplete

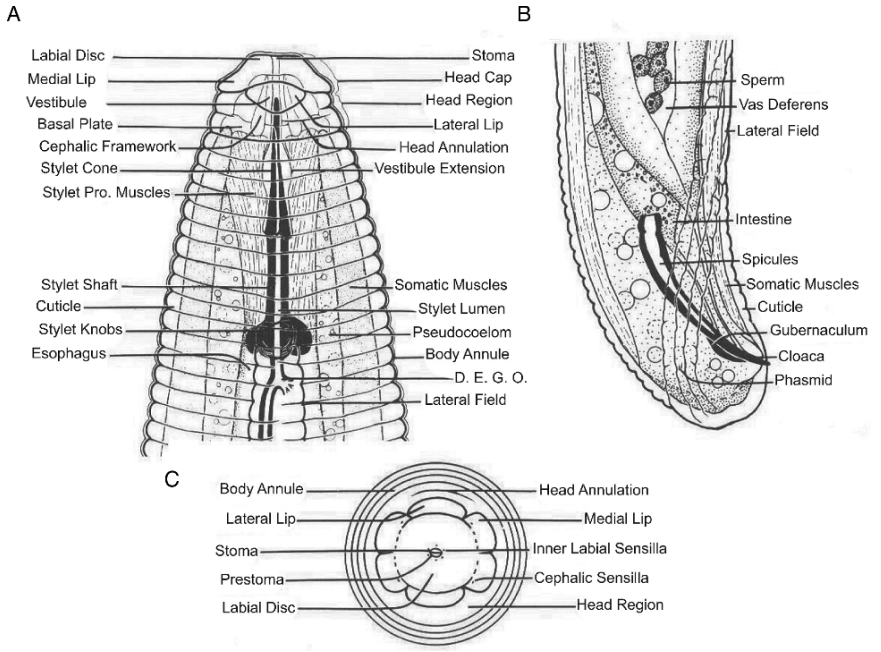

Fig. 6.2 Male morphology of root-knot nematodes (*Meloidogyne* sp.). (**A,C**) Anterior region in lateral and face views, respectively. (**B**) Posterior region (from Eisenback and Triantaphyllou, 1991, with permission)

annules can be present, which can be used to distinguish species and populations. The stylet has well-developed basal knobs; the stylet can be 13–30 μm long, although most *Meloidogyne* species have it in the range of 18–24 μm; this character presents a coefficient of variability of only 4%, which makes it a good character to differentiate species. The size and shape of the stylet cone, shaft and knobs are also excellent supporting characters for species identification. Males have a strong cephalic framework. The DEGO position is 2–13 μm; in general, this character exhibits much variation, although some species can be distinguished from it. The position of the excretory pore varies widely within species, being of limited value as a differential character. The hemizonid is usually located anterior to the excretory pore; thus, its positioning may help in identifying species that have it posterior to the excretory pore. Normal males present one gonad, whereas sex-reversed males have two. Males have gubernaculum (Fig. 6.2B). Spicule length ranges from 19 to 40 μm across the genus, with much overlap in its length among species. Slight differences in spicule structure have been described for some species, but in general spicule morphology is not of diagnostic value. The male tail is bluntly rounded and short, with little variation among the species.

Second-stage juveniles vary in body length from 290 to 912 μm across the genus. In many species this character ranges from 300 to 500 μm, which makes it inadequate for species identification. Due to J2's small size, discerning details precisely

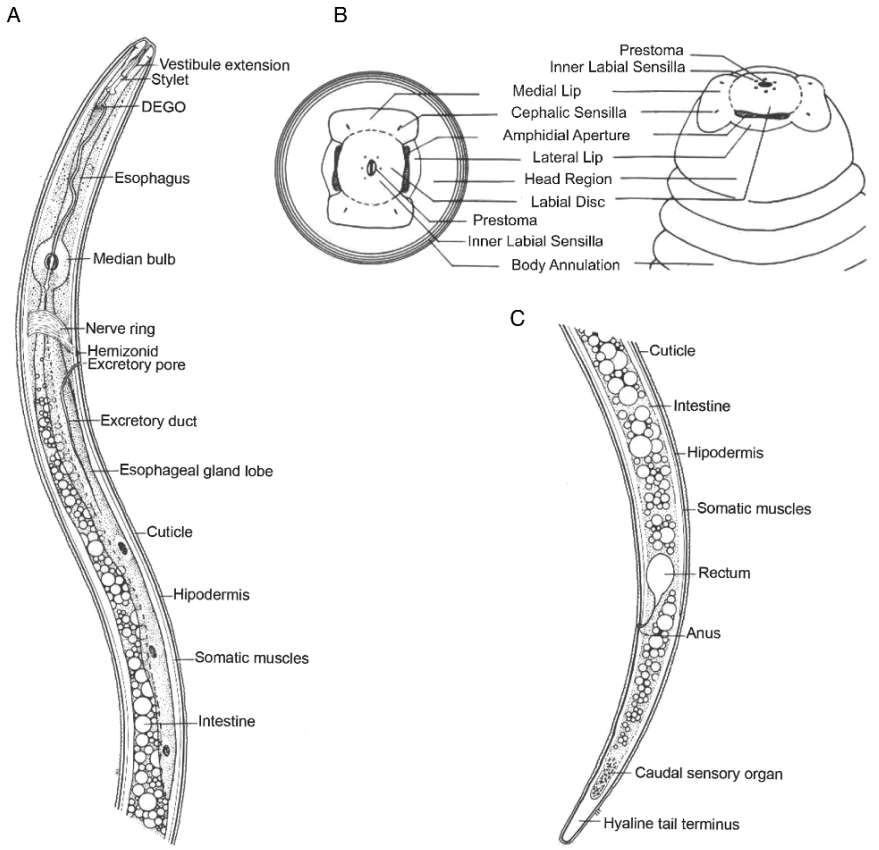

Fig. 6.3 J2 morphology of root-knot nematodes (*Meloidogyne* sp.). (**A,B**) Anterior region in lateral and face views, respectively. (**C**) Posterior region (from Eisenback and Triantaphyllou, 1991, with permission)

in the nematode's head (Fig. 6.3A,B) can only be done with the aid of a scanning electron microscope. Furthermore, head morphology is quite similar among most species, although some differ in the shape of the labial disk, details in the lateral and medial lips, format, size and positioning of labial and cephalic sensilla, and presence of head annulations. Second-stage juveniles have a delicate stylet that ranges in length from 8 to 18 μm across the genus. This character shows low variability among species, although it may be helpful in identifying certain species. The DEGO position is 2–8 μm, and it seems a good differentiating feature, with groups of species being distinguished based on it. The position of the excretory pore is variable. Hemizonid positioning can be a fairly useful diagnostic feature in those species in which it is located posterior to the excretory pore. Tail length varies considerably among species, from 15 to 100 μm. Due to its small intraspecific variation, it is a very useful measurement. In J2, the tail ends in a hyaline terminus (Fig. 6.3C),

which can be considered to identify those species in which it is always short or long. Whitehead (1968) and Jepson (1987) have grouped *Meloidogyne* species according to J2 tail lengths and shapes. The latter author has also stated that differences in tail measurements from populations of a single species can be larger than between species. Nevertheless, differences in mean tail length and/or mean length of the tail's hyaline terminus are large enough to distinguish species in groups.

6.3 The Status of Coffee-Parasitic *Meloidogyne* Taxonomy

Meloidogyne sp. comprises more than 90 species. Nineteen have been associated with coffee in many countries worldwide, including very damaging ones that cause great losses to coffee growers and to the economy of developing countries.

In this review 17 species are recognized as valid (see below). *M. thamesi* (Chitwood in Chitwood et al.) Goodey has been synonimized to *M. arenaria* (Neal) Chitwood by Jepson (1987), and confirmed by Eisenback and Triantaphyllou (1991). These authors have also synonymized *M. inornata* to *M. incognita*, but the former has been revalidated by Carneiro et al. (2008).

M. göldii has been described by Santos in his DS thesis (1997); nonetheless, this species' description and diagnosis have never been published. According to the International Code for Zoological Nomenclature, any publication that mentions *M. göldii* Santos, 1997 should refer to it as a *nomen nudum*.

6.3.1 Nominal List of Coffee-Parasitic Meloidogyne *Species*

6.3.1.1 Valid Species

M. exigua Göldi, 1887, type species
M. africana Whitehead, 1960
M. arabicida López, 1989
M. arenaria (Neal, 1889) Chitwood, 1949
  Syn. *M. thamesi* (Chitwood in Chitwood et al., 1952) Goodey, 1963
M. coffeicola Lordello and Zamith, 1960
M. decalineata Whitehead, 1968
M. hapla Chitwood, 1949
M. incognita (Kofoid and White, 1919) Chitwood, 1949
M. inornata Lordello, 1956
M. izalcoensis Carneiro, Almeida, Gomes and Hernandez, 2005
M. javanica (Treub, 1885) Chitwood, 1949
M. kikuyensis de Grisse, 1960
M. konaensis Eisenback, Bernard and Schmitt, 1994
M. mayaguensis Rammah and Hirschmann, 1988
M. megadora Whitehead, 1968
M. oteifae Elmiligy, 1968
M. paranaensis Carneiro, Carneiro, Abrantes, Santos and Almeida, 1996

6.3.1.2 Nomen Nudum

M. göldii Santos, 1997

6.4 Diagnostic Features and Distribution of Coffee-Parasitic *Meloidogyne* Species

6.4.1 M. exigua

The females are small (L = 387.5 − 496 μm), being characterized by the perineal pattern round to hexagonal, with the dorsal arch varying from low and rounded to somewhat high and squarish, with striae coarse and widely spaced (Fig. 6.4K, L, M). In the perineal pattern, the lateral fields are usually inconspicuous and only indistinctly forked; however, the inner lateral line regions may have coarse, raised, looped, and folded striae which also cover the anus (Chitwood, 1949; Lordello and Zamith, 1958; Cain, 1974; Jepson, 1987). The female stylet is 12–14 μm long, its shaft is cylindrical, but occasionally it narrows at the junction with the knobs. The DEGO position is usually 4–8 μm (Fig. 6.4F). In males, the head contour accompanies the contour of the body's first cuticle annules, thus being called not offset (Fig. 6.4A). The medial lips are often divided medially by a shallow groove. Stylets are 18–20 μm long; the shaft is straight and cylindrical, and it narrows at the junction with the knobs. The DEGO position is variable (3–5 μm). In J2, the moderately long tail (44–46 μm) ends in a bluntly rounded tip (Fig. 6.4I). A few narrow constricting annulations close to the tail terminus are typical of this species (Eisenback and Triantaphyllou, 1991). Although *M. exigua* populations are very similar morphologically (Lima and Ferraz, 1985), recent molecular studies have showed a high genetic variability among coffee-parasitic populations (Muniz et al., 2008).

M. exigua can be distinguished by its esterase phenotypes (Est E1 and E2, Fig. 6.21) (Carneiro et al., 2000; 2005b) and PCR-SCAR markers (Randig et al., 2002; 2004). It reproduces by meiotic parthenogenesis, with haploid chromosomal number (n) equal to 18 (Tryantaphyllou, 1985).

Coffee-parasitic populations of *M. exigua* have been reported from Brazil, Guatemala, Dominican Republic, Nicaragua, Costa Rica, Puerto Rico, Colombia, Peru, El Salvador, Venezuela, Bolivia, Honduras and Panama (Campos and Villain, 2005).

6.4.2 M. africana

Females are 660–910 μm long, and present a typical perineal pattern which is roughly circular, without punctations (Fig. 6.5B). The dorsal arch is low and the phasmids are located close to the wide tail terminus, which is often marked by short disordered striae. The wide lateral fields are unmarked by incisures, but they present tiny, disordered striae. The female stylet is 15 μm long and the DEGO position is

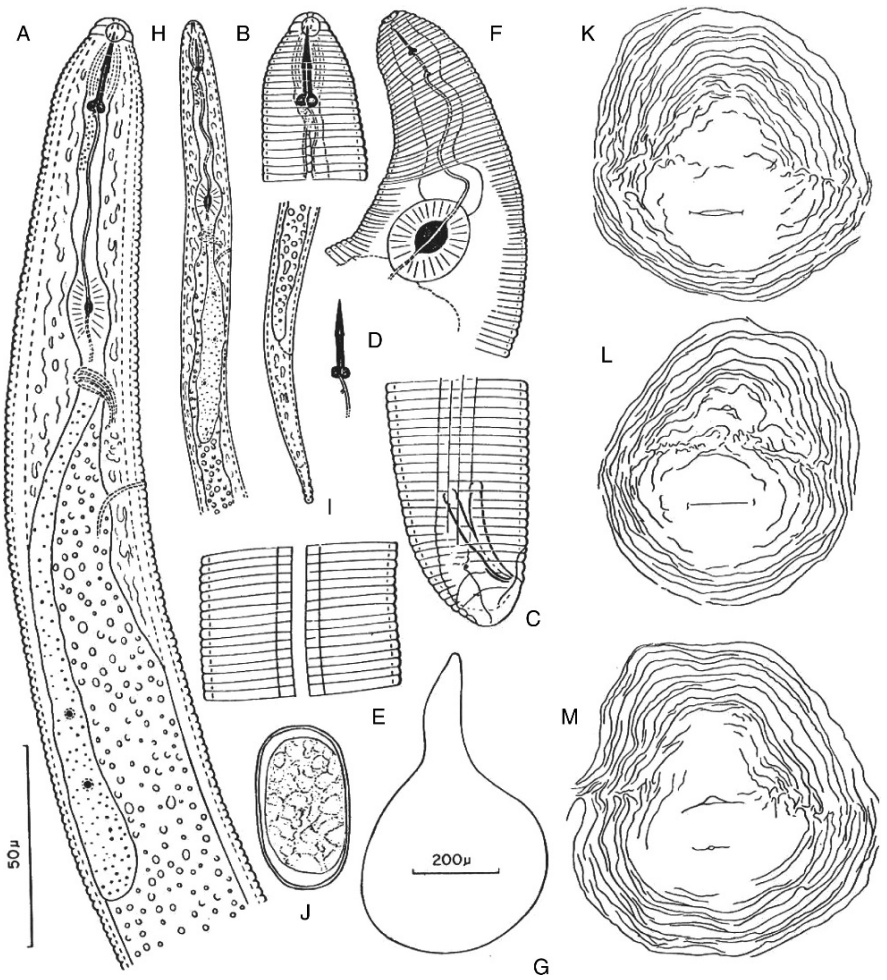

Fig. 6.4 *Meloidogyne exigua*. (**A–E**) Male anterior and posterior regions, stylet and lateral field, respectively. (**F,G**) Female anterior region and body shape. (**H,I**) J_2 anterior and posterior regions, respectively. (**J**) Egg. (**K–M**): Perineal patterns (from Lordello and Zamith, 1958, with permission)

4–9 µm. Males are 1,200–1,850 µm long, presenting one head annule behind the head cap; their stylet knobs are spherical and prominent (Fig. 6.5C,D). In males, the stylet is 19–22 µm, and the DEGO position is 4–6 µm. The spicules have a medial flange; the gubernaculum is crescent in lateral view (Fig. 6.5E). The J2 are 380–470 µm long and their stylet measures 12–18 µm; they present a fairly broad tail (Fig. 6.5A), which gradually tapers to a blunt, rounded terminus, generally without any cuticular constrictions in the hyaline region; instead, their tail presents fine striae extending close to the tail terminus (Whitehead, 1960; Jepson, 1987).

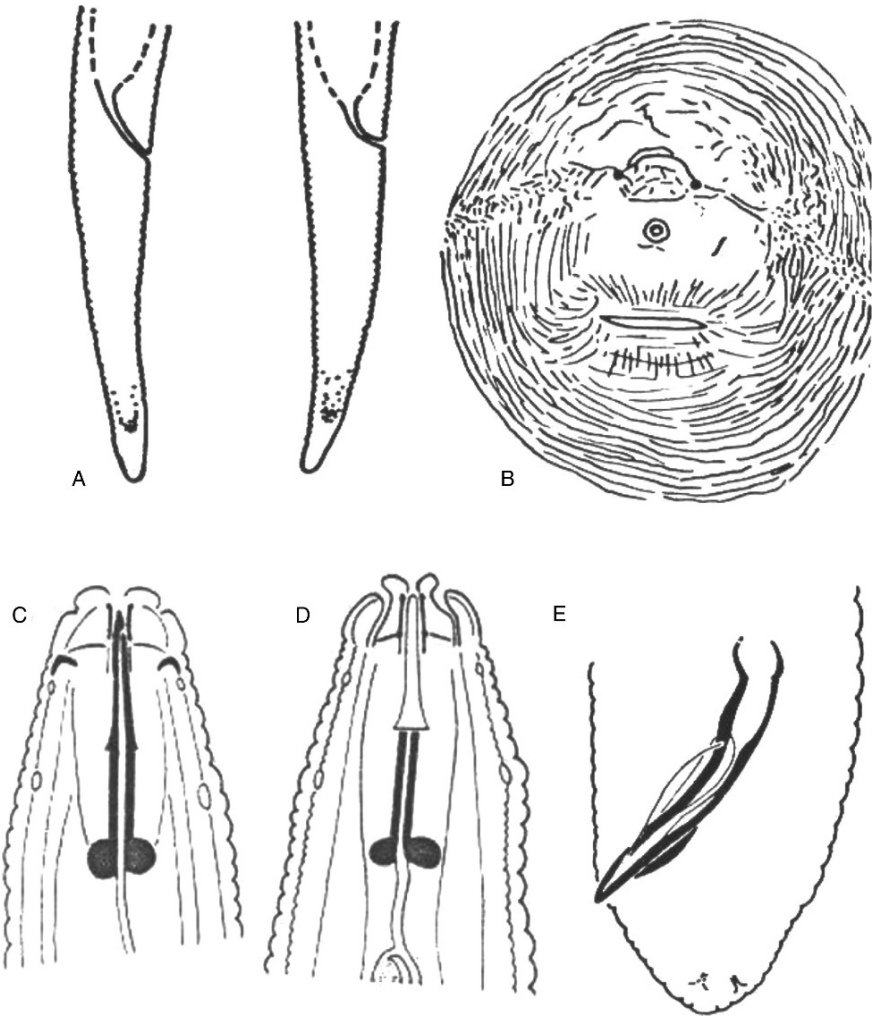

Fig. 6.5 *Meloidogyne africana*. (**A**) J_2 tails. (**B**) Perineal pattern. (**C,D**) Male anterior region. (**E**) Male posterior region (from Whitehead, 1968, with permission)

No esterase phenotype has been characterized for this species; its mode of reproduction and chromosome number are not known. On coffee, *M. africana* is known to occur in Kenya and Zaire (Campos and Villain, 2005).

6.4.3 M. arabicida

This species presents females 543–1,206 μm long, whose perineal pattern is very peculiar: it shows relatively angular contours with thick striae in the center and

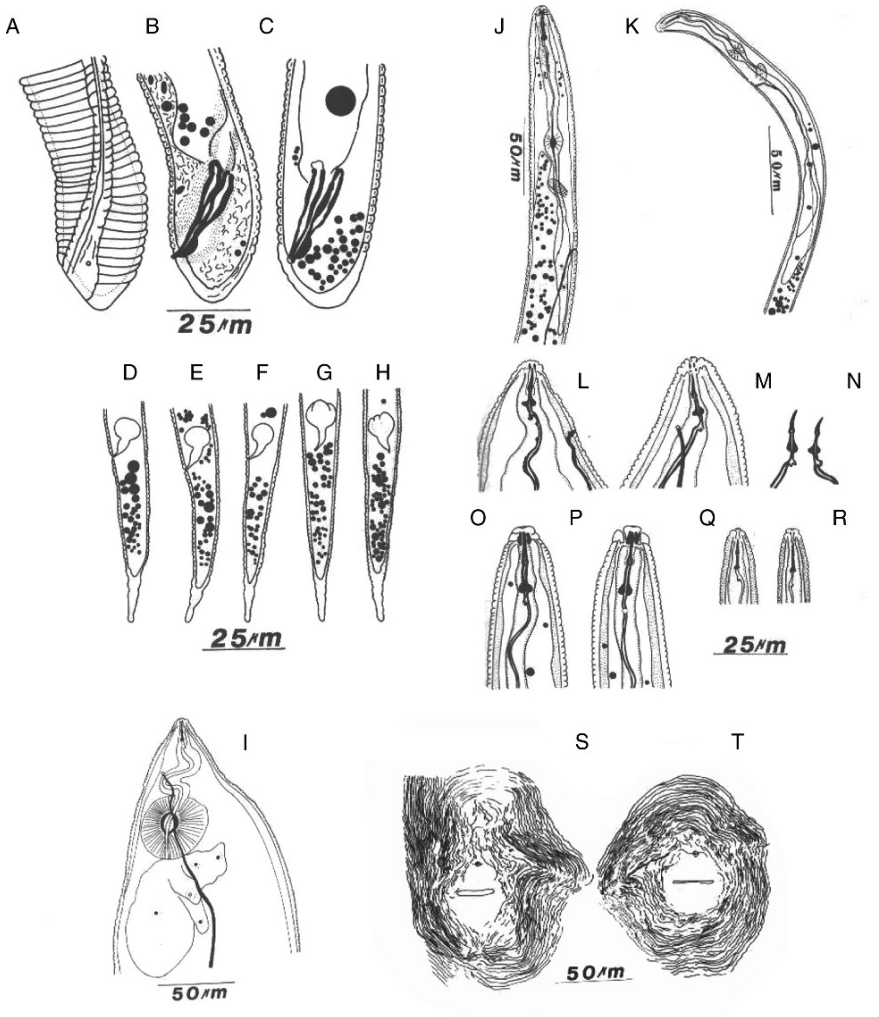

Fig. 6.6 *Meloidogyne arabicida*. (**A–C**) Male posterior end. (**D–H**) J_2 tails. (**J,K,Q,R**) J_2 anterior region. (**l,L,M**) Female anterior region. (**N**) Female stylet. (**O,P**) Male anterior region. (**S,T**) Perineal patterns. (from Lopez and Salazar, 1989, with permission)

thinner ones on the periphery; the dorsal arch is relatively high and rectangular (Fig. 6.6S,T). Most patterns have striae lateral projections ('wings'), which can be present on both sides or on just one. The vulva is elongated and smooth, without prominent striae originating from it. The female medial labial lips are separated by a small indentation in the center. Males are 905–1,881 µm long, with a smooth head region presenting just one annule ring (Fig. 6.6O,P) and areolated lateral fields (Fig. 6.6A). The 372–480 µm long J2 have a smooth head region with narrow lateral lips, slightly arcuate; one relatively short, incomplete striae is found in the lateral

area of the head region; the J2 present a dilated rectum (Fig. 6.6D–H) (López and Salazar, 1989).

This species can be diagnosed by its esterase phenotype (Est AR2, Fig. 6.21) (Carneiro et al., 2004; Hernandez et al., 2004). Its mode of reproduction and chromosome number are unknown. On coffee, *M. arabicida* has been reported from Costa Rica (Campos and Villain, 2005).

6.4.4 M. arenaria

This species is characterized by its female (510–1,000 μm long) perineal pattern, which is flattened to rounded (Fig. 6.7F). The striae in the arch are slightly indented at the lateral lines; often the dorsal and ventral striae meet at an angle at the lateral lines, and generally form a 'shoulder' on the arch. Some striae fork and are short and irregular near the lateral lines. The striae are smooth to wavy and some may bend towards the vulva. The pattern may also have striae that extend laterally to form one or two 'wings'. Some populations of *M. arenaria* present variant females which present perineal pattern similar to *M. incognita*'s. *M. arenaria* females have unique stylets: in general, their stylet is very robust, 13–17 μm long; the DEGO position is 3–7 μm (Fig. 6.7D,E). Stylet cone and shaft are broad. The shaft increases in width posteriorly and gradually merges with the stylet knobs; these are wide and rounded posteriorly. The males' head region is low and slopes posteriorly. It forms a smooth and continuous structure that is almost as wide as the head region (Fig. 6.7A,B). Two or three incomplete annulations are present on the head region. The stylet is 20–25 μm long, with the posterior portion of its cone much wider than the anterior portion of its shaft. The shaft is generally cylindrical, and it gradually merges with the very large stylet knobs. Typically, the J2 (398–605 μm long) present no annulations in the head region, although some specimens may have two or three annulations. The tail (44–69 μm long) is narrow, tapering to a subacute terminus (Fig. 6.7H).

M. arenaria can be distinguished by its esterase phenotypes (Est A2 and A3, Fig. 6.21) (Carneiro et al., 2000; 2004) and PCR-SCAR markers (Zijlstra et al., 2000a). It reproduces by mitotic parthenogenesis, with 36, 45 or 51–56 chromosomes. Coffee-parasitic *M. arenaria* populations have been found in Jamaica, Cuba and El Salvador (Campos and Villain, 2005).

6.4.5 M. coffeicola

This species is diagnosed by its brownish, very elongated females (992–1,348 μm), which have long necks (Fig. 6.8F). The stylet is 15.3–17.6 μm long and the DEGO position is 3.8–4.6 μm. The characteristic perineal pattern shows a low arch, which has very faint striae closely spaced, smooth to slightly wavy in the dorsal sector (Fig. 6.8G). Close to its tip, the tail is rather wide, being marked by faint striae

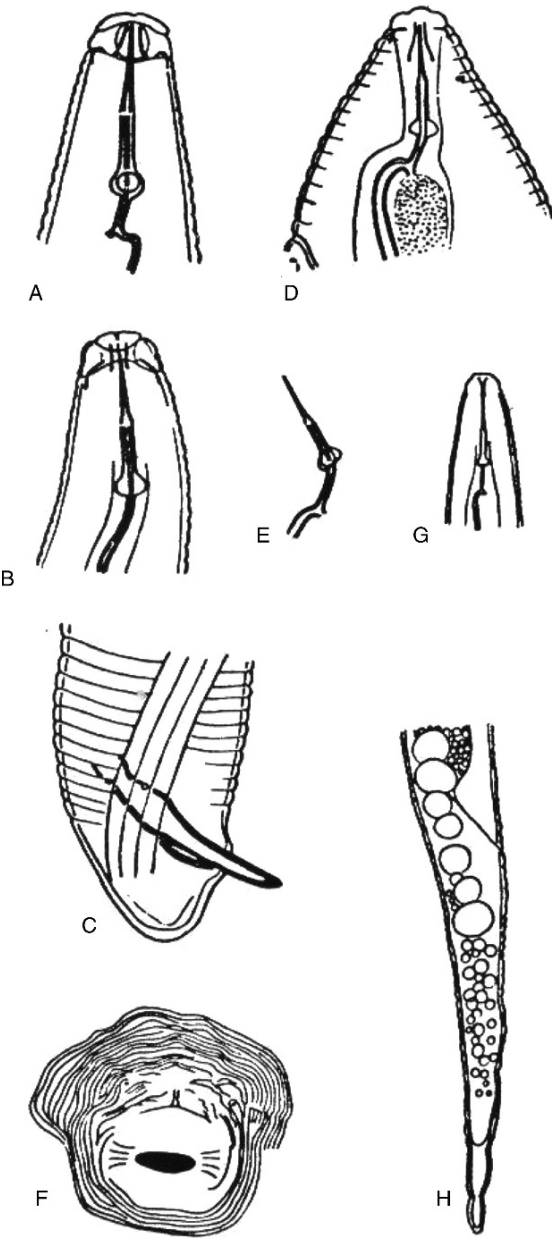

Fig. 6.7 *Meloidogyne arenaria*. (**A–C**) Male anterior and posterior regions. (**D**) Female anterior region. (**E**) Female stylet. (**F**) Perineal pattern. (**G**) J_2 anterior region. (**H**) J_2 posterior end (from Chitwood, 1949, with permission)

and surrounded by concentric circles; the phasmids are located close to the tail tip. The perineal pattern's lateral fields are very poorly defined; in some specimens, it is marked only by slight irregularities in the striae. Males ($L = 1,279 - 1,595\,\mu m$) present four aerolated lateral field incisures (Fig. 6.8D); the head is cupolate, and its contour extends beyond the body's contour (offset) (Fig. 6.8A), having one annule

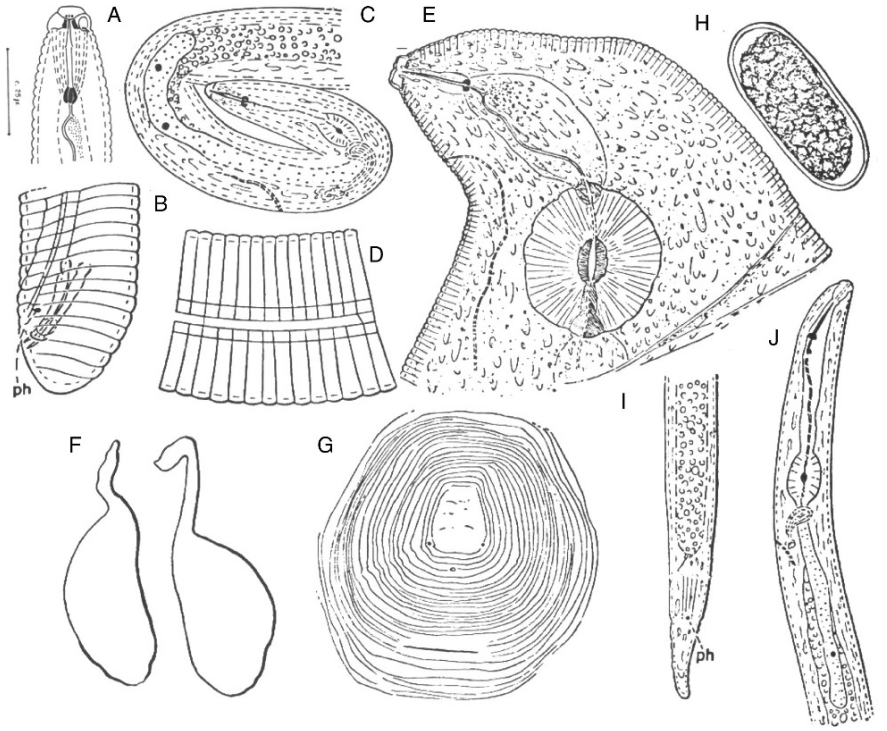

Fig. 6.8 *Meloidogyne coffeicola*. (**A–D)** Male. (**E**) Female anterior region. (**F**) Female body shapes. (**G**) Perineal pattern. (**H**) Egg. (**I,J**) J2 posterior and anterior regions, respectively. ph = phasmid (from Lordello and Zamith, 1960, with permission)

behind the head cap. The stylet knobs are longitudinally ovoid, not prominent. Male stylet length is 23–26 μm and the DEGO position is 3.8–4.6 μm. Phasmids are located before the cloaca (Fig. 6.8B). The J2 (L = 336.6 − 423.8 μm) present 9.2–10.7 μm long stylets, with weak, ovoid knobs; their tail is fairly short (29.1–33.6 μm) and bluntly rounded (Fig. 6.8I).

Care should be taken to differentiate *M. coffeicola* from *M. decalineata*, because these species may present similar perineal patterns. *M. decalineata* has smaller females (L = 649 − 1, 041 μm); males and J2 of these species are quite distinct (Whitehead, 1968).

M. coffeicola may be characterized by its esterase phenotype (Est C2, Fig. 6.21) (Carneiro et al., 2000). Its mode of reproduction and chromosome number are unknown. This species has only been reported in Brazil (Campos and Villain, 2005).

6.4.6 M. decalineata

This species is characterized by the length of female body (649–1,041 μm) and stylet (12–17 μm); the DEGO position is 3–4 μm. It also has a peculiar perineal

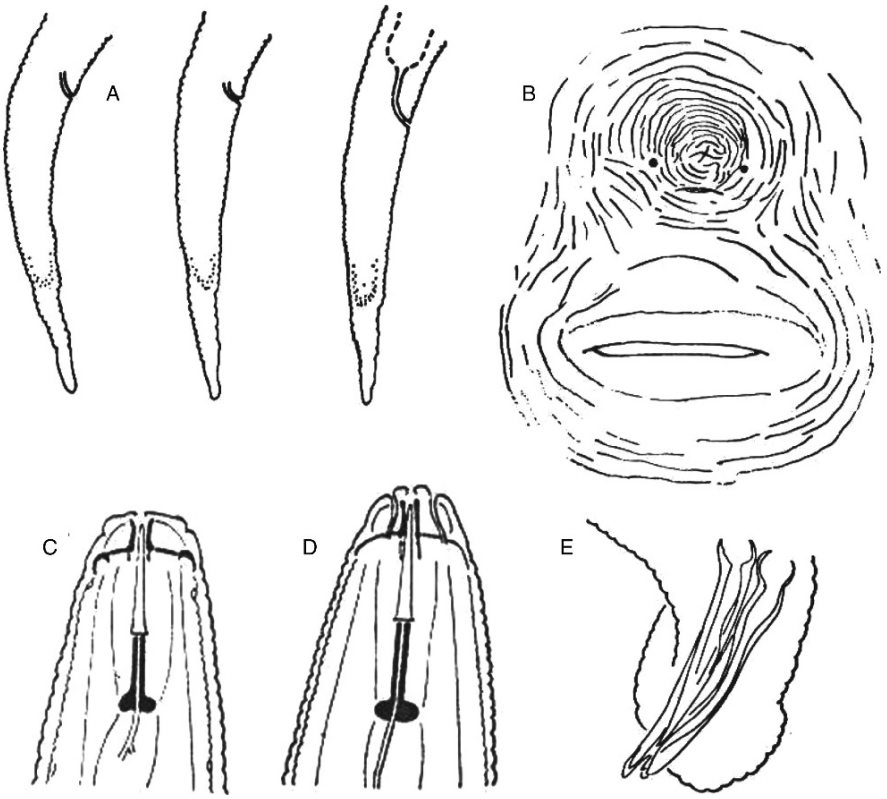

Fig. 6.9 *Meloidogyne decalineata*. (**A**) J_2 tails. (**B**) Perineal pattern. (**C,D**) Male anterior region. (**E**) Male posterior end (from Whitehead, 1968, with permission)

pattern, which shows striae fairly close and evenly spaced, which are often broken, especially at the lateral sides of the pattern (Fig. 6.9B). A distinct tail whorl is present, fairly distant from the vulva; the tail terminus is marked by short, disordered striae; numerous striae can be seen between the tail whorl and the vulva. Rudimentary lateral fields can be seen in some patterns, occasionally with minute disordered striae within the fields. Phasmids are located close to tail terminus. The body cuticle is often folded in the pattern's ventral region. Males are 649–1,041 μm long; their stylet is 12–17 μm long and the DEGO position is 3–4 μm. Males present head not offset, which in lateral view seems fairly low and shaped as a truncate cone, with a small head cap followed by a very short first head annule (Fig. 6.9C,D). Males present ten lateral field incisures. The J2 are 471–573 μm long, their stylet measure 10.7–13.7 μm long and they present their head slightly inflated, with three or four annules behind the head cap. The J2 present a narrow tapering tail, which is 44–52 μm long and ends in a broadly rounded terminus (Fig. 6.9A). The tail hyaline terminus is 15.5 μm long (Whitehead, 1968).

No esterase phenotype has been characterized for *M. decalineata*. The mode of reproduction and chromosome number are unknown. On coffee, this species has been found in Tanzania and São Tome and Principe (Campos and Villain, 2005).

6.4.7 M. hapla

The females are 550–790 μm long, and the DEGO position is 5–6 μm. The perineal pattern (Fig. 6.10J–N) is round hexagon to flattened oval, often with punctations in the tail terminal area. Lateral lines are indistinct. Some striae may extend laterally and form one or two 'wings'. Striae are smooth to wavy. The female stylet is short (10–14 μm), and its knobs are round and distinctly offset from the shaft. The stylet cone is slightly curved dorsally, and the shaft is broadest posteriorly (Fig. 6.10F,G). Males are 791–1,432 μm long and their stylet is 17.3–22.7 μm long. Their head is neither annulated nor offset from the body. The stylet is narrow and short, with round knobs, which are offset from the shaft (Fig. 6.10C,V–Z). The DEGO position is 4–6 μm. The J2 measure 312–355 μm long and their stylet is 10–12 μm long. The J2 head present a truncate cone shape, and a head cap that is small and circular. The tail is 33–48 μm long, tapering uniformly to a tip which is variable in shape, usually subacute but sometimes bifid (Fig. 6.10T,U).

This species can be distinguished by its esterase phenotype (Est H1, Fig. 6.21) and PCR-SCAR markers (Carneiro et al., 2000; Zijlstra et al., 2000). It reproduces by meiotic parthenogenesis (race A) or by mitotic parthenogenesis (race B). Race A has $n = 13 - 17$, while race B has $2n = 30 - 31$, although most populations present polyploidy and have $3n = 43 - 48$ (Tryantaphyllou, 1985). On coffee, *M. hapla* has been reported from Brazil, Tanzania, Zaire, India, Kenya, Congo, Guatemala and El Salvador (Campos and Villain, 2005).

6.4.8 M. incognita

The lengths of female body and stylet are 510–690 and 15–16 μm long, respectively. The DEGO position is 2–4 μm. This species is diagnosed by its perineal pattern, which has a high dorsal arch composed of smooth to wavy striae (Fig. 6.11F,G,M,R,S). Some striae fork near the lateral lines, but distinct lateral lines are absent. Striae that bend toward the vulva can often be seen. The female stylet cone is distinctly curved dorsally, and the shaft is slightly wider posteriorly. The stylet knobs are broadly elongated, offset from the shaft, and anteriorly indented. Males are 1,200–2,000 μm long. The male head shape is very characteristic, having a centrally concave labial disc, which is raised above the medial lips (Fig. 6.11A,K,J,N,O,P). The medial lips are as wide as the head region, which is generally marked by two or three incomplete annulations. The DEGO position is 1.4–2.5 μm. The stylet is 23–26 μm long, with a tip that is blunt and wider than the medial portion of the cone. The shaft is generally cylindrical and it often narrows near the stylet knobs. The stylet knobs are offset from the shaft, anteriorly indented,

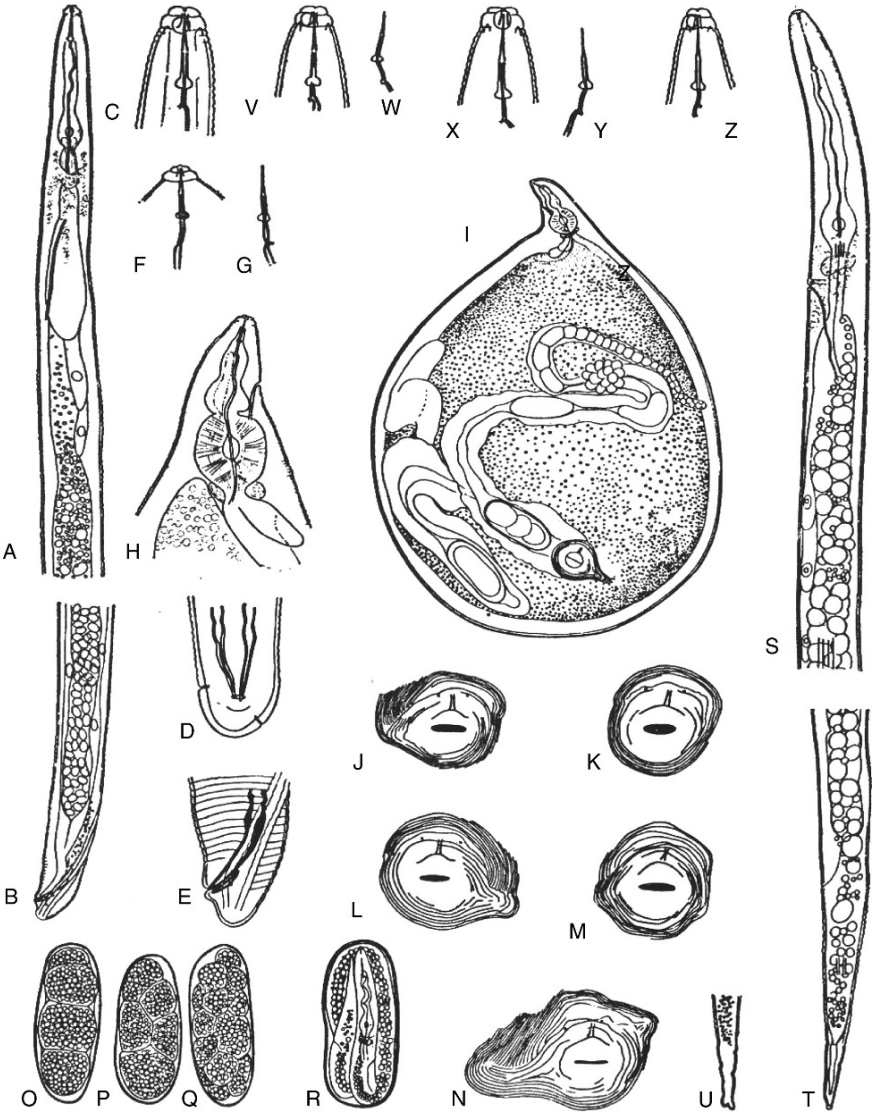

Fig. 6.10 *Meloidogyne hapla*. (**A–E,V,X,Z**) Male. (**F,G**) Female stylets. (**H–N**) Female anterior region, body and perineal patterns. (**O–R**) Eggs. (**S–U**) J_2 anterior region and tails (from Chitwood, 1949, with permission)

and broadly elongated to round (Fig. 6.11A,K). The J2 are 360–393 μm long, their DEGO position is 2.0–2.5 μm; the stylet is 10–12 μm long. The J2 present dumb bell-shaped labial disc and a medial disc. The labial disc is small and round, slightly raised above the medial lips. Lateral lips lie in contour with the head region, which usually bears two to four incomplete annulations. The J2 tail is 38–55 μm long, and it tapers steadily to a subacute terminus, with coarse posterior striae (Fig. 6.11U).

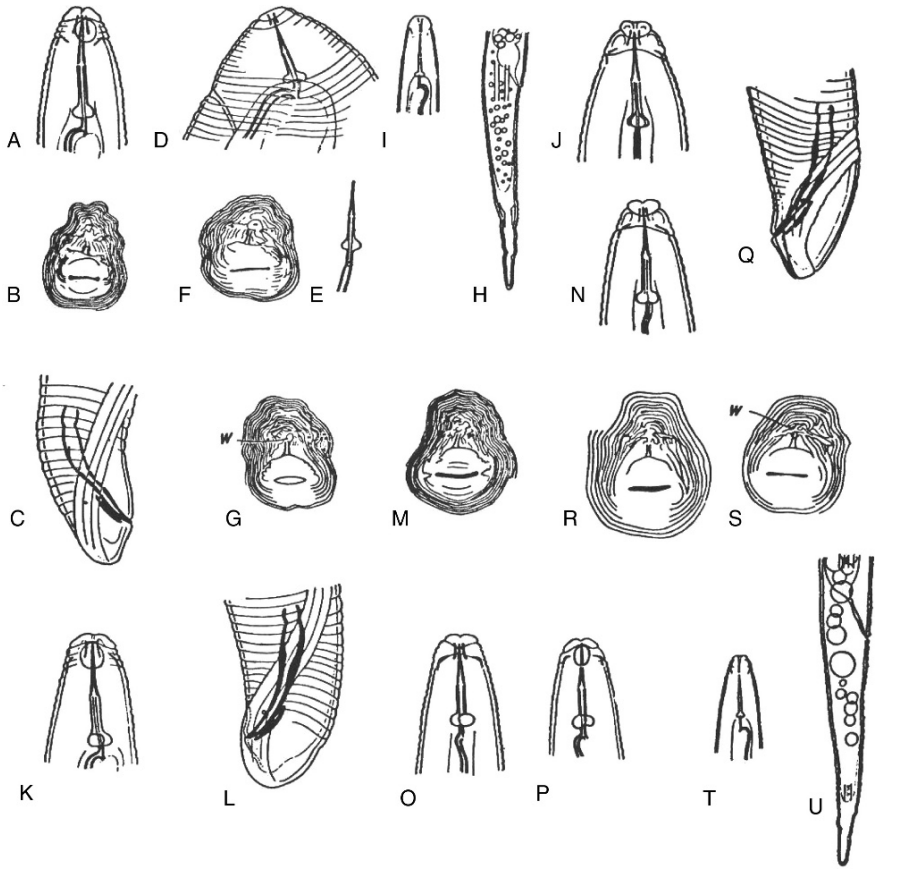

Fig. 6.11 *Meloidogyne incognita*. (**A,J,K,N,O,P**) Male anterior region. (**C,L,Q**) Male posterior end. (**D,E**) Female anterior region and stylet. (**B,F,G,M,R,S**) Perineal patterns. (**H,I,T,U**) J2 anterior and posterior regions. (from Chitwood, 1949, with permission)

M. incognita can be distinguished by its esterase phenotypes (Est I1 and I2) (Carneiro et al., 2000; Fig. 6.21) and PCR-SCAR markers (Zijlstra et al., 2000a; Randig et al., 2002). It reproduces by mitotic parthenogenesis, with $2n = 41 - 48$ (Tryantaphyllou, 1985). Coffee-parasitic *M. incognita* populations have been found in Brazil, Tanzania, Jamaica, Venezuela, Guatemala, the Ivory Coast, India, Costa Rica, El Salvador, Nicaragua, Cuba and the U.S.A. (Campos and Villain, 2005).

6.4.9 M. inornata

In its original description and in subsequent taxonomic reviews of *Meloidogyne* sp., *M. inornata* has been considered closely related to *M. incognita* (Whitehead, 1968; Hewlett and Tarjan, 1983). Jepson (1987) and Eisenback and Triantaphyllou (1991)

have synonymised *M. inornata* with *M. incognita* based on morphological features. Carneiro et al. (2008) have re-described and revalidated *M. inornata*.

The perineal pattern has a distinct, high dorsal arch composed of smooth to wavy striae, similar to those of *M. incognita* (Fig. 6.12H). The female stylet is 15–17 μm long, with the cone generally slightly curved dorsally and with well developed knobs. The DEGO position is 3.5–4.5 μm. Males have a high, rounded head cap, which is continuous with the body contour; it has a large, round, centrally concave labial disc, raised above the medial lips (Fig. 6.12A,B). The head region is

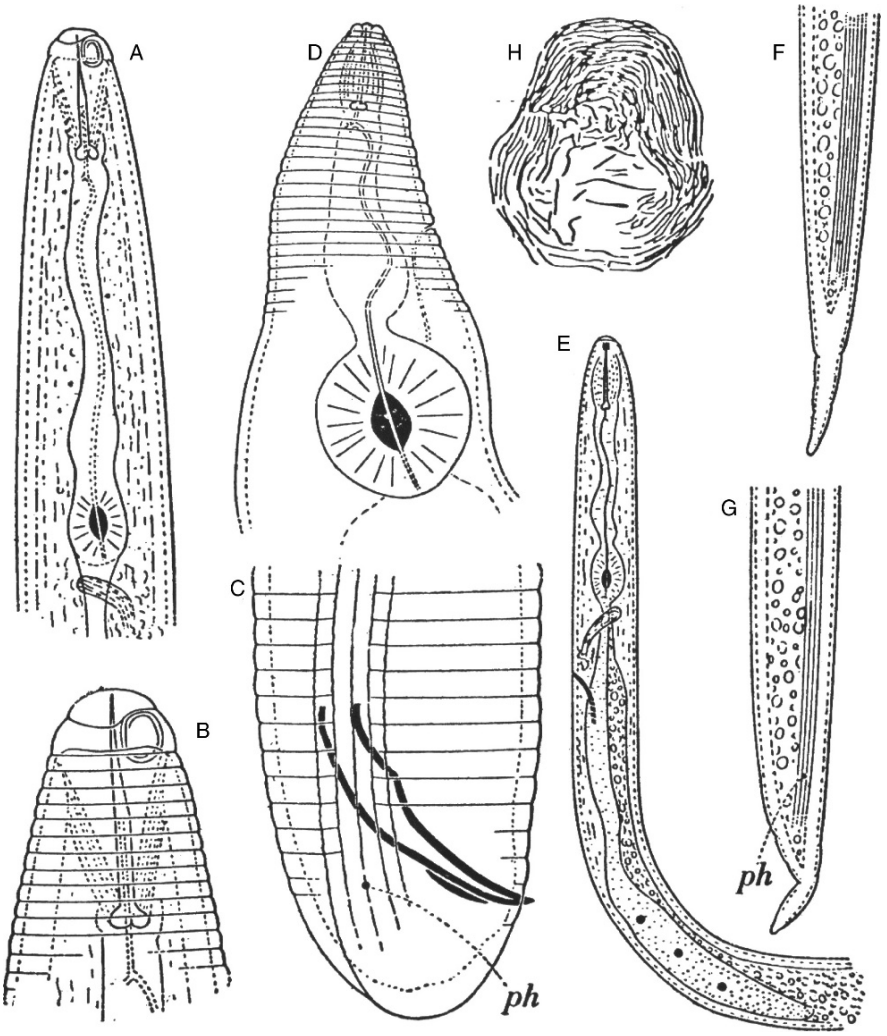

Fig. 6.12 *Meloidogyne inornata*. (**A–C**) Male stylet, anterior and posterior regions. (**D**) Female anterior region. (**E–G**) J_2 anterior and posterior regions. (**H**) Perineal pattern. ph = phasmid (from Lordello, 1956, with permission)

never marked by incomplete annulations. The stylet is robust (20–25 μm long) with a straight cone, cylindrical shaft with several small projections, and pear-shaped, backward-sloping knobs. The male lateral fields are composed of a variable number of crenate incisures in different parts of the body. The J2 stylet is 10–13 μm long and the DEGO position is 2.5–3.5 μm. The lateral fields are composed of four to six straight or undulate incisures (Fig. 6.12F,G), and the tail length is 35–58 μm.

The esterase phenotype I3 (Fig. 6.21) is species-specific, being the most useful character to differentiate *M. inornata* from other species. This species reproduces by mitotic parthenogenesis, with 3n = 54 − 58 (Carneiro et al., 2008). Coffee-parasitic *M. inornata* has been reported from Guatemala (Campos and Villain, 2005). Nonetheless, a recent survey conducted in Latin America with the aid of esterase phenotyping has not detected this species in Guatemala (Hernandez et al., 2004; Carneiro et al., 2004).

6.4.10 M. izalcoensis

The perineal pattern is similar to *M. incognita* and *M. paranaensis*. It presents a dorsal arch which can be moderately high or high, squarish to round. It also presents striae coarse, smooth to wavy, sometimes zigzaggy, usually without a distinct whorl (Fig. 6.13E). The female head region is offset from the body, sometimes annulated (Fig. 6.13C). The labial disc has two bumps on the ventral side, slightly raised above the medial lips. The female stylet is robust, 15–16 μm long; the DEGO position is 4.5–6 μm. Males have a high, round head cap which is continuous with the body contour (Fig. 6.13B,D). The labial disc is fused with the medial lips to form an elongated lip structure. The head region is never marked by incomplete annulations. The stylet is robust, 23–26 μm long and it has rounded knobs, backwardly sloping (Fig. 6.13B,D); the DEGO position is 4–7 μm. In J2, the stylet length is 12–13 μm and the DEGO position is 3–4 μm. The J2 tail is 45–48 μm long, conoid, with a round terminus (Fig. 6.13G–I).

The esterase phenotype I4 (Fig. 6.21) is unique and is the most useful character to differentiate *M. izalcoensis* from other species (Carneiro et al., 2005a). In molecular analysis, *M. incognita* and *M. izalcoensis* have appeared far apart in majority rule consensus dendrograms, which shows that these species are phylogenetically distant (Carneiro et al., 2004). *M. izalcoensis* reproduces by mitotic parthenogenesis, having 2n = 44 − 48. This species has been reported from El Salvador (Carneiro et al., 2005a).

6.4.11 M. javanica

The perineal pattern has a round to flattened dorsal arch, with distinct lateral lines which separate the pattern into dorsal and ventral regions (Fig. 6.14AA,BB,C,CC,D, G,N,O,Z). No or few striae cross the lateral incisures, while some striae bend

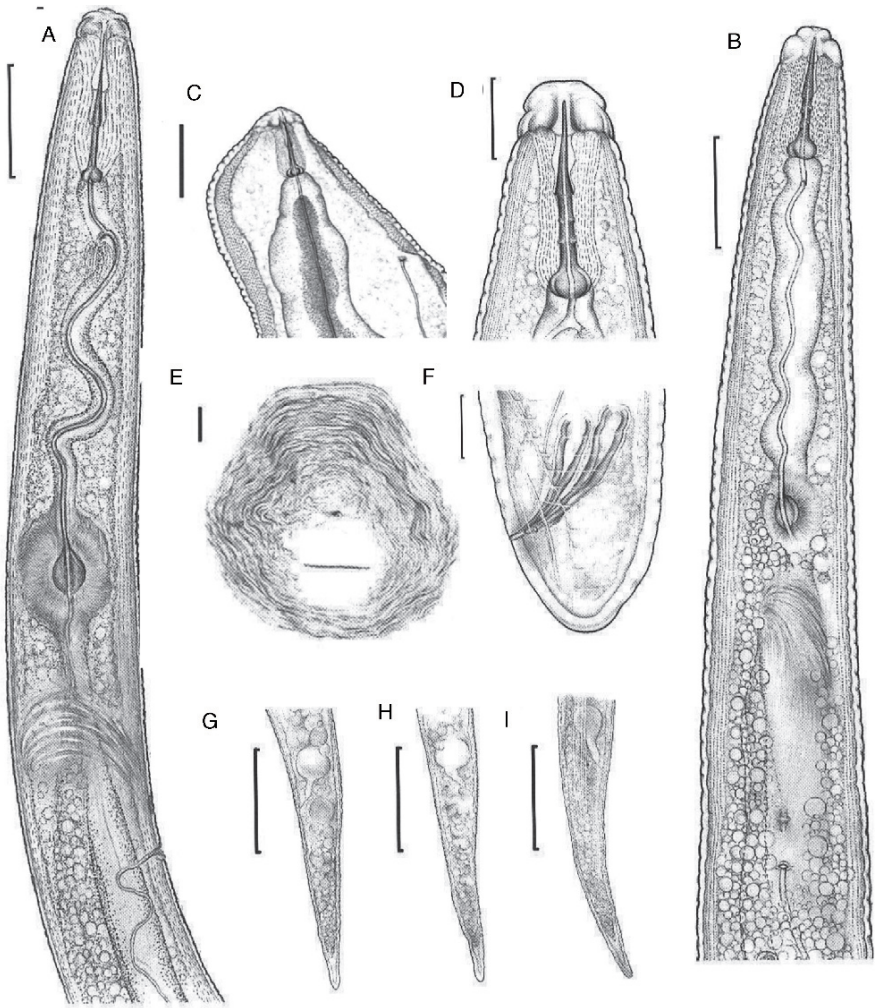

Fig. 6.13 *Meloidogyne izalcoensis*. (**A**) J_2 anterior region. (**B,D**) Male anterior region. (**C,E**) Female anterior region and perineal pattern, respectively. (**F**) male posterior region. (**G–I**) J_2 tails. Scale bars: A, B = 10 μm, C – I = 20 μm (from Carneiro et al., 2005a, with permission)

toward the vulva. Female stylet is 14–18 μm long and similar to *M. incognita*'s, except that its cone is not distinctly curved dorsally, and it gradually increases in width posteriorly (Fig. 6.14A,B,P). The DEGO position is 2–5 μm. Males are 940–1,440 μm long, and the head cap is high and almost as wide as the head region (Fig. 6.14E,H,R,S). The large smooth labial disc and the medial ones are fused. The stylet is 20–21 μm long, with a cone that is narrow anteriorly and very wide posteriorly; its shaft is cylindrical and it often narrows near the junction with the stylet knobs; these are low, wide and offset from the shaft (Fig. 6.14K–M). The DEGO position is 2–3 μm.

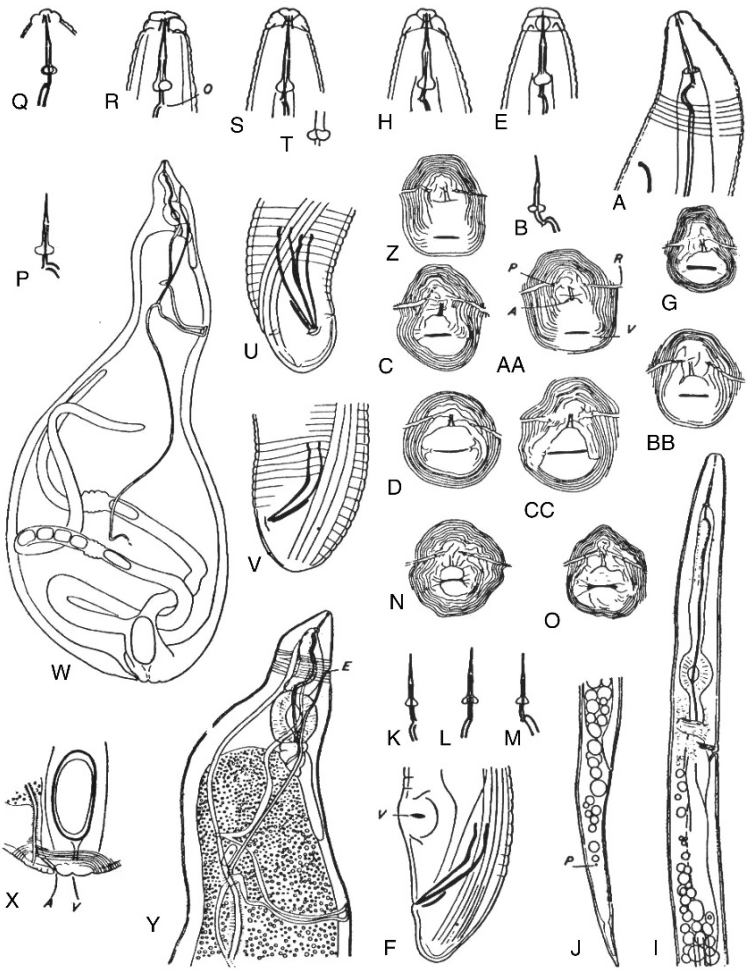

Fig. 6.14 *Meloidogyne javanica*. (**A**) Female anterior region. (**B,K,L,M**) Female stylet. (**AA,BB,C,CC,D,G,N,O,Z**) Perineal patterns. (**E,H,R,S**) Male anterior region. (**F**) Intersex male posterior region with rudimentary vulva. (**I,J**) J_2 anterior and posterior regions, respectively. (**P,Q**) Female stylet. (**U,V**) Male posterior end. (**W,X,Y**) Female body, posterior and anterior regions (from Chitwood, 1949, with permission)

Coffee-parasitic *M. javanica* has been reported from Brazil and other countries (see below). Nonetheless, experimental inoculations on susceptible genotypes have never confirmed that coffee is a suitable host for *M. javanica* (Santos, 1997; Oliveira et al., 1998; Carneiro et al., 2005b).

This species can be distinguished by its esterase phenotype (Est J3, Fig. 6.21) and by PCR-SCAR markers (Carneiro et al., 2000; Zijlstra et al., 2000a). *M. javanica* reproduces by mitotic parthenogenesis, with $2n = 41 - 48$ (Tryantaphyllou, 1985).

On coffee, *M. javanica* has been reported from Brazil, Tanzania, Zaire, El Salvador, India, Cuba and São Tome and Principe (Campos and Villain, 2005).

6.4.12 M. kikuyensis

This species is characterized by females 580–880 μm long, with a peculiar perineal pattern which has a low arch and prominent single lateral lines without incisures. The phasmids are located fairly close to the tail end, and characteristic striae with 'cheek-like' structures are seen on each side of the vulva (Fig. 6.15O,T). The female stylet is 13.5–16 μm and the DEGO position is 3.5–5 μm. Males are 810–1,650 μm long, with hexagonal head cap (Fig. 6.15C,D). The head has three annules behind the head cap. The stylet is 17–20 μm long and the DEGO position is 4.5–6 μm.

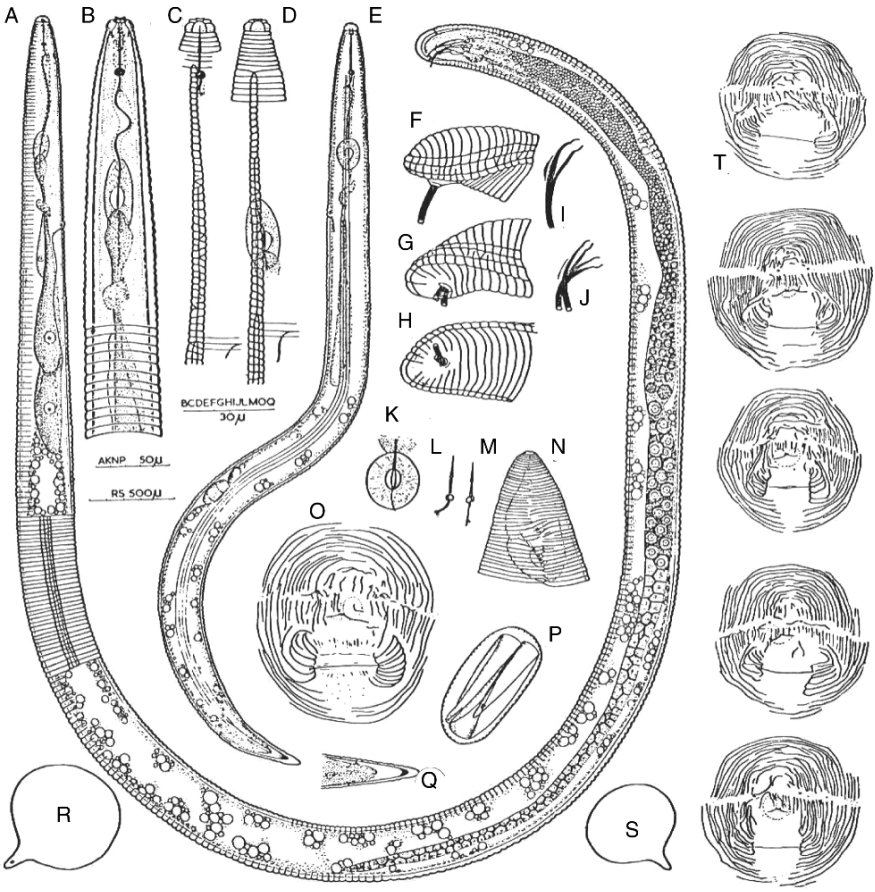

Fig. 6.15 *Meloidogyne kikuyensis*. (**A–D**) Male anterior region. (**E,Q**) J_2. (**F–J**) Male posterior end and spicules. (**K–N**) Female. (**O,T**) Perineal patterns. (**P**) Egg with J_2. (**R,S**) Female body shapes (from De Grisse, 1960, with permission)

The lateral fields present four incisures at mid-body (Fig. 6.15C,D). The J2 are 290–360 μm long, with stylet 12–15 μm long and the DEGO position is 3.5–5 μm. The tail is short (29.1 μm), tapering with a broad, rounded triangular hyaline area (Fig. 6.15E,Q). The short J2 tail differs in this species from all the others, except for *M. africana*. For a detailed morphological description of this species see De Grisse (1960), Whitehead (1968) and Jepson (1987).

No electrophoretic phenotype is available for this species. It reproduces by amphimixis, with n = 7 (Triantaphyllou, 1990). Cytogenetic studies have suggested that despite the small chromosome number, *M. kikuyensis* should be regarded as a true RKN (Triantaphyllou, 1990). The low chromosome number would represent the ancestral *Meloidogyne* condition from which all species have evolved. In comparison to the predominant parthenogenetic mode of reproduction found in *Meloidogyne* sp., the obligatory amphimitic mode of reproduction of *M. kikuyensis* further supports the hypothesis that this species represents the ancestral form of *Meloidogyne* sp. (Triantaphyllou, 1990). On coffee, this species has been reported from Kenya (Campos and Villain, 2005).

6.4.13 *M. konaensis*

In its original description (Eisenback et al., 1994), this species was diagnosed through the morphology of females ($L = 531.8 - 1,510\,\mu m$) and males ($L = 1,149 - 1,872\,\mu m$). Its perineal pattern is quite variable and similar to *M. incognita*'s and *M. arenaria*'s (Fig. 6.16M); thus, it is not a good taxonomic character. The morphology of female stylet is similar to *M. arenaria*'s; nonetheless, unlike the latter, the medial lips are divided into distinct lip pairs in *M. konaensis*. The most useful character to identify this species is male stylet morphology, which is 20.2–24.4 μm long, with 6–12 large projections surrounding its shaft (Fig. 6.16D,G); otherwise, the stylet is similar to *M. arenaria*'s. The male head cap is also similar to *M. arenaria*'s; however, the medial lip is often divided into distinct medial lip pairs in *M. konaensis* (Eisenback et al., 1994).

This species presents three different esterase phenotypes (Carneiro et al., 2000; 2004, Sipes et al., 2005), but only populations with the phenotype Est P1 (= Est F1) (Fig. 6.21) reproduce on coffee (Sipes et al., 2005). This species reproduces by mitotic parthenogenesis, with 2n = 44 (Eisenback et al., 1994). *M. konaensis* has only been reported from the USA (Hawaii) (Campos and Villain, 2005).

6.4.14 *M. mayaguensis*

In its original description (Rammah and Hirschmann, 1988), this species was diagnosed by the perineal pattern, which is round to dorso-ventrally ovoid (Fig. 6.17G,H). The dorsal arch is rounded, with striae that are fine, mostly continuous, widely spaced. The pattern's ventral region is rounded, with striae that are fine, closely

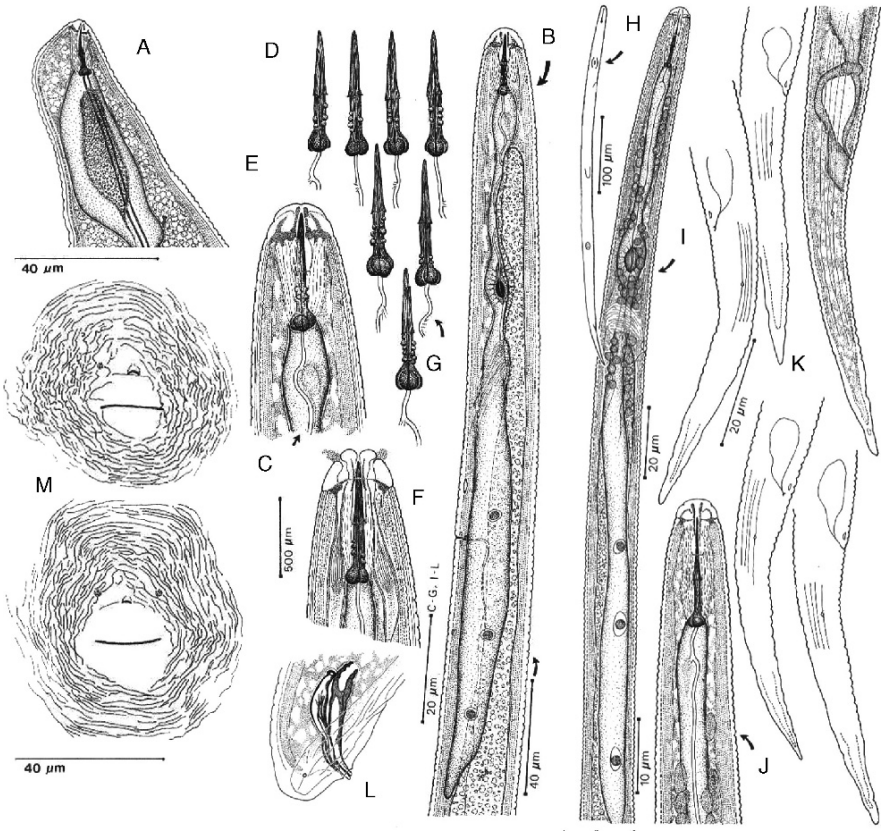

Fig. 6.16 *Meloidogyne konaensis*. (**A**) Female anterior region. (**B,C,F**) Male anterior region. (**D,G**) Male stylet. (**L**) Male posterior region. (**I,J**) J_2 anterior region. (**K**) J_2 tails. (**M**) Perineal patterns (from Eisenback et al., 1994, with permission)

spaced. Lateral lines are only seldom distinguishable; when seen, they break in striae; alternatively, a single lateral line may occur on one side of the pattern, at the junction of the dorsal and ventral arches. The tail tip area is large, circular, and usually free of striae. The female body is 518.4–769.5 µm long. Recently, Brito et al. (2004) have argued that the perineal pattern is not a good character for identification of *M. mayaguensis*, because it presents an accentuated variability and because many specimens show a pattern similar to *M. incognita*'s. The female stylet is 13.8–16.8 µm long, with knobs characteristically reniform in shape. In males, the high head cap is only slightly defined, is not offset from the body, and it lacks annulations. The stylet is 20.7–24.6 µm long, with knobs that are distinctly separated and not longitudinally divided by a groove; the base of the dorsal knob is concave. The stylet shaft is irregular in its diameter, with a wavy lumen, and it narrows near the junction with the stylet knobs. In J2, the tail measures 49.2–62.9 µm, and it tapers

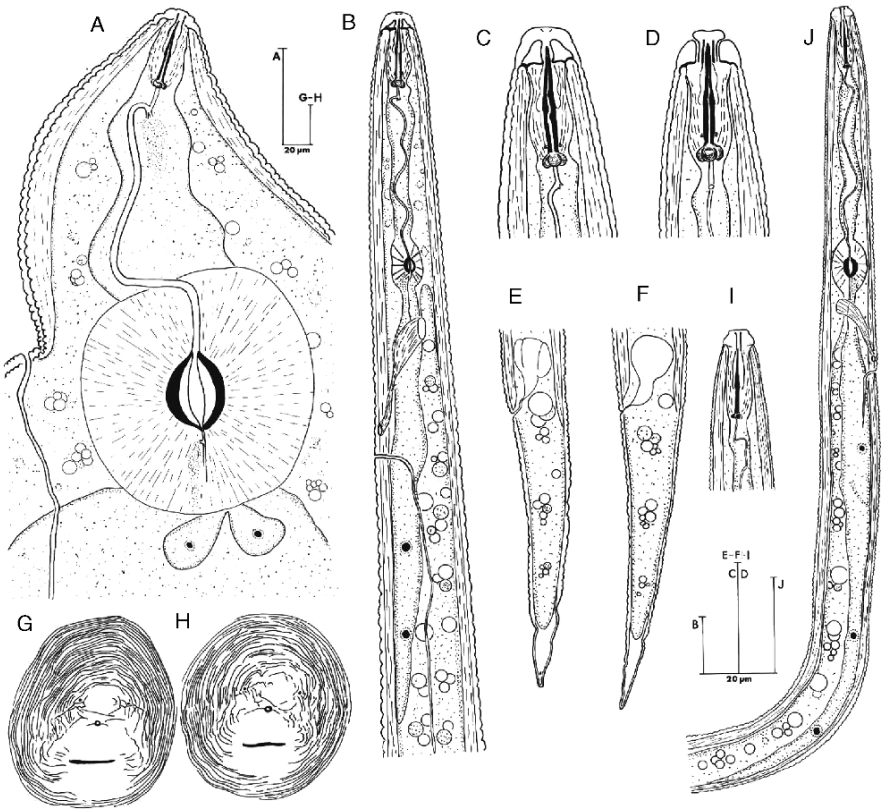

Fig. 6.17 *Meloidogyne mayaguensis*. (**A**) female anterior region. (**B–D)** Male anterior region. (**E,F**) J_2 tails. (**G,H**) Perineal patterns. (**I,J**) J_2 anterior region (from Rammah and Hirschmann, 1988, with permission)

gradually to its tip; the tail terminus is not distinctly narrow (Fig. 6.17E,F; Rammah and Hirschmann, 1988).

Considering the difficulty of characterizing *M. mayaguensis* on morphological grounds, the identification can be based on its esterase phenotype (Est M2, Fig. 6.21) (Carneiro et al., 2000; 2001) and DNA analysis (Block et al., 2002). *M. mayaguensis* reproduces through mitotic parthenogenesis, with $2n = 44 - 45$ (Esbenshade and Triantaphyllou, 1985a). On coffee, its geographical distribution includes Cuba, Costa Rica and Guatemala (Campos and Villain, 2005).

6.4.15 M. megadora

This species is diagnosed by its characteristic perineal pattern, which is more or less circular with very low dorsal arch; the pattern is also marked by short, thick

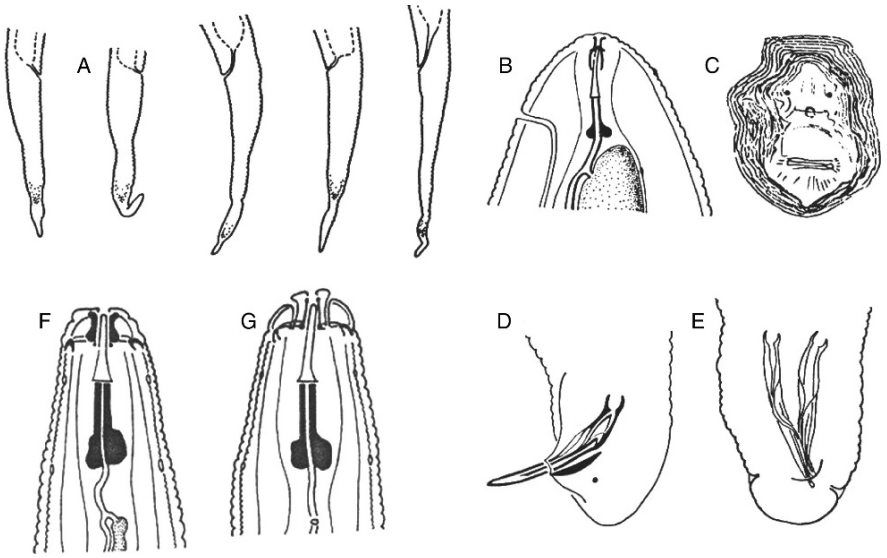

Fig. 6.18 *Meloidogyne megadora*. (**A**) J_2 tails. (**B**) Female anterior region. (**C**) Perineal pattern. (**D,E**) Male posterior region. (**F,G**) Male anterior region (from Whitehead, 1968, with permission)

striae, generally smooth but often broken (Fig. 6.18C). Phasmids are fairly close to tail terminus; the tail end is fairly wide. Lateral lines are not generally visible, but they are marked in the posterior region of the pattern by characteristic short coarse striae. In some patterns the tail whorl is seen distinct from the rest of the pattern. The female stylet is 13–17 μm long and the DEGO position is 4–9 μm. Males present a head that is low, shaped as a truncate cone, with one indented annule behind the head cap (Fig. 6.18F,G). In normal males, which are 905–2,277 μm long, the stylet is strong, 18.3–21.9 μm long, with knobs that are longer than wide, with outer margins longitudinally and transversely grooved (Fig. 6.18F,G). Dwarf males present reduced stylet with more rounded knobs. The DEGO position is 4–8.3 μm. The J2 are 413–548 μm long, with three annules behind head cap. Their tail is 47–58 μm long, subacute; it tapers irregularly in three 'sections', with its tip having various shapes (Fig. 6.18A; Whitehead, 1968).

No electrophoretic phenotype is available for *M. megadora*. Its reproduction mode and chromosome number are unknown. A review on this species has recently been prepared (I. Abrantes, U. Coimbra, personal communication). On coffee, this species' geographical distribution include Angola, Uganda and São Tome and Principe (Almeida and Santos, 2002; Campos and Villain, 2005).

6.4.16 M. oteifae

This species is diagnosed by small females ($L = 520 - 680$ μm) with short neck, and by the perineal pattern with low dorsal arch, very smooth and faint striae which are

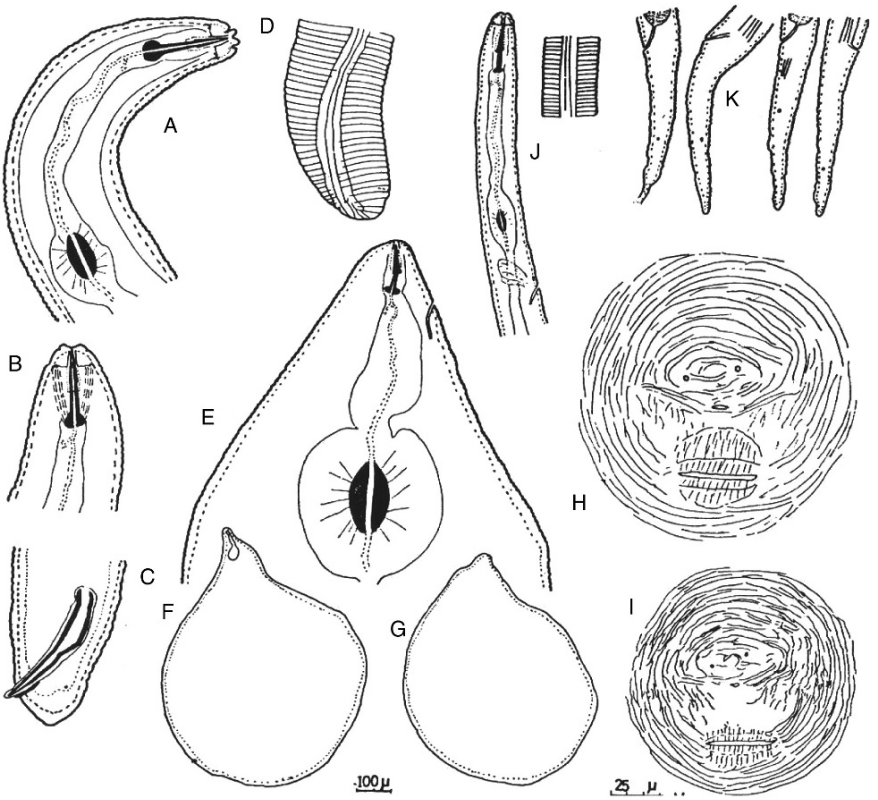

Fig. 6.19 *Meloidogyne oteifae*. (**A–D**) Male anterior and posterior regions, stylet and spicule. (**E–G**) Female anterior region and body shape. (**H,I**) Perineal patterns. (**J,K**) J_2 anterior region, lateral field and tails (from Elmiligy, 1968, with permission)

close together (Fig. 6.19H,I). The tail terminus is wide, covered by short, coarse striae and surrounded by concentric circles of striae, which form a distinct tail pattern that is not raised as a knob. The vulva is wide. *M. oteifa* and *M. africana*'s perineal patterns are similar, but the former has the vulva surrounded by circles of striae, which are themselves crossed by some striations radiating from the vulva; also, *M oteifa* does not have a wide, relatively clear area in the lateral field (Elmiligy, 1968). In *M. oteifa*, large phasmids are present, which are positioned closer than the vulva width. The female excretory pore is located posterior to the stylet knobs (Fig. 6.19E), at 18–23 µm from the anterior end of the body. The stylet is 13–14 µm long, slightly curved, and the knobs are round; the DEGO position is 3–4 µm. Males are 980–1,270 µm long, with one or two postlabial annules. The stylet is strong, 19–23 µm long, with elongated basal knobs (Fig. 6.19A,B). The tail is very short (Fig. 6.19C). The J2 (L = 320 − 400 µm) have stylet 11–13 µm long, tail tapering to a round terminus (Fig. 6.19K), and the lateral field is marked by four lines (Fig. 6.19J); the number of lines decrease towards the anterior and posterior ends of the body.

No electrophoretic phenotype is available for *M. oteifa*. Its mode of reproduction and chromosome number are unknown. On coffee, it has been reported only from Zaire (Campos and Villain, 2005).

6.4.17 M. paranaensis

This species can be distinguished from others by the combination of the following characters: the females (L = 512 − 780 μm) have labial and medial lips fused, asymmetric and rectangular. Their stylet is 15–17.5 μm long, with broad, distinctly offset knobs, and the DEGO position is 4.2–5.5 μm. The perineal pattern is similar to *M. incognita*'s (Fig. 6.20AA). Males (L = 983 − 2, 284 μm) have high, round head cap continuous with the body contour (Fig. 6.20B–D). The labial disc is fused with the medial ones, forming an elongated lip structure. Sometimes the head region is marked by an incomplete annulation. The stylet is robust (20–27 μm), usually

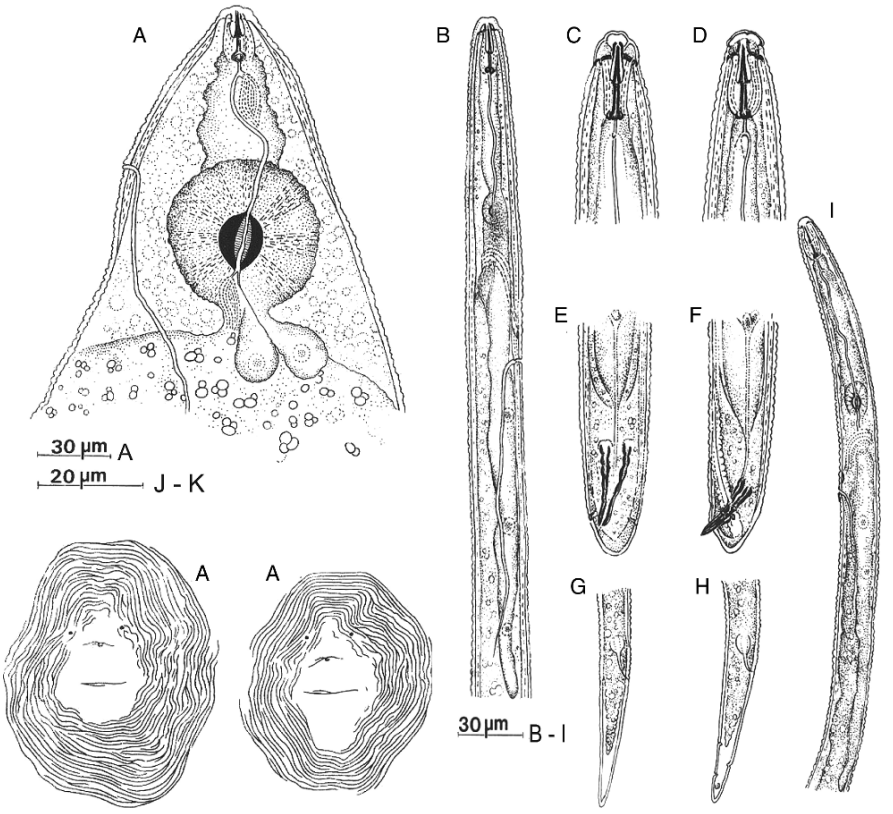

Fig. 6.20 *Meloidogyne paranaensis*. (**A**) Female anterior region. (**B–D**) Male anterior region. (**E,F**) Male posterior end. (**G,H**) J_2 tails. (**I**) J_2 anterior region (from Carneiro et al., 1996a, with permission)

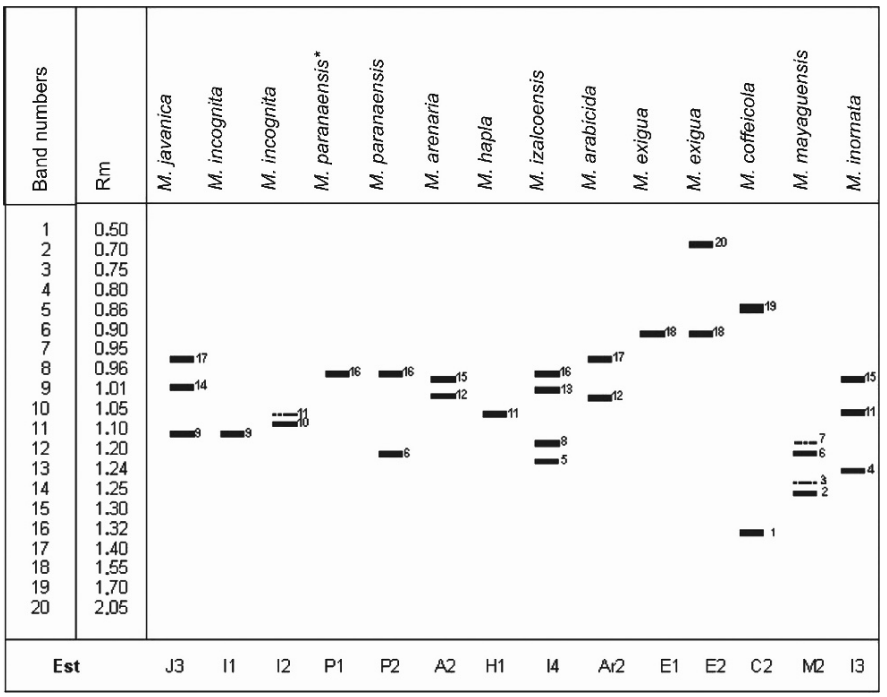

Fig. 6.21 Esterase (Est) phenotypes of coffee-parasitic *Meloidogyne* spp. Rm = ratio of migration in relation to the fastest band of *M. javanica*. Dotted lines indicate weak bands
* phenotype Est P1 (= Est F1) has been detected in *M. konaensis* from coffee

with rounded to transversely elongated knobs (Fig. 6.20C,D), and sometimes with one or two projections protruding from the shaft. The DEGO position is 3.5–5 μm. The J2 stylet is 13–14 μm long, and the DEGO position is 4–4.5 μm. The tail is 48–51 μm long, usually conoid and with a rounded terminus. The hyaline tail terminus is distinct (Fig. 6.20G,H). The rectal dilatation is large and the phasmids are small and located posterior to the anus.

M. paranaensis can also be distinguished by its esterase phenotypes [Est P1 (= Est F1) and P2] (Fig. 6.21; Carneiro et al., 2004) and PCR-SCAR markers (Randig et al., 2002; 2004). It reproduces by mitotic parthenogenesis, with $2n = 50 - 56$ (Esbenshade and Triantaphyllou, 1985a; Carneiro et al., 1996a). On coffee, it has been reported from Brazil, Guatemala and the USA (Hawaii) (Carneiro et al., 2004; Campos and Villain, 2005).

6.5 Electrophoresis-Based *Meloidogyne* Species Identification

The difficulties and benefits of identifying *Meloidogyne* species based on electrophoresis have been revealed by studies on about one thousand RKN populations

from different crops (Esbenshade and Triantaphyllou, 1985a; 1990; Carneiro et al., 1996b; 2000; 2004; Cofcewicz et al., 2004; 2005). These studies have demonstrated that several *Meloidogyne* species can be identified through enzyme phenotypes (esterase and malatodesidrogenase) revealed through polyacrilamide-gel electrophoresis. Through the methodology outlined by Esbenshade and Triantaphyllou (1985b) and Carneiro and Almeida (2001), the esterase phenotype of as many 20–25 individual females can be compared in the same gel.

Therefore, this biochemical taxonomic approach is a valuable tool in *Meloidogyne* research, specially (i) in extensive surveys, to determine the frequency and relative distribution of *Meloidogyne* species, (ii) to routinely identify RKN populations, and to detect atypical ones, and (iii) to purify RKN populations, prior to studies on DNA analyses, morphological characterization or others that need pure species (Carneiro et al., 1996b; 2000; 2005b; Cofcewicz et al., 2004; 2005; Esbenshade and Triantaphyllou, 1985a; 1990).

Unfortunately, there are no enzymatic phenotypes available for identification of all *Meloidogyne* species. Of the 17 coffee-parasitic *Meloidogyne* species, esterase phenotypes are available for the identification of 11 (Fig. 6.21). For each phenotype, the bands have their ratio of migration (Rm) calculated in relation to the fastest band of *M. javanica*, which is used as a reference.

The phenotypes available are: *M. incognita* (Est I1, Rm = 1.01; Est I2, Rm = 1.05 and 1.10); *M. exigua* (Est E1, Rm = 1.55; Est E2, Rm = 1.55 and 2.05); *M. coffeicola* (Est C2, Rm = 0.50 and 1.70); *M. javanica* (Est J3, Rm = 1.01, 1.25 and 1.40); *M. hapla* (Est H1, Rm = 1.10); *M. arenaria* (Est A2, Rm = 1.20 and 1.30); *M. paranaensis* (Est P1 (= F1), Rm = 1.32; Est P2, Rm = 0.90 and 1.32); *M. arabicida* (Est Ar2, Rm = 1.20 and 1.40); *M. mayaguensis* (Est M2, Rm = 0.70, 0.75, 0.90 and 0.95); *M. izalcoensis* (Est S4 (= I4), Rm = 0.86, 0.96, 1.24 and 1.32); and *M. inornata* (Est I3, Rm = 0.80, 1.10 and 1.30) (Carneiro et al., 2000; 2004; 2005b, 2008).

M. konaensis has been reported as presenting three different esterase phenotypes (Est F1, Est I1 and Est F1-I1), depending on the plant it is parasitizing (Sipes et al., 2005). According to these authors, only the Est F1 isolate parasitizes arabica coffee (*C. arabica* L.). In that publication, the morphological comparisons between Est F1, Est I1 and Est F1-I1 isolates are rather poor, and those authors have not convincingly shown that they all belong to *M. konaensis*. It is quite unusual that the same *Meloidogyne* species should present three esterase phenotypes when parasitizing different plants.

A coffee-parasitic RKN isolate from Hawaii (USA), reportedly belonging to *M. konaensis*, has been examined through morphological, isozyme and molecular approaches (Carneiro et al., 2004). This isolate presented the Est F1 (= P1) esterase phenotype and 90% genetic similarity with *M. paranaensis*. Thus, it is obvious that *M. konaensis* is not a clearly characterized species, as suggested by its variable esterase phenotype. The isolate studied by Carneiro et al. (2004) has indubitably been identified as *M. paranaensis* through a SCAR marker with a specific size fragment of 208 pb (Randig et al. 2004).

6.6 DNA-Based *Meloidogyne* Species Identification

The advent of PCR has allowed recent progress in nematode diagnostics. Through this technique, a single nematode or egg mass can be precisely identified at the species level.

Recently developed SCAR-primer sets have enabled sensitive and rapid identification of *M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi* and *M. fallax* (Zijlstra et al., 2000; Zijlstra et al., 2000). These SCAR primers were deduced from sequences of species-specific RAPD markers.

Randig et al. (2002) have developed a PCR-based assay to identify coffee-parasitic RKNs from Brazil. Three RAPD markers have been further transformed into SCAR markers specific for *M. exigua, M. incognita* and *M. paranaensis*. After the PCR procedure, the SCAR primers allow the initial polymorphism between those species to be retained as presence *vs* absence of DNA amplification. Moreover, multiplex PCR using the three pairs of SCAR primers in a single reaction allowed unambiguous identification of those *Meloidogyne* species, even when they were mixed in relative concentration as low as 1% (Randig et al., 2004).

Recently, 54 RKN populations from coffee fields in São Paulo and Minas Gerais States, Brazil, have been identified through esterase phenotyping and PCR reactions using the six SCAR primers altogether (Carneiro et al., 2005b). The multiplex PCR allowed unambiguous identification of *M. exigua, M. incognita* and *M. paranaensis* when present in the samples alone or in mixture; therefore, the potential of this approach for routine diagnostics has been confirmed. This coffee SCAR kit should be extended to include other important coffee-parasitic *Meloidogyne* species from Latin America, Africa and Asia.

Isolates of *M. mayaguensis* have also been identified through DNA-based methods, such as RFLP (Fargette et al., 1996), RAPD (Blok et al., 1997a), amplification of ribosomal DNA of the intergenic spacer region between the 18S and 5S genes (Blok et al., 1997b) and analysis of mitochondrial DNA with products of 705 bp from *COII* and lRNA region (Blok et al., 2002).

6.7 *Meloidogyne* Intraspecific Variability

The International *Meloidogyne* Project has summarized the response of nearly one thousand populations of the most common *Meloidogyne* species and their races to a list of differential hosts (Table 6.1; Hartman and Sasser, 1985).

As regards *M. incognita*, all four races have been found associated with coffee. In Paraná, one of the most important coffee-producing States in Brazil, race two is prevalent and race four the rarest (R. Carneiro, IAPAR, personal communication). Three *M. exigua* races have been detected in Brazil, two of them parasitizing coffee (Carneiro et al., 2000). No races have been detected on other coffee-parasitic *Meloidogyne* species in Brazil.

Table 6.1 Differential host test for the most common coffee-parasitic *Meloidogyne* species [Adapted from Hartman and Sasser (1985) and Carneiro and Almeida (2000)]

Species and races	Differential host plants[(a)] and results						Original host
	Cotton	Tomato	Tobacco	Pepper	Watermelon	Peanut	
M. incognita race 1	−[(b)]	+	−	+	+	−	coffee
M. incognita race 2	−	+	+	+	+	−	coffee
M. incognita race 3	+	+	−	+	+	−	coffee
M. incognita race 4	+	+	+	+	+	−	coffee
M. exigua race 1	−	−	−	+	−	−	coffee
M. exigua race 2		+	−	−	+	−	coffee
M. exigua race 3	−	−	−	−	−	−	rubber tree
M. paranaensis	−	+	+	−	+	−	coffee
M. coffeicola	−	−	−	−	−	−	coffee

[(a)] Cotton 'Deltapine'; tomato 'Rutgers'; tobacco 'NC95'; pepper 'Early California Wonder'; watermelon 'Charleston Gray'; peanut 'Florunner'.
[(b)] '−' indicates a resistant host; '+' indicates a susceptible one.

There have been few studies on diversity and phylogenetics of coffee-parasitic *Meloidogyne* species; these studies have focused only on meiotic or mitotic parthenogenetic species (Randig et al., 2002; Carneiro et al., 2004). A high level of intraspecific polymorphism has been detected in *M. arenaria, M. exigua* races two and three and *M. hapla*, in comparison to *M. incognita* and *M. javanica*. Phylogenetic analyses have showed that *M. hapla* and *M. exigua* are more closely related to each other than they are to other species; this suggests an early evolutionary divergence of these meiotically-reproducing species from those that reproduce mitotically, and supports the hypothesis that amphimixis is the ancestral reproductive state of *Meloidogyne* (Triantaphyllou, 1985).

A recent study on 18 RKN populations from coffee fields in Brazil, Central America and the USA (Hawaii) has revealed their diversity with respect to enzyme phenotypes, morphology and genome (Carneiro et al., 2004). An analysis of the dendograms deduced from RAPD data has allowed the definition of different clusters of species with high bootstrap support: (i) *M. paranaensis* and *M. arabicida*; (ii) *M. exigua* and *M. mayaguensis*; (iii) *M. arenaria, M. javanica* and *M. izalcoensis*. Intraspecific groups with a low degree of polymorphism have been observed in *M. paranaensis* (polymorphism of 20.3%) and in *M. incognita* (esterase phenotypes Est I1 and I2) (polymorphism of 11.2%). In *M. exigua*, the two coffee-parasitic races presented a genetic diversity of only 8.6%.

Recent studies by Muniz et al. (2008) using RAPD-PCR have showed a high variability among *M. exigua* populations belonging to different races and enzymatic phenotypes. No relationship was observed between races, enzymatic phenotypes and genetic polymorphism. This high genetic variability had been predicted to occur in *Meloidogyne* species that reproduce by facultative meiotic parthenogenesis, in comparison to mitotic parthenogenesis (Triantaphyllou, 1985). Indeed, previous investigations had showed the monophyly of *M. arenaria* and *M. incognita* races (Cenis, 1993; Baum et al., 1994).

These findings suggest that for a given *Meloidogyne* species, its races do not form monophyletic groups; this indicates that such intraspecific groups may not have a common ancestor. In other words, races do not have a genetic determinism, suggesting that this variability should be considered in breeding programs for RKN-resistance (Muniz et al., 2008).

6.8 Concluding Remarks

There have been considerable advances in recent years in the taxonomy of coffee-parasitic *Meloidogyne* species: misidentifications have been revised, species have been described or revalidated, and new identification methods have been developed or consolidated. Isozyme phenotyping, for example, is now well established for most RKNs associated with coffee, and it has become a fairly simple and inexpensive taxonomic tool. Furthermore, over the last few years nematologists worldwide have become aware of the complexity of *Meloidogyne* taxonomy, and the need for characterizing several morphological and morphometric features of RKN populations to accurately identify them.

Proper procedures should also be followed during surveys conducted in coffee fields and nurseries, so that precious time and resources are not wasted. Indeed, one should collect only non-rotten roots with typical RKN-symptoms (galls, swellings or crackings); in old, rotten roots the RKN females are unlikely to be useful for isoenzyme characterization. Roots should be packed in plastic bags, surrounded by moist soil collected from the same site. If samples cannot be examined and processed immediately, they should be maintained in cold chamber or refrigerator; samples should not be frozen or left in the sun or in hot locations.

Wherever possible, the first step in identifying RKN populations should be characterizing their esterase phenotype(s), according to the methodology outlined by Esbenshade and Triantaphyllou (1985b) and Carneiro and Almeida (2001). For each RKN population, at least 30 females should be individually submitted to esterase phenotyping.

In those nematology laboratories where esterase phenotyping cannot be performed, perineal patterning should be cautiously used for species identification. Morphological characterization will also be needed whenever the RKN population presents an unreported esterase phenotype. In these cases, the RKN population under investigation could be either a new species or a population of those five *Meloidogyne* species for which esterase phenotyping has not yet been performed.

Perineal patterning should be carefully done, making sure only mature, egg-laying females are collected, properly cut and mounted in glass slides for examination under the light microscope. Perineal patterns should be properly cleaned of body residues and carefully mounted to avoid the creation of artifacts that will make observation and judgment of perineal pattern characters difficult; special care should be taken to avoid deformation of the perineal pattern through the pressure (weight) of the coverslip. At least 10 perineal patterns should be examined *per* RKN population.

Perineal patterning should be complemented by observation of male and J2 morphology and/or morphometry, paying special attention to those features and/or measures that are typical of one or just a few *Meloidogyne* species.

Currently, five coffee-parasitic *Meloidogyne* species from Africa are not available in international nematology collections and/or their types are not in good conditions for examination: *M. africana, M. decalineata, M. kikuyensis, M. megadora* and *M. oteifae*. For these and new *Meloidogyne* species to be described, it would be extremely interesting to have live samples shipped to Embrapa/Genetic Resources and Biotechnology (Brasilia, Brazil), where a complete infrastructure is available to maintain RKN populations from across the globe, either alive or cryopreserved (Carneiro et al. 2005c). This collection has allowed morphological, physiological, electrophoretic and molecular studies on many coffee-parasitic RKN populations.

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