Identification of *Meloidogyne* : a general assessment and a comparison of male morphology using light microscopy, with a key to 24 species [1]

Susan B. Jepson

Rothamsted Experimental Station, Harpenden, Herts., England.

Summary

Quantitative characters utilised in the identification of *Meloidogyne* species are assessed for stability in terms of their coefficient of variation and interspecific overlap. Only eleven male, seven female and eleven second-stage juvenile characters aid species differentiation. Males of 24 species are differentiated in a key primarily based on qualitative morphology of the head and stylet observed in the light microscope. Twenty one species can be readily identified using only head and stylet shape, stylet length and distance of dorsal oesophageal gland orifice from stylet base. *M. incognita incognita*, *M. incognita wartellei* and *M. grahami* cannot be separated using male characters alone. In females, qualitative characters to be considered are stylet and perineal pattern morphology, and, in second-stage juveniles, tail shape. Qualitative characters should be used in preference to measurements for species identification, although combinations of these may serve to group species, particularly when the coefficient of variation ≤ 5 % and where the range in the genus is broad with limited species overlap.

Résumé

Identification des Meloidogyne : *considération générale et comparaison de la morphologie des mâles en microscopie optique, avec une clé de 24 espèces*

La stabilité des caractères quantitatifs utilisés pour l'identification des espèces de *Meloidogyne* est évaluée en se basant sur leur coefficient de variation et leur chevauchement interspécifique. Onze caractères des mâles, sept des femelles et onze des juvéniles de second stade sont utilisés dans la différenciation des espèces. Les mâles de 24 espèces sont différenciés dans une clé fondée, en premier lieu, sur des caractères qualitatifs concernant la morphologie de la tête et du stylet observés en microscopie optique. Vingt et une espèces peuvent être différenciées en n'utilisant que la forme de la tête et du stylet, la longueur du stylet et la distance entre le débouché de la glande œsophagienne dorsale et la base du stylet. *M. incognita incognita*, *M. incognita wartellei* et *M. grahami* ne peuvent être distinguées en n'utilisant que les caractères des mâles. Chez les femelles, les caractères qualitatifs à considérer concernent la morphologie du stylet et de la zone périnéale et, chez les juvéniles du deuxième stade, la forme de la queue. Dans l'identification des espèces, les caractères qualitatifs doivent être utilisés de préférence aux mensurations, bien que des combinaisons de ces dernières puissent servir à grouper des espèces, particulièrement si le coefficient de variation est ≤ 5 % et si l'étendue du caractère, dans l'ensemble du genre, est large avec des chevauchements interspécifiques limités.

Identification of *Meloidogyne* species has been based on the female perineal pattern, although often this has been supplemented by up to 140 other characters from the different life stages (see major references : Whitehead, 1968 ; Esser, Perry & Taylor, 1976 ; Franklin, 1978 ; Taylor & Sasser, 1978). The perineal pattern is variable in most species, but especially so in the evolutionarily advanced species, such as *M. incognita*, which have broad host ranges and reproduce entirely by parthenogenesis (Trianta-phyllou, 1979). A number of species have perineal patterns of a similar type (e.g. *M. incognita* and related species) and may be grouped on this basis, while others are unique. Many early descriptions are inadequate with poor illustrations and place much emphasis on measurements of specimens from single populations. Comparisons of different populations are essential to assess intraspecific variation and to adequately characterise a new species. Only those characters which exhibit least variation in the genus as a whole should be used.

The use of morphological characters of male heads of *Meloidogyne* species for their identification has been highlighted by Eisenback and Hirschmann (1980) and Eisenback *et al.* (1981). In a scanning electron microscope (SEM) study it was shown that races A and B of *M. hapla* and their cytological forms, *M. arenaria*, *M. incognita* and *M. javanica*, showed differences in labial and cephalic sensilla, shape and proportion of labial disc and lips, and markings on the head region. A similar study of the same species included a comparison of scanning and light micrographs of the male and female stylet and showed these four species to be morphologically distinct (Eisenback, Hirschmann & Triantaphyllou, 1980 ; Eisenback *et al.*, 1981).

The characters currently used for differentiating *Meloidogyne* species are assessed. Qualitative characters are shown to be more useful than measurements and their value is illustrated using males of 24 species, which are differentiated in a key.

Materials and methods

Character assessment

Thirty *Meloidogyne* species were observed ; fourteen (21 populations) were in culture at Rothamsted, and others in the Rothamsted and USDA slide collections. Mean, range, coefficient of variation and degree of overlap between species for each character were determined ; published data were also consider-ed. Quantitative and qualitative characters studied are listed below* :

Males : a, b, b', c, c', o, T, d, m, max. body width, body length, head shape, head height, head width, head height/head width, number of post labial annules, stylet length, stylet cone length, stylet shape, stylet knob width, stylet knob length, distance dorsal oesophageal gland orifice (DGO) from stylet base, stylet knob + stylet shaft length, length median bulb, width median bulb, distance anterior end to base median bulb, position of hemizonid relative to excretory pore, distance excretory pore to base median bulb, distance anterior end to excretory pore, distance anterior end to centre median bulb, length median bulb "valves", width median bulb "valves" distance anterior end to hemizonid, length oesophagus, number of lateral field incisures, width lateral field, areolation of lateral field, length testis, tail width at anus, length/width tail, distance anterior end of testis to tail terminus, distance phasmids to tail terminus, annule width, spicule length, gubernaculum length.

Second-stage juveniles : a, b, b', b", c, c', o, d, m, max. body width, body length, head height, head width, head height/head width, lateral cheek length, number of post labial annules, stylet length, stylet base to anterior end, stylet knob + stylet shaft length, stylet knob width, stylet knob length, DGO from stylet base, length median bulb, width median bulb, excretory pore to base median bulb, anterior end to excretory pore, position of hemizonid relative to excretory pore, anterior end to centre median bulb, length median bulb "valves", width median bulb "valves", anterior end to hemizonid, length oesophagus, number of lateral field incisures, width lateral field, areolation of lateral field, tail length, tail width at anus, length/width tail, tail shape, caudal ratio A**, caudal ratio B**, inflation of rectum (included because many authors have used it ; it is now known (Bird, 1979) to be an artifact, distance

---

* b = body length : distance from anterior end to posterior end of œsophageal glands ; b" = body length : distance from anterior end to centre of median bulb ; c' = tail length : body width at anus or cloaca ; d = length tail : body width seen laterally at level of anus ; m = length stylet cone × 100 : stylet length ; o = distance from stylet base to DGO × 100 : total stylet length ; T = distance from cloaca to anterior-most part of testis × 100 : body length.

** caudal ratio A = length of hyaline tail terminal : its width at its beginning ; caudal ratio B = length of hyaline tail terminal : width at a point 5 μm from its terminus.

phasmids to tail terminus, hyaline tail terminus length, annule width, anterior end to centre genital primordium, genital primordium to tail terminus.

Females : a, b, b', o, m, max. body width, body length, head width, stylet length, stylet shape, DGO from stylet base, stylet knob width, stylet knob length, neck length, neck width, body length/neck width, length median bulb, width median bulb, head to base median bulb, excretory pore to base median bulb, *position of excretory pore relative to stylet base*, anterior end to centre median bulb, length, median bulb "valves", width median bulb "valves", annule width ,vulval slit length, interphasmidal distance, level of phasmids to vulva, phasmids to anus, centre of vulva to anus, length of perivulval area free of striae, width perivulval area free of striae, perivulval length/perivulval width, R_1*, R_2*, perineal pattern.

Eggs : length, width, length/width.

To assess intraspecific variation, measurements were made by one operator on freshly fixed males of fifteen species and subspecies including three populations of *M. graminicola*. Fresh material was used because some characters are less easily seen in older material due to the fixative used and age. Second-stage juveniles of eleven species and subspecies including four populations of *M. graminicola*, two of *M. graminis* and two of *M. incognita incognita* were also measured.

Principal coordinate analysis, a method for finding coordinates for individuals referred to principal axes while preserving defined distances between them (Gower, 1966), was used as an objective assessment of the most discriminating characters in males and juveniles and to show the interrelationships of the species. Such an analysis makes no assumptions about the distribution of the variates in a population.

LIGHT MICROSCOPY OF MALES

Males of 24 species were examined (Tab. 1). With species which readily produce them, males were more easily obtained from fresh egg masses of mature females, rather than by extraction from soil. One or several males, depending on the species, were found to be associated with egg sacs. Males of some species

are rare in field collections (e.g. *M. incognita*) but can readily be produced in culture by stressing the host plants by one of the following methods : *i*) isolation, over a period of days, in a beaker, of part of a washed infected root system. The roots sprayed with water and males washed to the bottom of the beaker ; *ii*) isolation, over a period of days, in a beaker of water, of a complete, washed infected root system with the aerial parts intact. The water sampled regularly for males ; *iii*) removal of the aerial parts of an infected plant leaving the roots in soil. The soil and roots sampled regularly.

Live males were fixed in TAF (Courtney, Polley & Miller, 1955) at about 70° and mounted in TAF. Fixation and mounting in lactophenol was avoided because it sometimes caused the stylet to be obscured and deterioration occurred with age. Many older specimens had been mounted in glycerol and the stylet was obscured.

Head and stylet morphology were examined only in lateral view, and micrographs taken using differential interference contrast illumination (DIC). For some rare species the only material available for photography was limited and in poor condition but all the essential features are adequately shown. A key was constructed using mainly qualitative characters but with a limited number of measurements ; stylet length, stylet cone length, distance of dorsal oesophageal gland orifice (DGO) from stylet base and head width.

Results and discussion

CHARACTER ASSESSMENT

The most differential characters, with low coefficients of variation as opposed to those with overlapping ranges and/or coefficients of variation > 20 % are listed in Table 2.

Males

Body length : Intraspecific variation is about 12% on 1 600 μm (Tab. 2), however, the use of means and 95 % confidence limits enables groups of species to be roughly separated into size classes (Tab. 3).

Stylet length : This is the most differentiating character because of the broad range in the genus (16-27 μm) and low coefficient of variation (4 %, Tab. 2). The use of 95 % confidence limits in bivariate plots with linear characters of limited coefficient of variation reduces the overlap between species (Fig. 1).

* R_2 = length of dorsal arch base line horizontally through tail terminus : vertical distance between tail terminus and dorsal arch line.
R_2 = length of dorsal arch base line horizontally through tail terminus : vertical distance between base line and highest point of dorsal arch line.

Table 1

Male specimens observed by light microscopy.

Species	Population	Source
acronea Coetzee, 1956	Malawi	RES
africana Whitehead, 1960	Kenya	
ardenensis Santos, 1968	Bristol, U.K.	RES
arenaria (Neal, 1889)		
artiellia Franklin, 1961	Wells, Norfolk, U.K.	RES
camelliae Golden, 1979	(Japan) Beltsville, USA	RES ; paratypes 77/8/2, 3, 4.
chitwoodi Golden, O'Bannon, Santo & Finley, 1980	(Quincy, Washington, USA) Beltsville, USA	paratypes 77/24/3 RES ; USDA paratypes T. 2627-T. 2628
decalineata Whitehead, 1968	Kenya	holotype 77/10/1
exigua Goeldi, 1887	Bolivia	
grahami Golden & Slana, 1978	Florence, S. Carolina, USA Beltsville, USA	RES ; paratypes 77/23/7
graminicola Golden & Birchfield, 1965	Bangladesh	RES
	India	RES
	Nepal	RES
	Thailand	RES
	USA	RES
graminis Sledge & Golden, 1964	Florida, USA	RES
	Maryland, USA	RES
hapla Chitwood, 1949	Thetford, Norfolk, U.K.	RES
	Montreal, Canada	RES
incognita incognita (Kofoid & White, 1919)		RES
incognita wartellei Golden & Birchfield, 1978	NCSU, Washington, Louisiana, USA	RES
indica Whitehead, 1968	India	holotype 77/12/1
javanica (Treub, 1885)	Nepal	RES
	Iraq	RES
microtyla Mulvey, Townshend & Potter, 1975	Ontario, Canada	
megatyla Baldwin & Sasser, 1979	Elizabethtown, N.C., USA	USDA paratypes T. 2398
naasi Franklin, 1965	Tytherington, Glos., U.K.	RES
oryzae Maas, Sanders & Dede, 1978	Surinam	RES ; paratype 77/22/2
ovalis Riffle, 1963	Wisconsin, USA	paratype
propora Spaull, 1977	Aldabra	paratypes 77/21/10
undescribed species *	Elva River, Russia	

All material from Rothamsted Slide Collection unless otherwise noted. RES = in culture at Rothamsted ; NCSU = North Carolina State University.

* *M. kralli* Jepson, 1983. *Revue Nématol.* this issue.

Table 2

Differential characters, with coefficients of variation ≤ 17 %,
for species determination in *Meloidogyne*.

Character	Average coefficient of variation	Number of species	Order of size for character ± average variation coefficient
Males			
*Body length	12 %	15	1 600 ± 192 μm
*Stylet length	4 %	15	20 ± 0.8 μm
*Stylet cone length			
*Head shape			
*Stylet shape			
Stylet knob width	8 %	10	4 ± 0.3 μm
Stylet knob length	11 %	9	3 ± 0.3 μm
Distance of dorsal oesophageal gland orifice (DGO) from stylet base	17 %	9	3 ± 0.5 μm
Median bulb 'valve' length	10 %	8	5 ± 0.5 μm
Number post labial annules			
Number lateral field incisures			
Position of hemizonid relative to excretory pore			
Distance from excretory pore to anterior end	10 %	15	100 ± 10 μm
Second-stage Juveniles			
Body length	4 %	11	400 ± 16 μm
Stylet length	4 %	9	12 ± 0.5 μm
Stylet knob width	12 %	8	2 ± 0.2 μm
Stylet knob length	14 %	6	1.5 ± 0.2 μm
Distance of DGO from stylet base	12 %	9	4 ± 0.5 μm
Tail length	5 %	8	50 ± 2.5 μm
Distance from excretory pore to anterior end	4 %	8	70 ± 2.8 μm
*Tail shape			
Number lateral field incisures			
Position of hemizonid relative to excretory pore			
Hyaline tail length	14 %	9	15 ± 2.1 μm
*Stylet base to anterior end	5 %	8	15 ± 0.8 μm
Females			
*Stylet shape			
Distance of DGO from stylet base			
Stylet knob width			
Stylet knob length			
Position of excretory pore relative to stylet base			
*Perineal pattern			

* Most differential characters.

Table 3

Body length size classes in *Meloidogyne* males.

800-1 300 μm	1 300-1 700 μm	> 1 700 μm
artiellia	acronea *	ardenensis *
brevicauda Loos, 1953	africana *	arenaria *
chitwoodi *	coffeicola Lordello & Zamith, 1960	camelliae *
exigua	ethiopica Whitehead, 1968	deconincki Elmiligy, 1968
javanica	grahami *	grahami *
kikuyensis de Grisse, 1960	graminicola *	incognita wartellei •
litoralis Elmiligy, 1968	graminis *	megadora Whitehead, 1968
megatyla	hapla *	lucknowica Singh, 1969
megriensis (Pogosyan, 1971)	incognita incognita •	spartinae (Rau & Fassuliotis, 1965)
microtyla *	incognita wartellei *	
naasi	kirjanovae Terenteva 1965	
oteifae Elmiligy, 1968	mali Itoh, Oshima & Ichinohe, 1969	
ottersoni (Thorne, 1969)	megatyla	
propora	oryzae	
tadshikistanica Kirjanova & Iva-nova, 1965	ovalis	
undescribed species (Russia) *	querciana Golden, 1979	
	sewelli Mulvey & Anderson, 1980	

Stylet cone length : This is correlated with stylet length and in most species the cone is about half the stylet length. *M. acronea, M. africana* (Fig. 1) and *M. oleifae* Elmiligy, 1968 are distinguished by a cone length of much less than half the stylet length.

Stylet knob width and length : There is much variation in both characters (8 % and 11 % on 3 and 4 μm respectively, Tab. 2), partly due to imprecision in resolving small differences in size (even with high resolution optics, which are essential). Measurements may aid identification in some instances, particularly in those species with much larger knobs than most *Meloidogyne* (e.g. *M. megadora, M. megatyla* and *M. acronea*) but the overall shape of the knobs is more important as a distinguishing feature (e.g. transversely ovoid ; rounded ; backwardly sloping ; tapering onto stylet shaft ; set off from stylet shaft).

Distance of DGO from stylet base : Again measurements are subject to inaccuracy which partly accounts for the large coefficient of variation (17 % on 3 μm, Tab. 2). While there is much intraspecific variation, in most species the DGO lies between 3 and 6 μm behind the stylet knobs. *M. acronea* and *M. arenaria* are distinct, with the DGO more than 6 μm($\bar{x}$) behind the stylet knobs. It appears that *M. mali* and

M. megadora are similar in this respect with an average distance from the DGO to the stylet base of 8 μm and 6.5 μm respectively (Whitehead, 1968 ; Ito *et al.,* 1969).

Median bulb "valve" (= feeding pump lining) length : In most *Meloidogyne* species this value is about 5 μm with a coefficient of variation of about 10 % (Tab. 2). However, *M. acronea, M. africana, M. hapla, M. javanica* and *M. arenaria* have somewhat longer "valves" of up to 11 μm.

Number of post-labial annules : There is a single post-labial annule in most species but because of the presence of incomplete annules the condition in *M. incognita* and *M. arenaria* may be variable with the apparent number depending on their position and the precise orientation of the head. One to three annules may be seen and each side of the head may be dissimilar (Eisenback & Hirschmann, 1980).

Lateral field incisures : Throughout the genus the basic number is four, although in the mid region of the body additional, incomplete incisures may occur : e.g. *M. africana* (5), *M. ardenensis* (5), *M. graminicola* (6), *M. decalineata* (10) and the undescribed species (Elva River, Russia) (5).
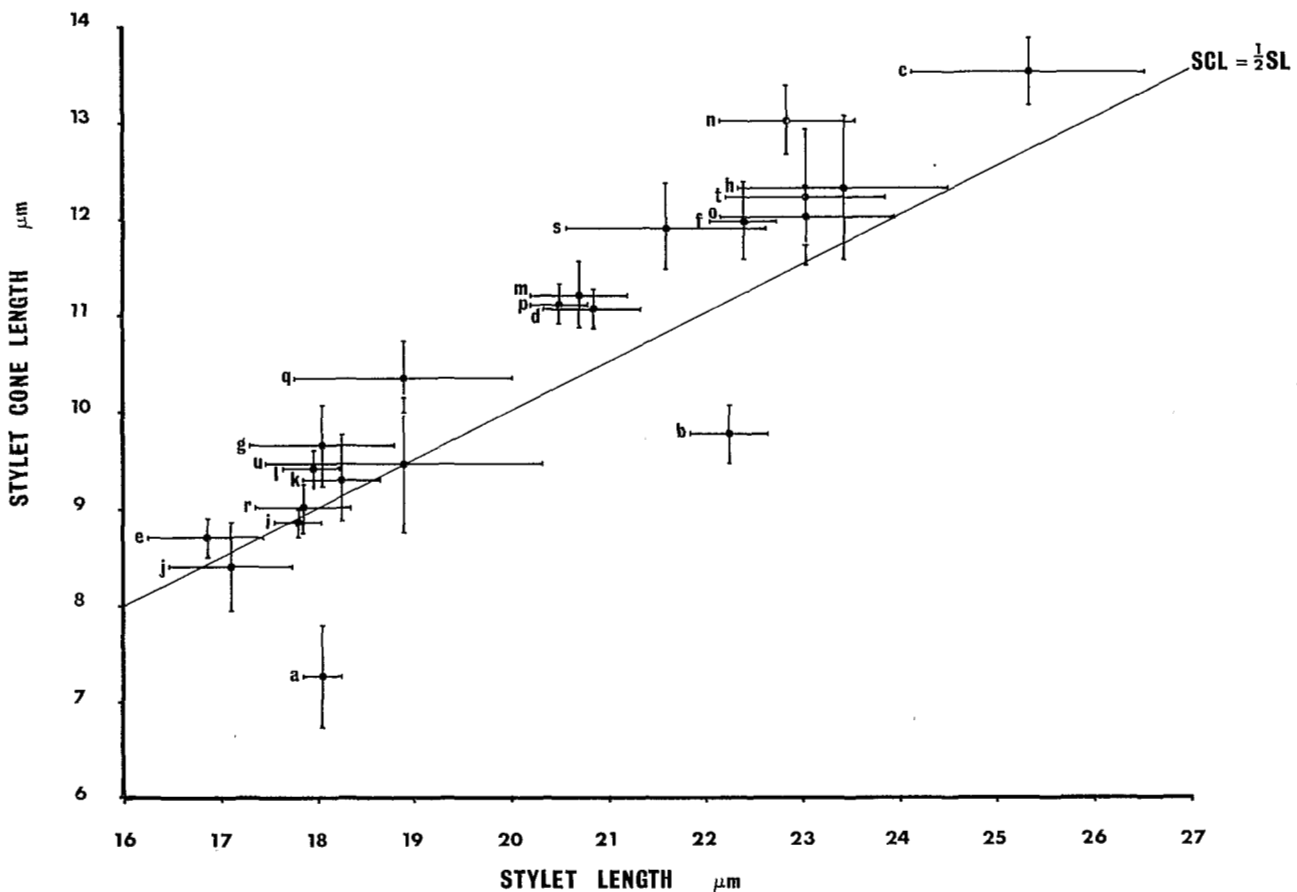
* Author's measurements, others from literature.

Fig. 1. Bivariate plot of stylet length/stylet cone length showing means and 95 % confidence limits for both variates in 19 species of *Meloidogyne*. a : *acronea*, b : *africana*, c : *ardenensis*, d : *arenaria*, e : *artiellia*, f : *camelliae*, g : *chitwoodi*, h : *grahami*, i : *graminicola* Bangladesh, j : *graminicola* India, k : *graminicola* Thailand, l : *graminis*, m : *hapla*, n : *incognita incognita*, o : *incognita wartellei*, p : *javanica*, q : *microtyla*, r : *naasi*, s : *ovalis*, t : *propora*, u : indescribed species (Russia).

Position of hemizonid relative to excretory pore : The hemizonid is anterior to the excretory pore in most species, although it may be close, or posterior to it as in *M. graminis* (some individuals), *M. hapla* (Norfolk population) and *M. graminicola* (some individuals).

. *Distance from excretory pore to anterior end :* This distance is correlated with body length, but exhibits greater intraspecific variation (Tab. 2) and is therefore less valuable as a species differentiating character.

Principal coordinate analysis : An analysis using nine characters and seventeen populations showed that the most important discriminating characters are, in decreasing order of importance, stylet length,

distance from stylet base to anterior end, distance from excretory pore to anterior end and body length. The distribution of species about the first four principal coordinates reinforced the view that quantitative characters are not exclusively discriminating.

Second-stage juveniles

Body length : Juvenile length is less variable than that of males (4 % on 400 μm as opposed to 12 % on 1 600 μm, Tab. 2) but there is a narrower overall range for the genus (275-650 μm, except for *M. spartinae* whose range extends to 912 μm) and overlap between species is considerable. While the use of 95 % confidence limits sometimes reduces this overlap, differentiation of species remains inadequate.

Stylet length : Stylet length, with a small coefficient of variation (4 % on 12 μm, Tab. 2) is a good character only when it is sufficiently well seen for consistent, accurate measurement. In many specimens the head skeleton obscures the end of the stylet and stylet length may be underestimated so it is better not to use stylet length unless the stylets are made to protrude by the method of Hooper (1977).

Distance from stylet base to anterior end : This is a more reliable character than stylet length and also exhibits a small coefficient of variation (5 % on 15 μm, Tab. 2). Careful preparation is essential to avoid displacement of the stylet by contraction of its musculature.

Stylet knob width and length : Measurement of these characters is of little aid in species determination. Overall shape is likely to be of more practical value as found in males and females, although this has not been investigated so far.

Distance of DGO from stylet base : Despite considerable variation (12 % on 4 μm, Tab. 2), species mean values fall between 2-3 μm, 3-4 μm and 4-5 μm and to this extent the use of 95 % confidence limits may differentiate groups of species. *M. sewelli* is so far unique with the DGO 7-8 μm behind the stylet base.

Distance of excretory pore to anterior end : While this character exhibits the same variation as body length (4 % on 70 μm and 4 % on 400 μm respectively, Tab. 2) there is a broader overall range in the genus and the use of 95 % confidence limits may improve differentiation of species.

Position of hemizonid relative to excretory pore : In most species the hemizonid is near or just anterior to the excretory pore, although it is posterior in some species (e.g. *M. ardenensis*, *M. graminis*).

Number of lateral field incisures : As in males the basic number of lateral field incisures is four, and likewise in the mid region of the body there may be additional incisures : e.g. *M. graminicola* (6), *M. hapla* (Norfolk population, some individuals) (5) and the undescribed species (Elva River, Russia) (6).

Tail length : Tail length is a good differentiating character with a small coefficient of variation (5 % on 50 μm, Tab. 2). Some species are clearly distinct from one another in overall range (e.g. *M. graminicola* and *M. naasi* with long tails, and *M. acronea* and *M. ardenensis* with short tails).

Tail shape : Tail shape is the most useful qualitative character and intraspecific variation is limited. The following species and subspecies are examples of groups with similar tail morphology (Jepson, in

press.) : *M. arenaria, M. chitwoodi, M. hapla, M. javanica, M. incognita incognita* and *M. incognita wartellei* ; *M. graminicola, M. oryzae* and *M. naasi* ; *M. indica, M. artiellia* and *M. propora*. Other species are unique such as *M. ardenensis*. Small differences occur within the groups which, when considered with other differential characters of limited variation such as tail length, distinguish component species.

Hyaline tail length : The hyaline tail length is often very variable, although in some species it is clearly short (e.g. *M. acronea* and *M. incognita incognita*) and in others long (e.g. *M. graminicola* and *M. naasi*).

Principal coordinate analysis : An analysis using ten characters and fourteen populations showed that, of discriminating characters the most important is tail length and less important are hyaline tail length and stylet knob width. As in a similar analysis with males, the distribution of species about the first four principal coordinates showed that even using a combination of characters most species are not mutually exclusive.

Females

Females have not yet been subjected to the same morphometric treatment as males and juveniles ; however, general views on characters used are presented here. Stylet length appears to be useful for differentiation because it exhibits limited intraspecific variation and there is a broad range, from 10-25 μm, in the genus. From SEM studies by Eisenback and Hirschmann (1980) and Eisenback *et al.* (1981) of *M. arenaria, M. incognita, M. javanica,* and *M. hapla* races, and my own which also include *M. ardenensis, M. chitwoodi* and *M. graminicola,* the female stylet cone, shaft and knobs shape appear to be species-specific. The differences in stylet knob dimensions are too small to be of practical value. The range of the distance from the DGO to the stylet base is broad in the genus (2-9 μm) and as in second stage juveniles, this character may aid differentiation of species groups. The position of the excretory pore relative to the base of the stylet may be of use in some species and subspecies, although it is very variable in others (e.g. *M. incognita incognita*).

The perineal patterns have been stressed in the past as the most important feature of a species. This continues to be the case but I consider that no suitable methods for assessing the pattern (such as that by Esser, Perry & Taylor, 1976) have yet been developed. If perineal patterns are to continue as major diagnostic features, it will be crucial to identify the essential features of a pattern which are stable, despite overall variation which may be considerable.

Summary of character assessment

The assessment of the characters listed in Table 2 indicates that combinations of measurements are useful as supporting data but are never unequivocal. The quantitative characters proposed are those of greatest practical value in terms of intraspecific variation and differentiation between species, the aim being to reduce the number of characters required for identification to a minimum. A realistic assessment of the morphological variation in a species should be made in terms of component isolates or populations. Where possible, in this study, quantitative measurements of more than one population of a species were made and, on these terms, only the characters most stable and apparently least influenced by environmental factors are suggested for use in identification. There is not a single character which can be consistently used to the exclusion of all others to immediately identify a species but combinations of those with low coefficients of variation and a broad range throughout the genus will serve to differentiate groups of species. Many species are alike in some of the characters, e.g. stylet knob width, stylet knob length, median bulb valve length ($\male\male$ & J2), position of hemizonid relative to excretory pore ($\male\male$ & J2), number of lateral field incisures ($\male\male$ & J2), and position of excretory pore relative to stylet base ($\female\female$), and it is in the few exceptions that the value of the character is manifest.

Conversely, qualitative characters are frequently species-specific and good examples are to be found in all three stages : male head shape, male and female stylet shape, perineal pattern and juvenile tail shape. *Additional characters of this type have been found with the SEM* (Eisenback & Hirschmann, 1979, 1980 ; Eisenback, Hirschmann & Triantaphyllou, 1980 ; Eisenback *et al.*, 1981) although these are of limited value in the field because the SEM is not available to all workers.

It is essential, as the number of species in the genus increases beyond 50, that future descriptions are standard in the characters used, the way in which these are measured and the illustration of certain features (particularly with the use of DIC light micrographs and SEM photographs), and that direct comparison is made by illustration, with other populations and species known to be closely related or of similar morphology.

LIGHT MICROSCOPY OF MALES AND A KEY TO THEIR IDENTIFICATION

The characters illustrated are typical for the species and provide a basis for comparison. Where possible

the stability of qualitative characters was confirmed by examination of specimens from several different populations. The key uses mainly qualitative characters but with a limited number of measurements. The range of variation in quantitative characters is often broad with considerable species overlap although 95 % confidence limits are narrow with much less overlap. For this reason, measurements are quoted with confidence limits. Figure 1 showing 95 % confidence limits of stylet length/stylet cone length of males illustrates this, although it should be stressed that except for the three populations of *M. graminicola*, species means are taken from single populations. Addition of data from other populations may extend the range of each species as occured with *M. graminicola*.

On the basis of head shape alone, the male heads of the species of *Meloidogyne* considered here form three groups with the exception of four of the 24. The exceptions are three very distinct species *M. acronea*, *M. javanica* and *M. propora*, and also *M. arenaria*, although this resembles species in one of the larger groups.

M. acronea is distinguished by the unique form of the stylet (Fig. 8). The cone is broad and the shortest of any known *Meloidogyne* species at < 8 μm long (95 % c.l.), with a mean stylet length of 18.06 ± 0.19 μm (95 % c.l.). The stylet knobs are large and pear shaped, resembling those of *M. ardenensis*, but taper onto a much broader shaft (Figs 28 & 29). The distal opening of the stylet lumen is frequently obvious. The head outline is ill-defined with a shallow labial cap and single deep post-labial annule.

M. javanica (Figs 9 & 10) is recognised by the broad, flattened labial cap. The median lips are strongly rounded and meet the labial disc at a small indentation on either side of the raised rim of the stoma. The stylet knobs are transversely ovoid and the stylet length 20.5 ± 0.29 μm.

The distinguishing features of *M. propora* (Fig. 11) are the deep, dome shaped labial cap with the rim of the stoma raised, the shallow single post-labial annule and the long slender stylet (23.5 ± 0.83 μm) with large, broad rounded knobs.

The main groups will be referred to as Group 1-5 respectively. Group 1 is distinguished by an offset head (Fig. 3), in which the outline of the head does not form a smooth contour with the rest of the body and the neck is more constricted than in those individuals without the head offset. The post-labial annule is approximately three times deeper than the labial annule. Three species constitute the group. *M. indica* and *M. artiellia* can be distinguished from *M. camelliae* using stylet length (≤ 18.0 μm in *M. indica* and *M. artiellia*, and > 21.0 μm in *M. camelliae*), also the narrower head (< 9.0 μm) in
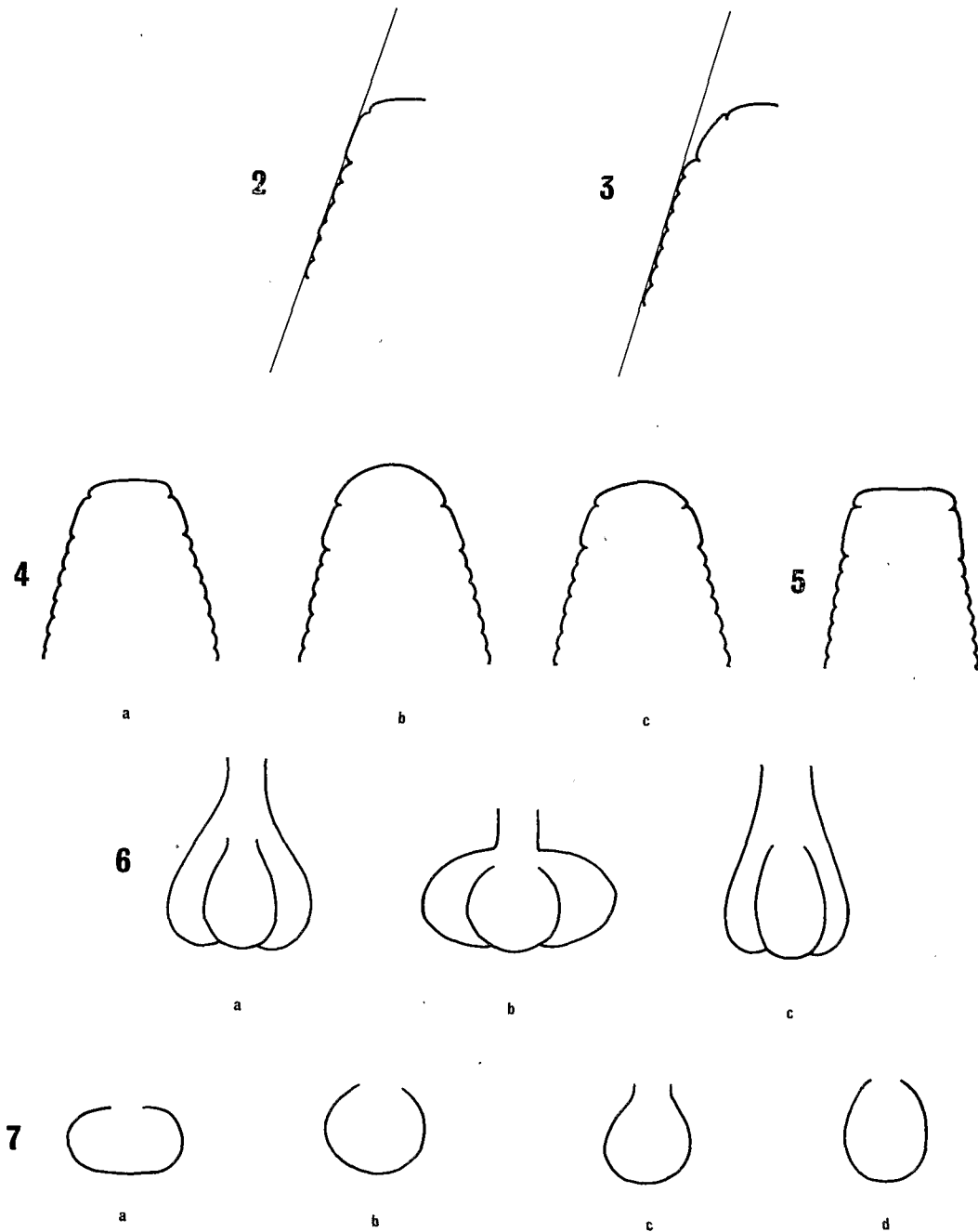
Figs. 2-7. Character of male head and stylet knob shape. 2 : head not offset ; 3 : head offset ; 4 : labial cap much narrower across than post-labial annule, post-labial annule distinctly tapering anteriorly, head generally rounded, a : labial cap flattened ; b : labial cap distinctly domed ; c : labial cap shallowly rounded ; 5 : head truncate ; 6 : stylet knobs and shaft, a : knobs backwardly sloping ; b : knobs set off from shaft ; c : knobs gradually tapering onto shaft ; 7 : shape of individual stylet knobs, a : transversely ovoid ; b : rounded ; c : pear shaped ; d : elongate.
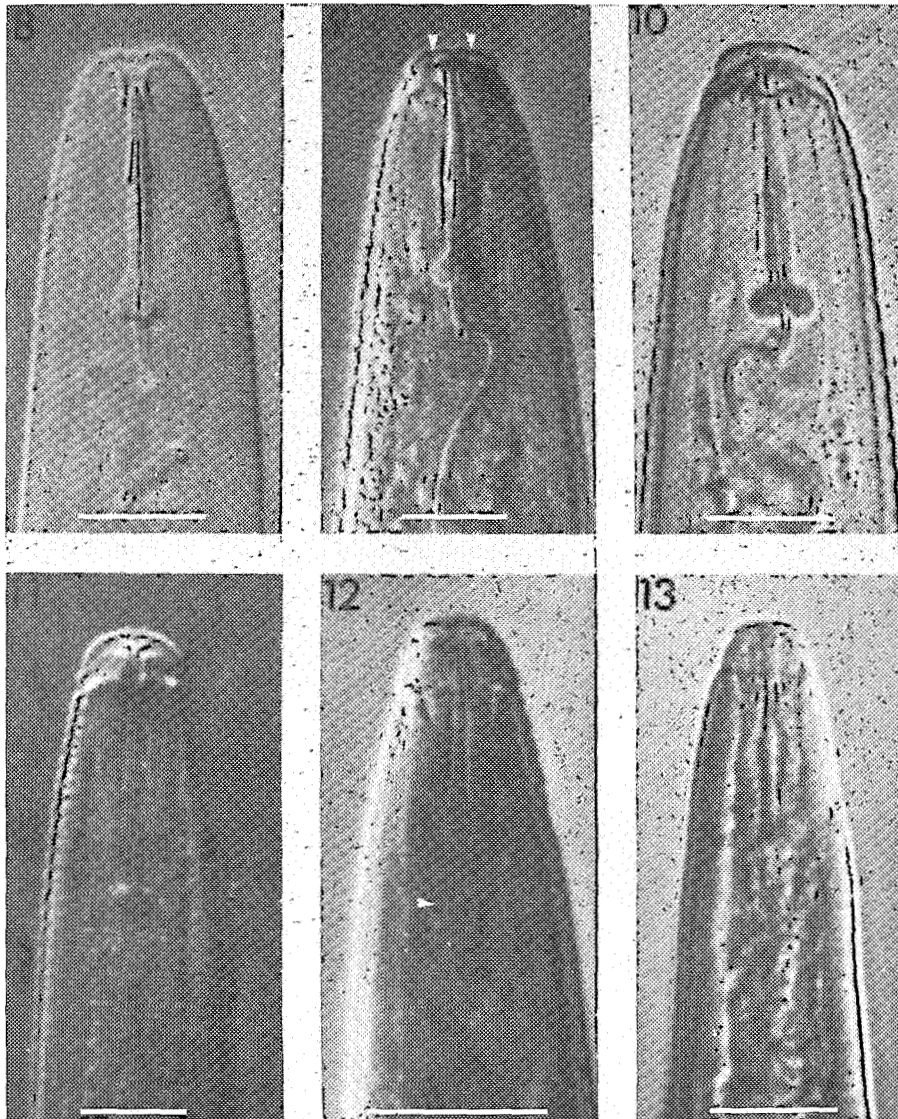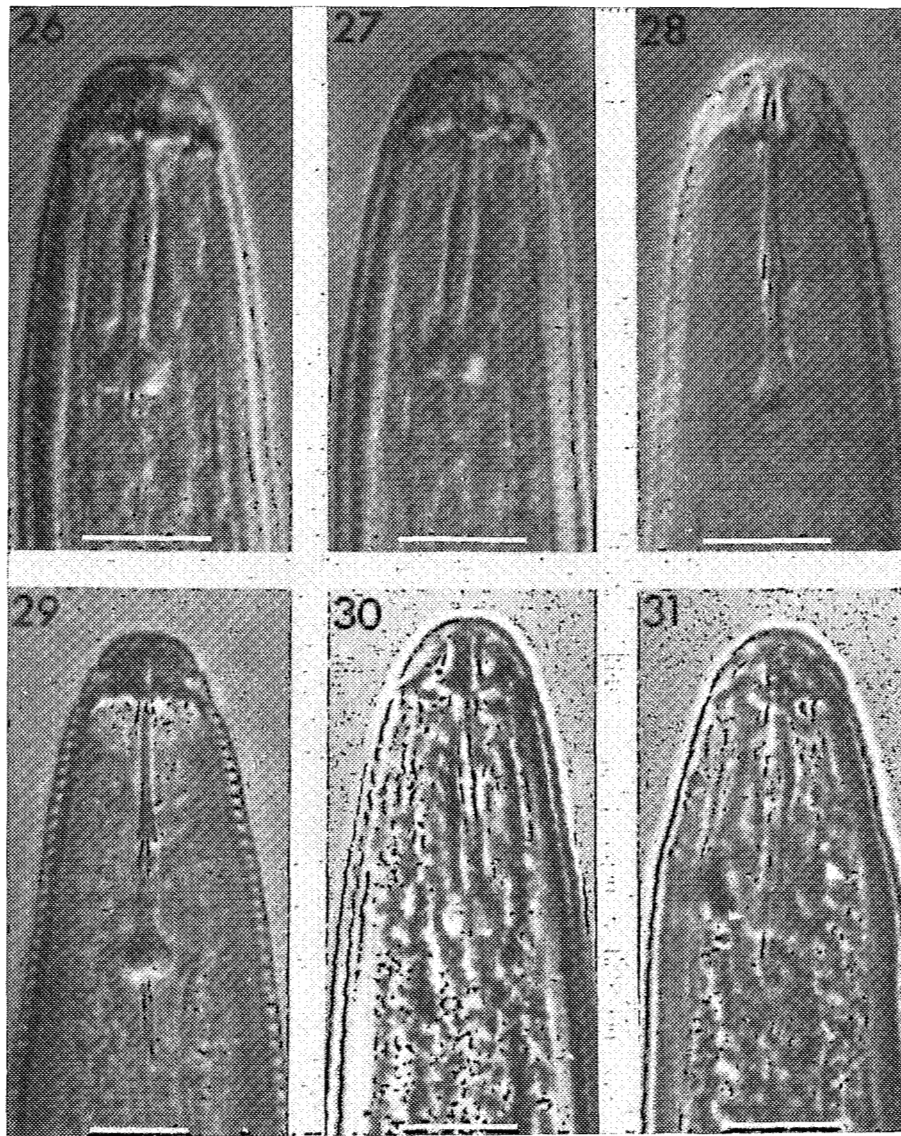
Figs. 8-13. Anterior ends of *Meloidogyne* males. 8 : *M. acronea ;* 9 : *M. javanica ;* arrows = indentations on labial cap ; 10 : *M. javanica ;* 11 : *M. propora ;* 12 : *M. indica,* arrow = stylet knobs ; 13 : *M. artiellia.* Bar = 10 μm.
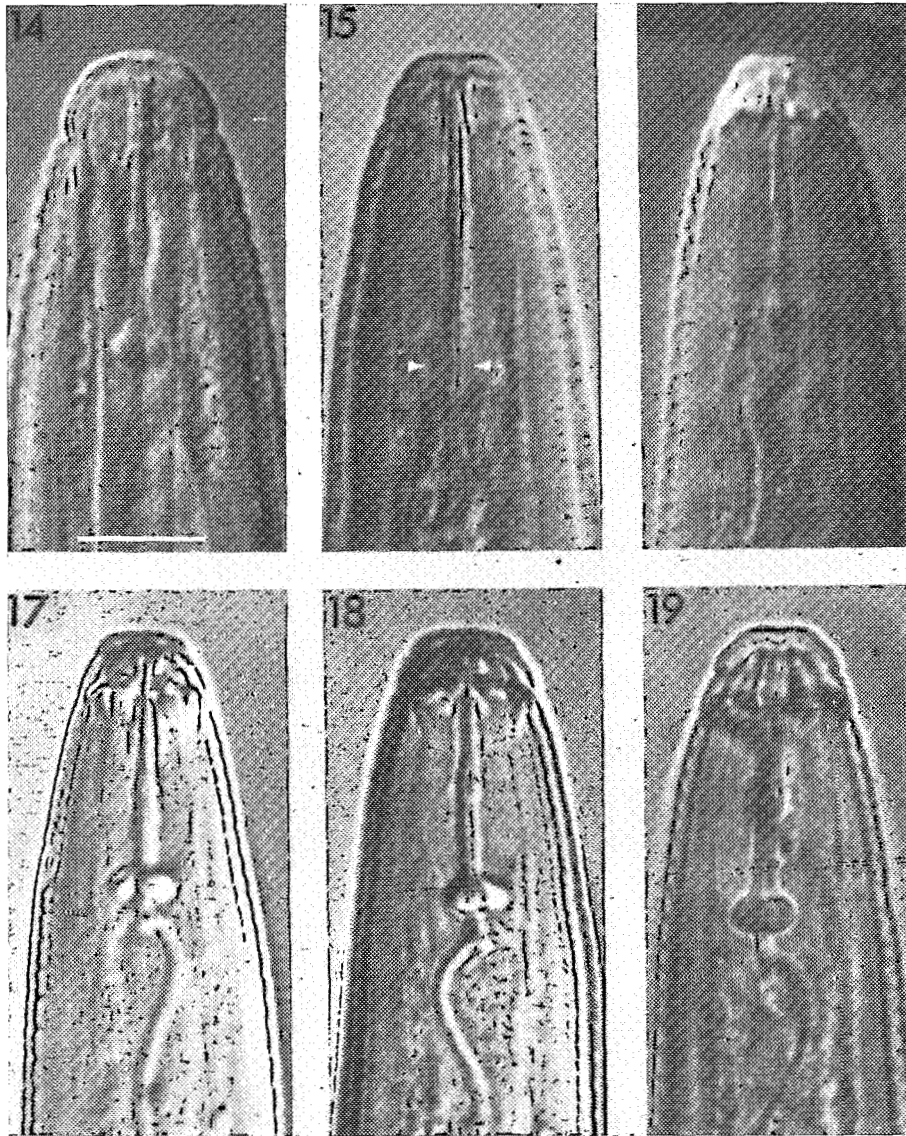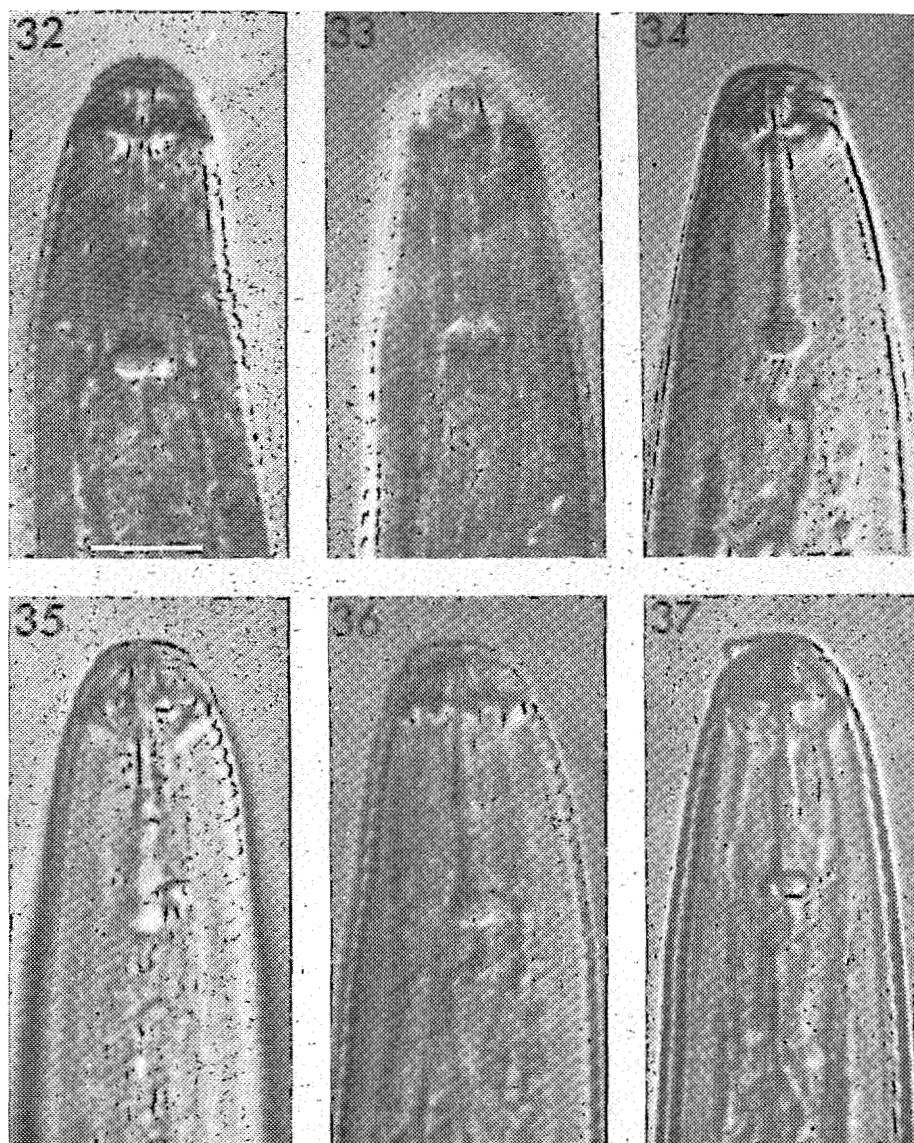
Figs. 14-19. Anterior ends of *Meloidogyne* males. 14 : *M. camelliae* ; 15 : *M. africana* ; arrows = stylet knobs ; 16 : *M. microtyla* ; 17 : *M. graminis* ; 18 : *M. graminis* ; 19 : *M. incognita incognita*. Bar = 10 μm.
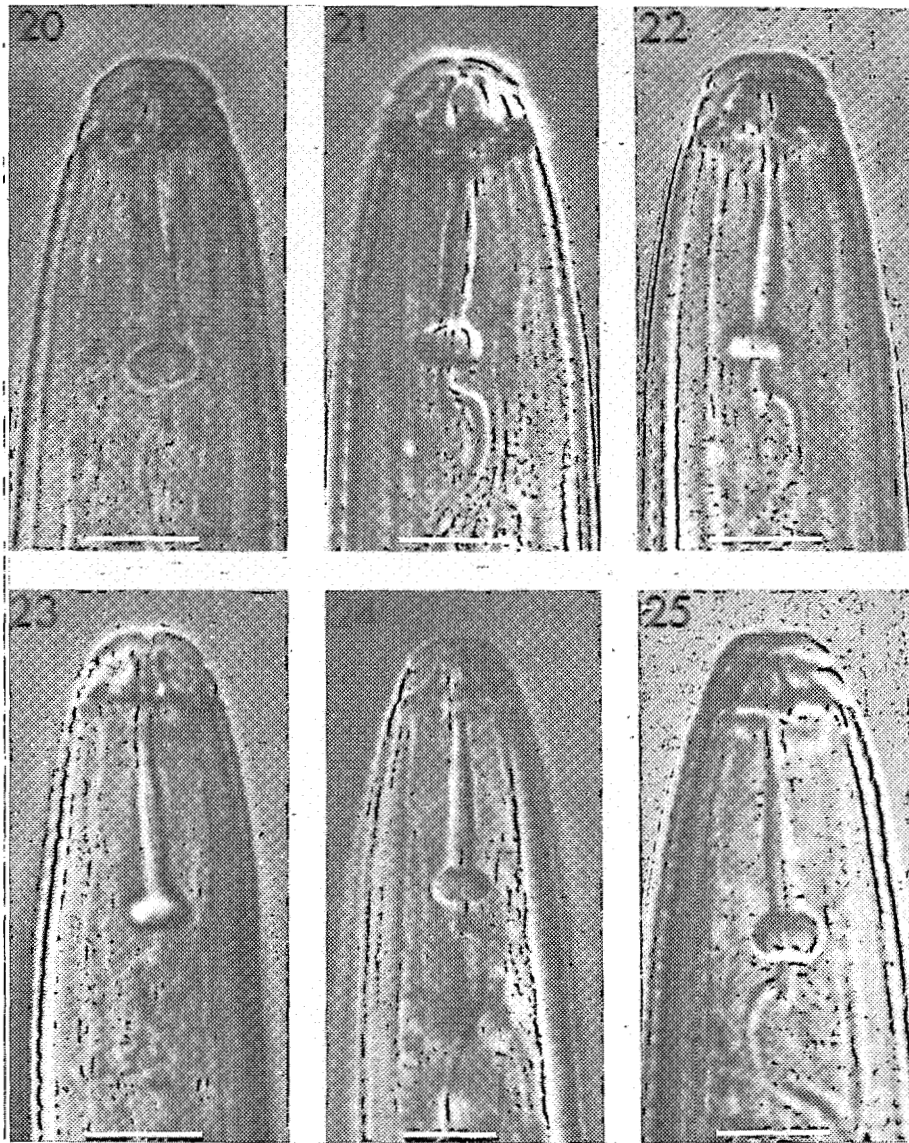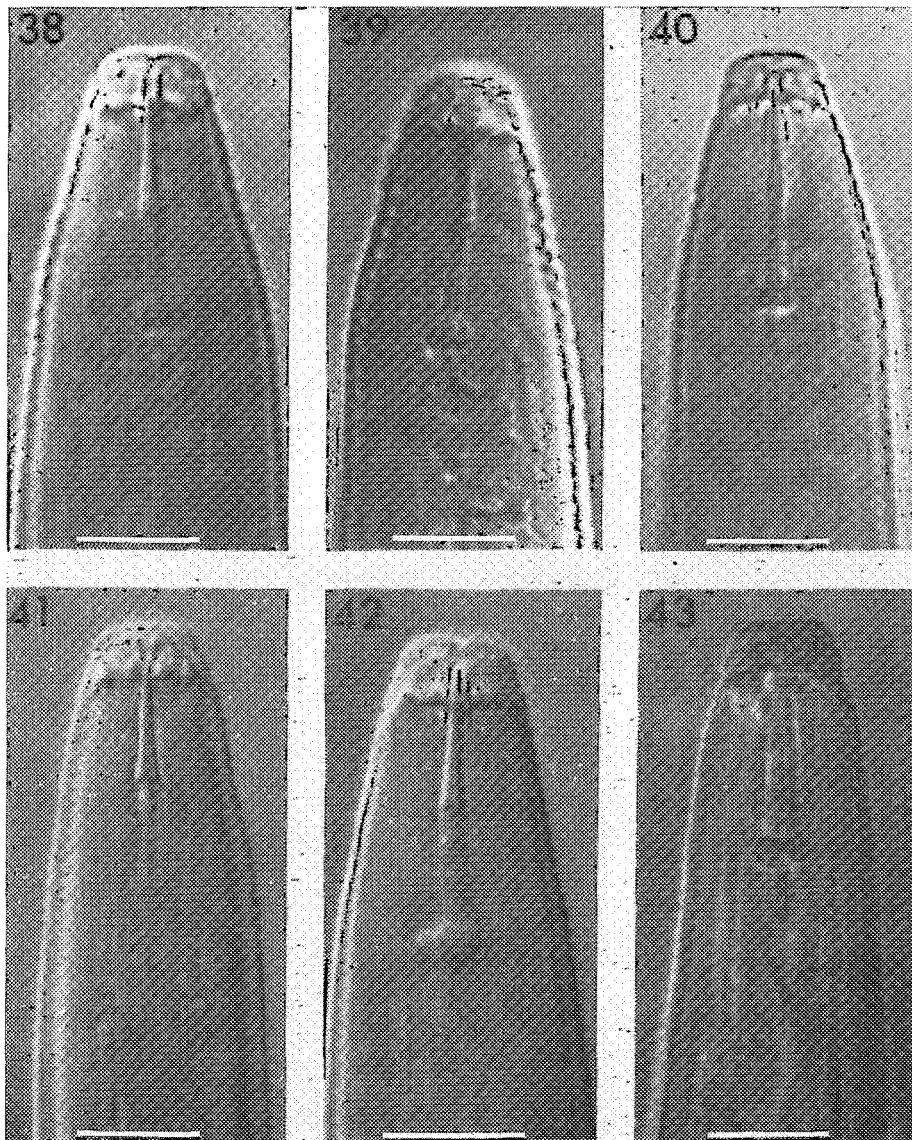
Figs. 20-25. Anterior ends of *Meloidogyne* males. 20 : *M. incognita incognita* ; 21 : *M. incognita incognita* ; 22 : *M. incognita incognita* ; 23 : *M. incognita wartellei* ; 24 : *M. incognita wartellei* ; 25 : *M. incognita wartellei*. Bar = 10 μm.

Figs. 26-31. Anterior ends of *Meloidogyne males*. 26 : *M. grahami* ; 27 : *M. grahami* ; 28 : *M. ardenensis* ; 29 : *M. ardenensis* ; 30 : *M. megatyla* ; 31 : *M. megatyla*, arrow = stylet knobs. Bar = 10 μm.

Figs. 32-37. Anterior ends of *Meloidogyne* males. 32 : *M. ovalis ;* 33 : *M. ovalis,* to show stylet knobs ; 34 : *M. arenaria ;* 35 : *M. hapla* (Norfolk), to show head shape ; 36 : *M. hapla* (Norfolk) ; 37 : *M. hapla* (Canada). Bar = 10 μm.
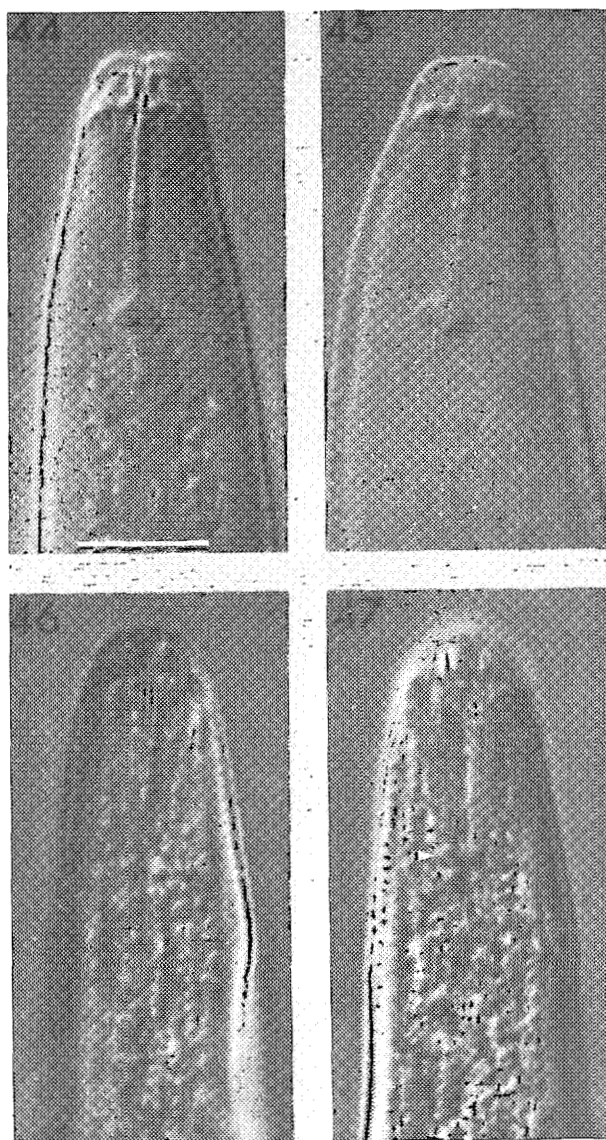
Figs. 38-43. Anterior ends of *Meloidogyne* males. 38 : *M. chitwoodi*, to show head shape ; 39 : *M. chitwoodi*, to show stylet knobs ; 40 : *M. chitwoodi* ; 41 : *M. naasi* ; 42 : undescribed species (Russia) ; 43 : *M. oryzae*. Bar = 10 μm.

M. indica and *M. artiellia*, and the shape of the post-labial annule which is distinctly rounded in *M. camelliae. M. artiellia* and *M. indica* are very similar but are distinguished by one post-labial annule in *M. artiella* and two in *M. indica* (Figs 12 & 13).

Twelve species and subspecies of Group 2 have a similar head outline which is anteriorly tapering and rounded (Fig. 4a, b, c) : *M. africana, M. microtyla, M. graminis, M. oryzae, M. incognita incognita, M. incognita wartellei, M. grahami, M. hapla, M. chitwoodi, M. ardenensis, M. megatyla* and *M. ovalis*. This group is further subdivided into three subgroups on the shape of the labial cap, *i.e.* flat ; rounded ; domed. Using qualitative characters of the head and stylet, as well as stylet length and distance from the DGO to the stylet base, most of the species can be readily distinguished. *M. graminis, M. microtyla* and *M. oryzae* are particularly close but *M. graminis* and *M. oryzae* can be separated from *M. microtyla* using stylet knob shape (Figs 16, 17, 18 & 43). *M. graminis* is distinguished from *M. oryzae* by the distance from the DGO to the stylet base (2.5 μm in *M. graminis* and 5.0 μm in *M. oryzae*). It is notable that these three species are parasites of Graminae, *M. graminis* and *M. microtyla* also sharing very similar perineal patterns. *M. incognita incognita, M. incognita wartellei* and *M. grahami* appear together at two points in the key because the labial cap may be interpreted as being flat or shallowly rounded (Figs 19-27). These species are not separable from each other by qualitative characters alone and the slight quantitative differences between them may be due to intraspecific variation. The specific status of *M. grahami* should be further tested. In female chromosome compliment and behaviour it appears to be host race 4 and chromosome race A of *M. incognita incognita* (Triantaphyllou, 1981).

Group 4, again based on a similarity in the head outline, which is truncate, includes five species ; *M. graminicola, M.* sp. (undescribed, Russia), *M. naasi, M. decalineata* and *M. exigua*. Except for *M. decalineata* and *M. exigua* which parasitize *Coffea arabica* the others parasitize Graminae. Quantitative characters are difficult to use in the group because all the species are very similar in this respect. However, a combination of stylet length and body length may aid differentiation between *M. graminicola, M. graminis* and the rest, and *M. decalineata* from the rest. Members of the group are distinguishable on the basis of stylet knob shape. *M. graminicola* and *M. exigua* have almost identical pear shaped knobs (Figs 44 & 45). The undescribed species from Russia has rounded, but somewhat transversely ovoid knobs, set off from the shaft. *M. decalineata* has strongly transversely ovoid knobs (Fig. 47). *M. naasi* has very small rounded knobs (Fig. 41). If the host

Figs. 44-47. Anterior ends of *Meloidogyne* males. 44 : *M. graminicola ;* 45 : *M. exigua ;* 46 : *M. decalineata ;* to show head shape ; 47 : *M. decalineata*, arrow = stylet knobs. Bar = 10 μm.

is known then *M. exigua* and *M. decalineata* can be identified without resorting to other stages.

M. arenaria (Fig. 34) constitutes a separate category, Group 3, since the outline of the head exhibits features contained in Groups 2 and 4. The labial cap is broad as in Group 4 but without the truncate appearance, and is rounded as in Group 2 but without tapering anteriorly.

Key to *Meloidogyne* males
(Laretal views only)

1. Head offset and narrower than body (Fig. 3), post-labial annule about three times deeper than labial annule : Group 1 2
— Head not offset (Fig. 2) 4

2. Stylet length ⩽ 18.0 μm, stylet cone length < 9.0 μm, head width at base < 9.0 μm 3
— Stylet length > 21.0 μm, post-labial annule distinctly rounded at sides ....
.... *camelliae* (Fig. 14) (Group 1)

3. Two post-labial annules ..... *indica* (Fig. 12) (1)
— Single post-labial annule .... *artiellia* (Fig. 13) (1)

4. Labial cap much narrower across than post-labial annule, post-labial annule distinctly tapering anteriorly, head generally appearing somewhat rounded : Group 2 (Fig. 4) 5
— Labial cap almost as broad across as post-labial annule 17

5. Labial cap flattened (Fig. 4a) 6
— Labial cap rounded or domed (Fig 4b & c) 12

6. Stylet cone distinctly shorter than shaft 7
— Stylet cone not distinctly shorter than shaft ... 8

7. Stylet cone > 9.0 μm and < 10.2 μm, stylet length > 20.0 μm, stylet knobs transversely ovoid (Fig. 7a) .... *africana* (Fig. 34) (2)
— Stylet cone broad and < 8.0 μm, stylet knobs large and pear shaped (Fig. 7c) ..... *acronea* (Fig. 8)

8. Stylet length < 20.0 μm 9
— Stylet length > 21.0 μm 11

9. Stylet knobs very small and rounded (Fig. 7b) ....
.... *microtyla* (Fig. 16) (2)
— Stylet knobs transversely ovoid (Fig. 7a) 10

10. DGO 2.5 μm ($\bar{x}$) ..... *graminis* (Figs 17 & 18) (2)
— DGO 5.0 μm ($\bar{x}$) .... *oryzae* (Fig. 43) (2)

11. Sides of labial cap chamfered ....
.... *incognita incognita* (Figs 19-22) (2)
.... *incognita wartellei* (Figs 23-25) (2)
.... *grahami* (Figs 26 & 27) (2)
— Sides of labial cap not chamfered 14

12. Labial cap shallowly rounded (Fig. 4c) 13
— Labial cap distinctly domed (Fig. 4b) 15

13. Stylet length > 20.0 μm 14
— Stylet length < 19.0 μm, stylet knobs rounded (Fig. 7b) and backwardly sloping (Fig. 6a) (sometimes irregular), median lips rounded, labial annule and post-labial annule of almost equal depth, post-labial annule straight sided ....
.... *chitwoodi* (Figs 38-40) (2)

14. Stylet length < 21.5 μm, stylet knobs rounded (Fig. 7 b) and set off from shaft (Fig. 6b) ....
.... *hapla* (Figs. 35-37) (2)
— Stylet length > 22.0 μm ....
.... *incognita incognita* (Figs 19-22) (2)
.... *incognita wartellei* (Figs 23-25) (2)
.... *grahami* (Figs 26 & 27) (2)

15. Stylet length > 24.0 μm, stylet knobs pear shaped (Fig. 6c) .... *ardenensis* (Figs 28 & 29) (2)
— Stylet length < 24.0 μm 16

16. Stylet length ⩾ 22.8 μm and stylet knobs large, rounded and slightly elongate (Fig. 5d) ....
.... *megatyla* (Figs 30 & 31) (2)
— Stylet length < 22.8 μm ....
.... *ovalis* (Figs 32 & 33) (2)

17. Deep, dome shaped labial cap with rim of stoma slightly raised, shallow single post-labial annule, long slender stylet with large broad knobs ....
.... *propora* (Fig. 11)
— Labial cap not distinctly domed, labial and post-labial annule of about equal depth 18

18. Labial cap rounded, stylet knobs rounded and gradually tapering onto shaft (Fig. 6c), DGO > 5.0 μm : Group 3 ...... *arenaria* (Fig. 34) (3)
— Labial cap flat with slight depression at stoma ....
.... 19

19. Median lips strongly rounded and distinct indentation on either side of the raised rim of the stoma, stylet knobs transversely ovoid (Fig. 7a), stylet length > 20.0 μm .... *javanica* (Figs 9 & 10)
— Median lips not strongly rounded, head appearing truncate (Fig. 5) stylet length ⩽ 21.0 μm : Group 4 20

20. Graminaceous host 21
— Coffee host (N.B. *M. exigua* may also parasitize other non-graminaceous hosts) 23

21. Stylet knobs very small (overall width ⩽ 3.2 μm ($\bar{x}$)) and round .... *naasi* (Fig. 41) (4)
— Stylet knobs wider than 4.5 μm ($\bar{x}$) 22

22. Stylet knobs pear shaped and backwardly sloping (Fig. 7c & 6a) .... *graminicola* (Fig. 44) (4)
— Stylet knobs set off from shaft, rounded and somewhat transversely ovoid ....
.... undescribed species (Russia) (Fig. 42) (4)

23. Stylet knobs pear shaped (Fig. 7c) ....
.... *exigua* (Fig. 45) (4)
— Stylet knobs strongly transversely ovoid (Fig. 7a).
.... *decalineata* (Figs 46 & 47) (4)

REFERENCES

BIRD, A. F. (1979). Ultrastructure of the tail region of the second stage preparasitic larva of the root-knot nematode. *Int. J. Parasit.*, 9 : 357-370.

COURTNEY, W. D., POLLEY, D. & MILLER, V. L. (1955). TAF, an improved fixative in nematode technique. *Pl. Dis. Reptr*, 39 : 570-571.

EISENBACK, J. D. & HIRSCHMANN, H. (1979). Morphological comparison of second-stage juveniles of six populations of *Meloidogyne hapla* by scanning electron microscopy. *J. Nematol.*, 11 : 5-15.

EISENBACK, J. D. & HIRSCHMANN, H. (1980). Morphological comparison of *Meloidogyne* males by scanning electron microscopy. *J. Nematol.*, 12 : 23-32.

EISENBACK, J. D., HIRSCHMANN, H. & TRIANTAPHYLLOU, A. C. (1980). Morphological comparison of *Meloidogyne* female head structures, perineal patterns and stylets. *J. Nematol.*, 12 : 300-313.

EISENBACK, J. D., HIRSCHMANN, H., SASSER, J. N. & TRIANTAPHYLLOU, A. C. (1981). A guide to the four most common species of root-knot nematodes *(Meloidogyne)*. A Coop. Public. Depts Pl. Pathol. & Genetics N. Carolina St. Univ. and U.S.A.I.D. : 1-48.

ELMILIGY, I. A. (1968). Three new species of the genus *Meloidogyne* Gœldi, 1887 (Nematoda : Heteroderidae). *Nematologica*, 14 : 577-590.

ESSER, R. P., PERRY, V. G. & TAYLOR, A. L. (1976). A diagnostic compendium of the genus *Meloidogyne* (Nematoda : Heteroderidae). *Proc. helminth. Soc. Wash.*, 43 : 138-150.

FRANKLIN, M. T. (1978). *Meloidogyne*. In : Southey, J. F., (Ed.), *Plant Nematology*, London, MAFF, HMSO, 98-124.

GOWER, J. C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*, 53 : 325-338.

Accepté pour publication le 16 février 1982.

HOOPER, D. H. (1977). Spicule and stylet protrusion induced by ammonia solution in some plant and soil nematodes. *Nematologica*, 23 : 126-127.

ITO, Y., OHSHIMA, Y. & ICHINOHE, M. (1969). A root knot nematode *Meloidogyne mali* n. sp. on Apple Tree from Japan (Tylenchida : Heteroderidae). *Appl. Entom. Zool.*, 4 : 194-202.

JEPSON, S. B. (in press). The use of second stage juvenile tails as an aid in the identification of *Meloidogyne* species. *Nematologica*.

SOUTHEY, J. F., Ed., (1970). *Laboratory methods for work with plant and soil nematodes.* London, MAFF, HMSO, Tech. Bull. n° 2, p. 1-148.

TAYLOR, A. L. & SASSER, J. N. (1978). Biology, identification and control of root-knot nematodes *(Meloidogyne* species). A Coop. Public. of the Depart. Pl. Pathol. N. Carolina St. Univ. and USAID, 111 p.

TRIANTAPHYLLOU, A. C. (1979). Cytogenetics of root-knot nematodes. In : Lamberti, F. & Taylor, C. E., (Eds), *Root knot nematodes* (Meloidogyne *species*), *Systematics, biology and control.* New York, Academic Press : 85-109.

TRIANTAPHYLLOU, A. C. (1981). Oogenesis and the chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita. J. Nematol.*, 13 : 95-104.

WHITEHEAD, A. G. (1968). Taxonomy of *Meloidogyne* (Nematodea, Heteroderidae) with descriptions of four new species. *Trans. zool. Soc. London*, 31 : 263-401.