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Morphological and morphometrical characterization of *Meloidogyne incognita* from different host plants in Kerala, India

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Abstract

The study has been focused on the intra specific morphological variability of *Meloidogyne incognita* from different host plants in Kerala. On the basis of different levels of coefficient of variability for morphometric characters of mature females and perineal pattern, the characters body length, body width, neck length, length of median bulb, width of median bulb, length of vulval slit and anus to tail terminus were rated as moderately variable characters (CV 4.31% to 18.41%). In second stage juveniles, the characters anal body width and ratio c' (tail length/ABW) were found to be moderately variable (CV 2.25% to 10.15%) whereas ratio c (length/tail length) was found to be highly variable (CV 4.43% to 12.15%). The characters like stylet length, ratio a (body length/body width), anus to vulval slit and interphasmidial distance of mature females and body length, stylet length, head to median bulb and tail length of second stage juveniles were recorded as least variable characters (CV 1.36% to 10.54%) and found useful in characterizing the species. Most of the characters were in conformity with the type description of *M. incognita* except for some variations, which have been discussed.

Keywords: intraspecific; morphological; morphometrical; *Meloidogyne incognita*; Kerala

Introduction

Root knot nematodes (*Meloidogyne* spp.) are considered to be the most wide spread and destructive plant parasites which cause a yield loss of 25 to 50 per cent over large areas of cultivated land ^[1]. In India, vegetables and banana are adversely affected by these nematodes and cause a yield loss of 16.675 per cent, 14.10 per cent, 27.21 per cent and 12.3 per cent in brinjal, okra, tomato and banana respectively ^[2]. The attacks by these nematodes also pay way to infection by secondary pathogens which further increase the severity of damage.

The lack of awareness about the existence of these nematodes and the characteristic symptom they cause, make these nematodes often neglected by the farmers. The knowledge about the species often involves identification of the species and better species descriptions to tackle the problem of unawareness. Morphological analyses including perineal patterns are considered important for differentiating *Meloidogyne* species. Morphological and morphometric studies are useful in identification of root-knot nematodes from different crops and locations which in turn help in species specific management. Moreover, it will also help in documenting the root knot nematode diversity. Varietal resistance is reported in some crops and identification of nematode species in an area will help in selection of plant varieties resistant to that species for cultivation and thus, effective management practices can be developed by precise identification of nematode species and variation within single nematode species ^[3]. Thus, the objective of this study was aimed at the intraspecific morphological diversity of *M. incognita* populations in Kerala in order to assess the stability of various taxonomic characters of this species.

Materials and Methods

Root knot infested root and soil samples were collected from brinjal, bhindi, tomato and banana in Thiruvananthapuram, Idukki and Thrissur districts of Kerala. About 250 cc of soil sample and 5 g of root were collected from the rhizosphere area of crops in polythene covers and labelled carefully. Nematodes were extracted from soil using Cobb's decanting and sieving method followed by Baermann's funnel technique. The juveniles were then killed and fixed ^[4]. Permanent slides of processed nematodes were prepared.

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The roots were washed thoroughly, cut into small pieces and stained in lactophenol-acid fuchsin [5] and left overnight immersed in clear lactophenol for destaining. The adult females were teased out from stained roots and each specimen was placed in a drop of lactophenol on a glass slide. The posterior portion of the female was cut with a sharp blade and the inner tissue was carefully removed using a fine bristle [6]. The perineal pattern was transferred to a drop of glycerol on a glass slide and covered with cover slip and sealed using a suitable sealant. Ten specimens from a population were examined.

The measurements of body length, width, neck length, stylet length, length of median bulb (LMB), width of median bulb (WMB) and ratio a (body length/body width) of females and length of vulval slit (LVS), distance from anus to vulval slit (AVS), distance from anus to tail terminus (ATT) and interphasmid distance (IPD) of perineal pattern were taken. For second stage juveniles, measurements of body length, stylet length, distance from head to median bulb (H-MB), anal body width (ABW), tail length, ratio c (length/tail length) and c' (tail length/ABW) were made under a 40X microscope with calibrated ocular micrometer. The arithmetic mean, standard error of mean (SEM), standard deviation (SD) and coefficient of variance (CV) for each measurement were computed. Based upon CV values, the characters were rated as least variable, moderately variable, and highly variable, using scale < 12%, 12% to 20%, and >20% for female, respectively, and <8%, 8% to 12%, and > 12% for juveniles (J₂), respectively [7].

Results and Discussion

The following are results on morphological and morphometric data of the mature females, perineal pattern and second stage juveniles of four populations of *M. incognita* from Kerala.

Description: Based on 10 females and 10 juveniles (J₂).

Measurements: Female: Body length= 546.42- 789.27 µm; body width= 388.96-570.41 µm; a= 1.28-1.56 µm; stylet length= 13.64-17.90 µm; neck length=151.47-206.72 µm; LMB= 30.55-42.96 µm; WMB= 26.46-42.02 µm.

Perineal pattern: LVS= 20.68-27.90 µm; AVS=17.82-20.81 µm; ATT=14.98-21.85µm; IPD= 23.70-30.79 µm.

Second stage juveniles (J₂): Body length=368.53-423.19 µm; stylet length=13.25-15.67 µm; H-MB=45.84-56.70 µm; ABW=9.88-12.17 µm; tail length=47.59-55.30 µm; c=6.85-10.00 µm; c'= 4.21-5.71 µm.

Host plant: Brinjal, okra, tomato and banana

Locality: Thiruvananthapuram, Idukki and Thrissur districts

1. Morphological characters of four populations of *M. incognita*

The following were the morphological characters of populations of *M. incognita* from Kerala

A. Mature female

The females were pear shaped to saccate shaped with no posterior terminal protuberance. The neck was straight to curved to sickle shaped, directed ventrally or at an angle to the body of nematode. The shape and size of females were variable within and between the populations. The stylet cone was curved to straight at dorsal region and the knobs were set off from the shaft.

B. Perineal pattern

The perineal pattern was characterized by high, squared dorsal arch. There was distinct whorl of striae near the tail terminal area. The strike was wavy in majority of population, whereas it was smooth in some populations.

C. Second stage juveniles (J₂)

In J₂, the character body length was least variable. The labial region was not set off. The basal knobs of stylet were rounded and set off. The shape and size of rectal sac was variable between the populations and the rectal sac was prominent in few populations.

ii. Morphometric characters of populations of *M. incognita* from Kerala.

A. Mature female (Table 1)

The range for mean values of body length and body width in the four populations were 618.49 to 676.47 µm and 420.23 to 478.21 µm, respectively, with the maximum length and width being in banana population. The character body length and width showed maximum variation within the population in okra which was up to 15.18 per cent for body length and 16.19 per cent for width.

The character neck length was moderately variable with maximum variation in okra population (CV 12.404%). The maximum mean value for neck length was observed in banana population (194.00 µm) and the least in tomato population (173.90 µm). Stylet length showed least variation among the populations in their mean values and the maximum mean value was observed in banana population (16.59 µm).

The size of the median bulb varied from 35.07 to 41.10 µm and 33.62 to 40.13 µm in length (LMB) and width (WMB) respectively, with maximum length and width in banana population. The ratio 'a' was least variable character with the maximum mean value for tomato population (1.47 µm) and least for brinjal population (1.41 µm).

Table 1: Morphometric characters of mature females of *M. incognita* in four crops in Kerala, [Mean ± SD ± SE; (range); CV%], N=10

Character (µm)	Populations*				CR**
	Brinjal	Okra	Tomato	Banana	
Body length	664.59±42.23±21.11 (620.14-714.18) 6.35%	657.60±99.84±49.92 (546.42-789.27) 15.18%	^a 618.49±56.25±25.15 (550.29-702.19) 9.09%	^b 676.47±58.57±26.19 (606.71-740.06) 8.65%	MV
Body width	470.24±34.98±17.49 (439.38-515.82) 7.44%	464.91±75.31±37.65 (396.54-570.41) 16.19%	^a 420.23±25.70±11.49 (388.96-453.24) 6.11%	^b 478.21±30.84±13.79 (447.93-525.77) 6.44%	MV
Neck length	186.76±14.04±6.28 (167.89-206.72) 7.52%	174.89±21.69±10.84 (151.47-201.24) 12.40%	^a 173.90±12.85±5.74 (161.50-195.43) 7.39%	^b 194.00±8.78±4.39 (183.24-201.34) 4.52%	MV
Stylet length	16.44±1.11±0.50 (15.44-17.90) 6.80%	^a 15.15±1.43±0.71 (13.64-16.88) 9.47%	15.68±1.04±0.46 (14.01-16.56) 6.66%	^b 16.59±0.71±0.35 (15.97-17.23) 4.28%	LV
LMB	35.48±2.13±0.95	^a 35.07±4.75±2.37	38.76±4.26±1.90	^b 41.10±1.89±0.94	MV

	(33.116-38.62) 6.00%	(30.55-39.97) 13.55%	(31.62-42.28) 11.00%	(38.90-42.96) 4.61%	
WMB	34.48±2.65±1.18 (31.44-38.13) 7.68%	^a 33.62±6.19±3.09 (26.46-39.52) 18.41%	37.97±4.71±2.10 (30.04-42.02) 12.41%	^b 40.13±1.57±0.78 (38.01-41.71) 3.92%	MV
a	^a 1.41±0.12±0.06 (1.29-1.56) 8.47%	1.43±0.05±0.02 (1.39-1.50) 3.67%	^b 1.47±0.06±0.02 (1.38-1.54) 4.31%	1.44±0.11±0.05 (1.28-1.54) 7.69%	LV

*For details, see text; a-Minimum mean value; b-maximum mean value **LV-least variable (<12%); MV-moderately variable (12-20%); HV-highly variable (>20%), CR=Character ranking. LMB=length of median bulb, WMB- width of median bulb, a- length/width ratio.

B. Perineal pattern (Table 2)

The characters LVS, AVS and ATT were highest in banana population (25.76 µm, 20.05 µm and 18.93 µm respectively). The coefficient of variability for the four characters of perineal patterns varied from population to population. The characters LVS and ATT were rated as moderately variable

with maximum variation in tomato population (CV 13.34% and 14.39% respectively). The character AVS and IPD were least variable with maximum variation in okra and brinjal population respectively. Okra population showed maximum mean value for IPD (27.74 µm).

Table 2: Morphometric characters of perineal pattern of *M. incognita* in four crops in Kerala, [Mean±SD±SE; (range); CV%], n=10

Character (µm)	Populations*				CR**
	Brinjal	Okra	Tomato	Banana	
LVS	25.15±1.78±0.79 (23.30-27.90) 7.79%	24.54±1.40±0.70 (22.93-26.34) 5.70%	^a 23.70±3.16±1.41 (20.68-27.49) 13.34%	^b 25.76±2.50±1.25 (22.01-27.34) 9.74%	MV
AVS	19.66±0.92±0.41 (18.49-20.48) 4.71%	19.39±1.09±0.54 (17.82-20.37) 5.63%	^a 19.38±0.97±0.43 (17.98-20.61) 5.02%	^b 20.05±0.71±0.35 (19.29-20.81) 3.57%	LV
ATT	18.84±2.29±1.02 (16.86-21.85) 12.18%	^a 17.79±1.54±0.773 (15.557-19.032) 8.691%	18.49±2.66±1.19 (14.98-21.19) 14.39%	^b 18.93±2.06±1.03 (17.01-21.32) 10.91%	MV
IPD	26.67±2.81±1.25 (23.70-30.79) 10.54%	^b 27.74±1.69±0.84 (26.370-30.21) 6.11%	^a 26.13±0.54±0.2 (25.326-26.64) 2.10%	27.34±0.80±0.40 (26.38-28.17) 2.93%	LV

*For details, see text; a-Minimum mean value; b-maximum mean value **LV- least variable (<12%); MV-moderately variable (12-20%); HV- highly variable (>20%), CR= Character ranking. LVS-length of vulval slit, AVS-anus to vulval slit, ATT-anus to tail terminus, IPD-interphasmid distance.

C. Second stage juveniles (J₂) (Table 3)

The average body length of second stage juveniles was 392.52 to 406.41 µm, with maximum body length recorded in banana population. The body length, stylet length, H-MB and tail length were the least variable characters and the maximum mean values being 406.41 µm, 14.37 µm, 50.82 µm and 52.51 µm respectively. The maximum stylet length was observed in banana population whereas minimum value was observed in

okra population (13.87 µm). The characters ABW and ratio c' were moderately variable with maximum variation in tomato and banana population respectively. Ratio 'c' was highly variable with maximum variation in banana population and maximum mean values being 8.43 µm. The tail was longer in tomato population and smaller in banana population (50.46 µm).

Table 3: Morphometric characters of second stage juveniles of *M. incognita* in four crops in Kerala, [Mean±SD±SE; (range); CV%], n=10

Character (µm)	Populations*				CR**
	Brinjal	Okra	Tomato	Banana	
Body length	401.49±19.21±8.59 (370.03-418.37) 4.78%	405.28±11.10±5.55 (390.86-417.60) 2.74%	^a 392.52±23.10±10.33 (368.53-421.72) 5.88%	^b 406.41±19.30±9.65 (378.76-423.19) 4.74%	LV
Stylet length	14.12±0.36±0.16 (13.72-14.53) 2.60%	^a 13.87±0.18±0.09 (13.66-14.10) 1.36%	14.09±0.77±0.34 (13.25-15.05) 5.52%	^b 14.37±0.92±0.46 (13.54-15.67) 6.45%	LV
H-MB	50.43±2.06±0.92 (47.93-53.69) 4.09%	^a 49.09±1.03±0.51 (48.01-50.02) 2.10%	^b 50.82±3.87±1.73 (45.84-56.70) 7.62%	49.22±3.52±1.76 (46.38-54.37) 7.16%	LV
ABW	10.94±0.34±0.15 (10.38-11.32) 3.16%	^a 10.59±0.50±0.25 (10.05-11.21) 4.77%	11.04±1.05±0.47 (9.88-12.67) 9.53%	^b 11.81±0.26±0.13 (11.53-12.17) 2.25%	MV
Tail length	50.52±2.01±0.90 (48.38-53.51) 4.01%	51.10±2.89±1.44 (47.60-54.55) 5.67%	^b 52.51±2.97±1.32 (47.59-55.30) 5.66%	^a 50.46±1.97±0.98 (48.18-52.98) 3.91%	LV
c	8.00±0.38±1.17 (7.66-8.64) 4.75%	8.00±0.35±0.17 (7.72-8.49) 4.43%	^a 7.61±0.86±0.38 (6.85-9.08) 11.38%	^b 8.43±1.05±0.52 (7.80-10.00) 12.51%	HV
c'	4.92±0.46±0.20 (4.57-5.71) 9.40%	^b 5.18±0.33±0.16 (4.82-5.60) 6.43%	^a 4.72±0.46±0.20 (4.21-5.29) 9.90%	4.78±0.48±0.24 (4.38-5.37) 10.15%	MV

*For details, see text; a-Minimum mean value; b-maximum mean value

**LV- least variable (<8%); MV-moderately variable (8%-12%); HV-highly variable (>12%), CR= Character ranking, H-MB-head to median bulb; ABW-anal body width; c- length/tail length; c'- tail length/anal body width.

Comparing the morphological characters of mature female and perineal pattern of nematodes from four different host plants in Kerala, it was found that mean value for body length, body width, neck length, stylet length, LMB, WMB, LVS, AVS, ATT and IPD were maximum in banana

population. Based on CV values, characters body length, body width, neck length, LMB (length of median bulb), WMB (width of median bulb), LVS (length of vulval slit) and ATT (anus to tail terminus) of females were rated as moderately variable characters (CV 4.31% to 18.41%) and the characters

like stylet length, ratio a body (length/body width), AVS (anus to vulval slit) and IPD (interphasmidial distance) were recorded as least variable (CV 1.36% to 10.54%).

In the present study, the gross range of female stylet was between 13.64 to 17.90 μm with mean value of 16.30 μm which was exceptionally longer than earlier reported populations [8, 9, 10]. The variation can be attributed to what said by Jepson (1983) [8] that the stylet structure is adapted to the feeding habit of nematode which usually varies with the host plant.

Intermodulation comparison of second stage juveniles of *M. incognita* with four different host plants showed that the maximum mean values for body length, stylet length, ABW,

tail length and ratio c were maximum for banana population. On comparing the CV values, it was found that the characters body length, stylet length, H-MB, ABW, tail length and ratio c' were stable characters (CV 1.36% to 10.54%) in the population and ratio c was not stable exhibiting high variability (CV 4.43% to 12.15%) (Table 4).

Comparing the morphometric characters of four populations with type description of *M. incognita* by Chitwood (1949) showed that these populations shared most of the characters except for slight variations. The populations studied were in conformity with the morphological characters of the type species. The differences were attributed as the intraspecific variations of *M. incognita*.

Table 4: Comparison of the gross range of morphometric data recorded for Kerala populations of *M. incognita* with that given by Chit wood (1949 [9] and Kaur and Attri (2013) [11].

Characters*	As per Chitwood (1949)	^a As per Kaur and Attri (2013).	^a Gross range in Kerala Populations
Females			
Body length	510-690	530-812 (717)	546.42-789.27 (658.66)
Body width	300-430	510- 692 (597)	388.96-570.41 (467.26)
Stylet length	15-16	16-27 (19)	13.64-17.90 (15.95)
Neck length	-	176-280 (233)	151.47-206.72 (183.60)
LMB	-	20-42 (33)	30.55-42.96 (37.252)
WMB	-	20-42 (33)	26.46-42.02 (35.91)
A	-	1.03-1.43 (1.30)	1.28-1.56 (1.43)
Perineal pattern			
LVS	-	16.5-26 (22)	20.68-27.90 (24.74)
AVS	-	16-29 (22)	17.82-20.81 (19.48)
ATT	-	12-18 (15)	14.98-21.85 (18.47)
IPD	-	17-26 (22)	23.70-30.79 (27.19)
Second stage juveniles(J ₂)			
Length	360-393	200-380 (281)	368.53-423.19 (398.63)
Stylet length	-	11-25 (17)	13.25-15.67 (14.19)
H-MB	-	35-68 (51)	45.84-56.70 (50.36)
ABW	-	8-17 (25)	9.88-12.17 (11.15)
C	-	4.6-16.6 (10)	6.85-10.00 (8.28)
c'	-	1-6 (4)	4.21-5.71 (4.99)

*For details, see text; a: Figures in parentheses are the mean values

Conclusion

Morphological and morphometric variations of *M. incognita* infecting brinjal, okra, tomato and banana from Thiruvananthapuram, Idukki and Thrissur districts has been documented, which in turn helps in use of resistant varieties and species specific management practices to control these nematodes. Intraspecific morphological variations were observed within the species with respect to shape of females, length and position of neck, perineal pattern morphology, tail characters including rectum dilation. The variations in morphological characters among the individuals may be geographical, Eco phenotypic or host induced. Based on the CV values, the characters length, width, neck length, stylet length, LMB, WMB and ratio a of females, LVS, AVS, ATT and IPD of perineal pattern and length, stylet length, H-MB, ABW, tail length, ratio c and c' were recorded as stable characters and found useful in characterizing species.

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