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Morphological characterization of root knot nematode, *Meloidogyne enterolobii* (Tylenchida:Meloidogynidae) in guava (*Psidium guajava* L.) from Tamil Nadu, India

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ABSTRACT: Root-knot nematodes (*Meloidogyne* spp.) are polyphagous plant parasites of global importance. Recently root knot nematode infection of guava trees has become serious problem in Tamil Nadu, India. Extensive surveys were carried out in nine districts (Coimbatore, Erode, Madurai, Theni, Villupuram, Thiruvannamalai, Dharmapuri, Krishnagiri and Dindigul) of Tamil Nadu, India, to confirm the presence of *Meloidogyne enterolobii*. Morphological and morphometrical characterization of females, males and second stage juveniles (J2) using characters *viz.,* body length, body width, stylet length, stylet knob height, stylet knob width and perineal patterns revealed similarity with those of the type description.

Keywords: *Maloidogyne enterolobii*, taxonomy, perineal patterns, morphological, characterization

INTRODUCTION

The most widespread and economically significant genus of plant parasitic nematodes is *Meloidogyne* (root-knot nematodes) with more than 97 identified species (Hunt and Handoo, 2009). Till recently, four species *viz., M. incognita* (Kofoid and White) Chitwood, *M.javanica* (Treub) Chitwood,*M. arenaria* (Neal) Chitwood and *M.hapla* Chitwood were frequently prevalent in Tamil Nadu, India. Yet another new species, *M. enterolobii* was reported recently as a serious pest among the guava growers in Tamil Nadu, India (Ashokkumar and Poornima, 2019) (Poornima *et al*., 2016). *M. enterolobii* is a highly virulent root knot nematode originally described from a population collected in Puerto Rico from eggplant (*Solanum melongena*) (Rammah and Hirschmann,1988). *Meloidogyne enterolobii* have been detected from different countries in North, Central and South America, Africa and Asia (CABI, 2000). In China, *M. enterolobii* was mainly isolated from guava (*Psidium guajava*) (Xu *et al.,* 2004). *Meloidogyne enterolobii* generally occurs in polyspecific communities, interacting dynamically with the host plant, environment and other organisms present in the rhizosphere. Considering the risk of introduction and dissemination of this pest in India *M. enterolobii* was recently added to the EPPO Alert List (EPPO, 2008) with EPPO code: MELGMY, Phytosanitary categorization: EPPO A2 list No. 361(OEPP/EPPO Bulletin (2014). Being a new species, *M. enterolobii* might have been misidentified in a number of surveys. The optimal temperature for development of *M. enterolobii* is 28°C and corresponds to the geographical distribution of this nematode in subtropical regions. At this temperature it takes approximately 3 weeks to complete one life cycle (Ashokkumar *et al*., 2019). The root knot nematode, *M.enterolobii* mostly allows the entry of the fungus, *Fusarium* spp. that ultimately causes death of plants (Ashokkumar *et al.*, 2019). Therefore this study was aimed at confirming the occurrence of *M.enterolobii* in Tamil Nadu, India *via* morphological and morphometeric characters.

MATERIALS AND METHODS

Survey and collection of soil and root samples

A random survey was conducted in major guava growing areas of different districts of Tamil Nadu *viz.,* Coimbatore, Erode, Theni, Madurai, Krishnagiri, Dharmapuri, Villupuram, Thiruvannamalai and Dindigul.

Soil samples were collected from 2-3-year-old guava trees at a depth of 30-40 cm at a distance of 120 cm from the tree trunk. A composite sample of 200 cm^3 soil and 5g of feeder roots were collected from each locality were collected and transported to the Nematology Laboratory, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for further processing.

Extraction of nematodes Extraction of nematodes from soil

Nematode were extracted from the soil using Cobb's decanting and sieving method (Cobb, 1918), - followed by the modified Baermann's technique (Schindler, 1961) were used for separation of nematodes from debris. The nematode samples were observed under a binocular stereo zoom microscope (Labomed, CZM6, USA). Nematode genera were identified using the characters given in Mai & Lyon (1975).

Extraction of nematodes from root

Acid fuchsin - Lactophenol method (Bybd Jr *et al.*, 1983)

Roots were thoroughly washed and immersed in 3% sodium hypochlorite (NaOCl) for 2 min to be free from residue. The washed roots were transferred to a glass beaker that contained boiling acid fuchsin + lactophenol solution. A stock solution was prepared by dissolving 1 g of acid fuchsin stain in 100 ml of distilled water, from this stock solution 5 ml was added to 100 ml lactophenol. The solution was heated and kept at boiling point for 3 minutes or until the formation of two to 3bubbles.

To remove the excess stain, the stained roots were removed from the warm lactophenol and placed in tap water to cool down. The roots were transferred to petridish containing clear lactophenol solution for 12 hrs. Stained adult females were selected for study and counted using a stereo zoom microscope (Labomed, CZM6, USA).

Processing and preparation of permanent slides

The specimens of both male and juvenile nematodes were processed by the Seinhorst Method (Seinhorst, 1959). Permanent slides were prepared for male, juvenile and perineal pattern of female and confirmed with morphological characters using the taxonomic keys.

Preparation of posterior cuticular pattern

The neck portion of adult root knot nematode was cut using a scalpel blade under a stereo microscope and the body content gently removed. The cuticle of the posterior end containing the anus and vulva were trimmed and placed in a drop of 45% lactic acid for 30 minutes. After the remaining body contents adhering to the cuticle on the inner side were carefully removed by using a fine pointed nylon pick,the cuticle was trimmed in to a square shape. The cuticle was then transferred to a drop of anhydrous glycerine and mounted on a microscope slide with the inner side of the pattern facing downwards. The head portion was also mounted on the same slide. A cover slip was then placed over the drop and sealed using transparent nail polish (Hartman and Sasser, 1985).

RESULTS

Nematode distribution

The survey observed from each of the infested guava orchard in different district showed typical symptoms such as bronzing of leaves with marginal necrosis, presence of simple and compound galls in the roots and browning of younger and older leaves with wilting of plants (Plate 1a and 1b). Survey results concluded that the incidence and population of *M.enterolobii* in roots was the highest in Theni (588) followed by Dindugul (467), Dharmapuri (454), Coimbatore (452), Erode (404), Thiruvannamalai (393) Krishnagiri (371), Madurai (268) and Villupuram (159). Whereas in soil, the highest population was found in Theni(414),Thiruvannamalai(317),Dharmapuri(305),Coimbatore(292),Krishnagiri(276), Erode (267), Madurai (245), and lowest in Villupuram (109) (Fig.1). Wherever the root knot infestation was combined with fungal infection, sudden death of plants were experienced.

Morphological characterization of root knot nematode, *M.enterolobii*

Description Mature female

Body size and shape

Length of mature female varied from 701.0-763.4 µm, pear to globular in shape, long and prominent neck without posterior protuberance that varied from other species of root knot nematode (Fig. 2 A-D). Head region continuous with the body (No off-set). Head cap comprising labial disc and medial lips. The position of excretory pore often varies and found near the metacorpus. Annules were clearly visible in the posterior region of female body. Thin stylet conus curved slightly and tapered towards the end of the stylet. DOGO length varied from 4.1-5.6 µm from base of stylet. A pair of small, rounded, oesophago-intestinal cells with single nucleus was located between metacorpus and intestine (Fig. 2E).

Perineal pattern was oval in shape with coarse and fine striae, with moderately high dorsal arch, often rounded to squarish in some females (Fig. 2 F-G). Lateral lines were absent; striations were absent in perivulval region but present in lateral sides of vulva tail tips.

Morphometrics

The maximum body length was observed from the CBE (741.0 µm) and KGI (611.3 µm) population. The neck length in DGL population was 216.0 µm whereas

Table 1. Morphometrics of mature female in different populations of *M. enterolobii* [Mean ± SD ± SE (range), CV%]

Character	Coimbatore (CBE)	Erode (ERD)	Krishnagiri (KGI)	Dharmapuri (DPI)	Theni (TNI)	Madurai (MDU)	Thiruvannamalai (TVM)	Villupuram (VPM)	Dindigul (DGL)
Body length (µm)	741.0±17.6±8.8 (721.3-763.4),2.5	723.1±19.9±10 (701.0-747.1),2.8	737.0±19.3±9.6 (710.1-752.3),2.6	730.0±7.5±7.5 (709.2-742.8),2.1	725.0±22.8±11.3 (705.2-757.7),3.14	729.2±18.7±9.3 (712.6-754.8),2.6	740.0±21.4±10.7 (710.6-759.9),2.9	724.7±17.6±8.8 (701.1-742.9),2.4	739.0±20.8±10.4 (714.0-763.0),2.8
Body width (µm)	609.3±30.4±15.2 (569-642.5),5.0	600.0±31.8±15.9 (558.2-631.4),5.3	611.3±31.4±15.7 (574-642),5.14	605.0±29.5±14.8 (570.2-632),4.9	604.0±29.8±14.9 (562.0-632.1),4.9	611.0±28.7±14.3 (573.0-639.4),4.7	608.4±35.2±17.6 (567.0-643.4),5.8	602.0±29.8±14.9 (568.3-630.7),4.9	609.3±31.8±15.9 (565.9-636.5),5.2
Neck length (µm)	211.0±21.3±10.6 (192-241),10.6	204.9±21.5±10.8 (184-232.3),10.5	213.3±21.4±10.7 (192.1-241.8),10.0	202.6±21.2±10.6 (180.2-230.2),10.5	204.3±20.9±10.5 (182.0-232.0),10.3	210.3±20.7±10.3 (190.0-234.2),9.8	209.0±20.1±10.0 (188.0-235.5),9.6	202.4±21.5±10.7 (180.0-229.7),10.6	216.0±21.9±10.9 (195.0-242.5),10.1
Stylet length (µm)	15.4±3.8±1.9 (12.2-20.9),24.9	15.1±3.08±1.5 (11-18.4),20.4	15.2±3.0±1.5 (11.4-18),20.4	15.1±3.03±1.5 (11.2-18.6),20.07	15.3±3.3±1.7 (11.9-19.7),21.7	15.6±3.0±1.5± (12.2-19.0),19.5	15.3±3.0±1.5 (12.4-19.0),19.8	15.4±2.9±1.5 (11.8-18.9),19.3	16.2±3.1±1.5 (14.1-19.1),18.9
Stylet knob height (µm)	2.3±0.5±0.2 (1.9-2.8),20.4	2.2±0.5±0.2 (1.6-2.6),22.3	2.4±0.4±0.2 (1.8-2.7),16.5	2.3±0.6±0.3 (1.5-2.8),24.2	2.3±0.4±0.2 (1.8-2.6),17.7	2.3±0.4±0.2 (1.9-2.8),19.0	2.2±0.3±0.1 (1.9-2.5),11.7	2.4±0.7±0.3 (1.4-2.9),28.6	2.4±0.6±0.3 (1.6-2.8),11.9
Stylet knob width (µm)	4.8±0.5±0.3 (4.2-5.4),11.01	4.7±0.4±0.2 (4.3-5.2),8.5	4.9±0.6±0.3 (4.1-5.4),11.8	4.8±0.5±0.3 (4.1-5.2),11.1	4.8±0.6±0.3 (4.0-5.1),11.9	4.6±0.4±0.2 (4.3-5.1),8.3	4.7±0.5±0.2 (4.2-5.4),10.6	4.9±0.5±0.3 (4.3-5.4),10.5	5.0±0.6±0.3 (4.5-5.8),11.9
DOGO (µm)	4.7±0.6±0.3 (4.2-5.4),13.08	4.6±0.6±0.3 (4.2-5.4),12.6	4.8±0.6±0.3 (4.1-5.5),11.8	4.7±0.5±0.2 (4.2-5.3),9.5	4.6±0.5±0.3 (4.2-5.1),11.4	4.7±0.5±0.3 (4.2-5.4),11.3	4.6±0.6±0.3 (4.1-5.3),12.2	4.8±0.5±0.2 (4.3-5.4),9.5	5.0±0.6±0.3 (4.4-5.6),11.1
Excretory pore to head end (µm)	61.8±7.7±3.8 (54.1-72.4),12.4	60.7±5.8±2.9 (52-64.1)9.6	63.9±4.6±2.3 (57.1-66.2),7.1	62.4±5.1±2.6 (56.2-68.5),8.3	60.1±7.8±3.9 (53.1-71.0),13.1	63.4±6.4±3.2 (54.0-65.3),10.0	60.8±3.4±1.7 (56.0-61.0),5.5	62.5±4.2±2.1 (58.2-68.0),6.7	64.9±6.0±3.0 (58.0-72.0),9.3
Inter phasmidial length (µm)	29.3±3.1±1.6 (26.0-33.3),10.7	28.7±3.9±2.0 (24.0-32.3),13.6	29.5±5.4±2.7 (24.3-35.8),18.3	30.0±2.5±1.2 (26.8-32.7),8.3	27.5±3.3±1.7 (24.6-31.5),12.0	32.0±2.6±1.3 (29.0-35.3),8.3	29.5±5.9±2.9 (24.3-35.8),19.9	28.0±2.9±1.5 (25.0-31.1),10.6	30.2±2.9±1.4 (27.0-34.0),9.6
Vulval length (µm)	27.3±1.7±0.9 (25.4-29.3),6.3	27.4±1.9±1.0 (25.3-29.8),7.03	28.0±2.3±1.1 (24.6-29.4),8.1	27.5±2.4±1.2 (25.1-30.7),8.7	27.6±2.3±1.1 (25.0-30.0),8.3	28.4±1.8±0.9 (26.0-30.2),6.2	27.8±2.3±1.1 (24.6-30.0),8.2	26.6±1.7±0.9 (25.0-29.0),6.4	28.5±2.1±1.0 (26.0-31.0),7.3
Vulva anus distance (µm)	21.9±1.7±0.8 (19.7-23.4),7.5	21.7±1.8±0.9 (20.2-24.2),8.10	21.8±2.3±1.1 (18.3-23.1)10.6	21.8±1.8±0.9 (19.8-23.7),8.5	21.8±1.9±0.9 (19.0-23.1),8.9	21.9±1.6±0.8 (20.5-24.1),7.3	22.0±2.3±1.2 (18.5-23.0),10.7	20.9±1.9±0.9 (19.6-23.0),9.1	22.3±2.2±1.1 (19.2-24.0),9.8
a value	1.22±0.4±0.2 (0.99-1.8),31.8	1.21±0.4±0.2 (0.96-1.8),33.8	1.25±0.4±0.2 (1.0-1.8),30.3	1.20±0.8±0.1 (1.1-1.6),22.6	1.20±0.3±0.2 (0.9-1.6),26.4	1.19±0.4±0.2 (0.9-1.8),35.4	1.22±0.3±0.2 (1.0-1.7),26.9	1.20±0.3±0.2 (1.0-1.7),28.1	1.21±0.4±0.2 (1.0-1.7),28.7

in VPM population it was 202.4 µm with a CV percent of 10.10 to 10.6.The character of stylet length was slightly variable (CV 18.9% to 20.4%). For DGL population stylet length was a maximum of 16.2 µm while at DPI and ERD it was at a minimum of 15.1 µm. DOGO in nine populations ranged from 5.0 µm (DGL) and 4.6 µm (ERD, TNI, TVM) with CV per cent of 11.1 (DGL), 12.6 (ERD), 11.4 (TNI) and 12.2 (TVM). These results revealed that the morphometric characters observed from nine different locations of Tamil Nadu had very slight or no variations. The length of vulva ranged from maximum of 28.5 µm for DGL and minimum of 26.6 µm for VPM population. The CV were 7.3 and 6.4 per cent respectively. The vulva anus distance was maximum in the females of DGL district (22.3 µm) with a CV of 9.8 per cent. The ratio 'a' (BL/BW) was slightly variable (CV 22.6 to 35.4 %) with mean value minimum in MDU population (1.19 µm) and maximum in KGI population (1.25 µm). Mean values of four population taken for study was more, hence the CV value of each location was moderately variable (Table1).

Males

Body size length of male varied from 1563.8-1616.3µm, translucent white, vermiform in nature. Head region was slightly set off from the body (Fig. 3 A). Cephalic framework was moderately developed. Stylet pointed, robust and the conus was straight with a cylindrical shaft. Knobs bigger, round and set off from the shaft (Fig. 3 B-D). Distance of DOGO to stylet base was long (4.1-4.9 µm). Excretory pore was far distant from head region. Testis outstretched. Spicules arc shaped with a round base (Fig. 3 E-G). Tail short and rounded (Fig. 5 C,D).

Morphometrics

Body length and width ranged from1563.8-1616.3 µm and 40.7-42.1 µm respectively. The minimum stylet length of 21.3 µm was recorded in MDU (CV 6.8 %) and maximum of 23.4 µm in TVM and DGL (CV 6.4 % and 7.1 % respectively). Stylet knob height ranged from 3.2, 3.4, 3.1, 2.9, 3.1, 3.0, 3.7, 3.4 and 3.8 µm, respectively in CBE, ERD, KGI, DPI, TNI, MDU, TVM, VPM and DGL districts (Table 2). The character of DOGO was highly variable within the populations from nine districts (CV 3.3 % to 13.1%). The distance from head to excretory pore was in similar range in all the population (162.4-182.3 µm) (Table 2).

The spicule length had a similar mean value of (29.8, 30.7, 30.1, 31.0, 30.4, 29.8, 31.2, 30.6 and 31.3 µm). The length of testis ranged from minimum 789.6 in VPM and maximum of 817.4 in DGL. The 'a' ratio for all population was similar with the CV per cent from 9.3 to 10.9% and for 'c' ratio was 17.8% in population of TNI, TVM and VPM and maximum of 18.9 per cent in CBE (Table 2).

Second stage juveniles (J2) Description

Body: Transparent white, vermiform in nature, body long, tapered towards both the ends (422.4-439.8 µm) with a narrow tail. Head region continuous, slightly set off from the body (Fig. 4 A-B). Lateral field began from procarpus and ended at tail terminus as 2 lines, irregularly areolated. Stylet fine with a sharply pointed, straight conus and posteriorly wider shaft. Knobs separate and set off from the shaft. Distance of DOGO from stylet was 2.6-3.2 µm. Procarpus was visible and metacarpus oval shaped (Fig.4 C). Tail tip hyaline, very thin with few fat droplets. Phasmids indistinct (Fig.5 A, B) (Fig. 4 D-E).

Morphometrics

The mean body length of J_2 varied from 422.4 µm in VPM to 439.8 µm in DPI. Body width was maximum in DPI (15.3 µm) and minimum in VPM (13.9 µm). The stylet length (10.8 to 11.5µm) from 9 districts showed slight variability with CV 3.1-4.8 per cent. Meanvalues of stylet knob height were 1.5, 1.5, 1.4, 1.6, 1.2, 1.3, 1.3, 1.0 and 1.6 µm and widthwere 2.6, 2.5, 2.3, 2.5, 2.3, 2.3,2.3, 2.2, 1.7 and 2.4 µm, respectively in CBE, ERD, KGI, DPI, TNI, MDU, TVM, VPM and DGL districts with slight or no variations (Table 3). The distance of DOGO from stylet base was smaller in VPM (2.6 µm) and greater in ERD and CBE (3.2 µm) with higher variations. The 'a' ratio for 9 locations ranged from 28.7-30.3 and is slightly variable. The 'c' ratio ranked as moderately varied as the CV value as ranged from 9.4-12.1% (Table3).

Perineal pattern

Oval in shape with coarse and fine striae, anus was located anteriorly from (AVS) at 19.20-21.17µm distance from vulval slit and anus located 9.70 to 11.68 µm distance from tail tip (ATT). Tail smooth with 2 phasmids. The interphasmidal length observed in populations from the nine different locations varied slightly with the CV ranging from 4.69 per cent to 9.49 per cent. (Table 4; Fig.6).

DISCUSSION

Survey and distribution of *M.enterolobii*

The *Meloidogyne enterolobii* was first reported from guava from Tamil Nadu, India in 2016 (Poornima *et al.*, 2016). Root knot nematode has been found to cause massive decline in guava orchards across India and other parts of the world. Ansari and Khan (2012)

Table 2. Morphometric of different populations of males of *M. enterolobii* [Mean ± SD ± SE (range), CV%]

Character	Coimbatore (CBE)	Erode (ERD)	Krishnagiri (KGI)	Dharmapuri (DPI)	Theni (TNI)	Madurai (MDU)	Thiruvannamalai (TVM)	Villupuram (VPM)	Dindigul (DGL)
Body length(µm)	1580.5±132.9±66.5	1602.3±135.4±67.7	1599.1±121.2±60.6	1582.4±100.0±50.0	1574.3±89.6±44.8	1563.8±88.0±44.0	1614.9±70.6±35.3	1588.7±62.5±31.3	1616.3±86.8±43.4
	(1395.9-1697.1),8.4	(1402.2-1694.7),8.4	(1454.3-1742.6),7.6	(1442.2-1672.6),6.3	(1493.2-1696.5),5.7	(1475.3-1654.3),5.7	(1526.3-1689.2),4.4	(1524.3-1671.4),3.9	(1538.1-1732.9)5.4
Body width(µm)	41.8±3.9±1.9	42.1±3.7±1.8	42.0±3.9±1.9	40.8±3.8±1.9	41.1±3.6±1.8	40.7±2.7±1.3	41.4±3.7±1.8	40.9±3.7±1.8	42.0±3.3±1.6
	(39.2-47.4),8.4	(39.0-47.3),8.8	(39.2-47.8),9.5	(36.2-44.5),9.2	(38.2-46),5.7	(38-44.3),6.6	(39-46.8),8.8	(36.4-44.5),8.9	(39.1-45.7),7.8
Tail length(µm)	12.3±3.2±1.6	12.5±3.3±1.7	12.4±3.3±1.7	12.2±3.1±1.6	11.9±1.9±0.9	11.8±2.0±1.0	12.1±2.0±2.0	11.9±2.09±1.05	12.3±2.0±1.0
	(7.9-14.2),25.8	(8.0-15.8),26.7	(7.9-15.6),26.7	(7.9-15.2),25.4	(9.3-13.6),15.9	(9.0-13.4),16.8	(9.6-14.4),16.2	(9.2-14.2),17.7	(9.8-14.6),16.3
Stylet length(µm)	22.1±0.8±0.4	23.0±0.7±0.4	22.6±1.1±0.6	23.0±0.8±0.4	22.8±0.9±0.5	21.3±1.5±0.7	23.4±1.5±0.8	22.5±1.1±0.6	23.4±1.7±0.8
	(21.0-23.0),3.8	(22.8-24.0),3.3	(21.5-24.0),4.9	(22.0-24.0),3.5	(21.8-24),4.03	(20.2-23.2),6.8	(21.5-25.0),6.4	(22.0-24.0),4.9	(21.2-25.2),7.1
Stylet knob height(µm)	3.2±0.6±0.3	3.4±0.5±0.2	3.1±0.6±0.3	2.9±0.3±0.1	3.1±0.8±0.4	3.0±0.4±0.2	3.7±0.5±0.3	3.4±0.4±0.2	3.8±0.6±0.3
	(2.5-3.8),18.3	(2.9-4.0),13.1	(2.6-3.9),19.2	(2.6-3.2),8.9	(2.0-3.8),26.07	(2.7-3.6),8.6	(2.9-4.0),13.8	(2.9-3.8),11.6	(3.0-3.8),16.2
Stylet knob width(µm)	5.3±0.3±0.2	5.5±0.5±0.3	5.3±0.5±0.2	4.9±0.2±0.1	5.3±0.5±0.3	5.1±0.5±0.3	5.5±0.5±0.2	5.3±0.5±0.2	5.7±0.5±0.3
	(4.9-5.7),6.4	(4.9-6.1),9.9	(4.8-5.9),8.6	(4.7-5.2),4.4	(4.6-5.9),10.2	(4.5-5.8),10.5	(4.8-5.9),8.6	(4.7-5.8),8.6	(4.9-6.0),8.9
DOGO(µm)	4.5±0.2±0.1	4.7±0.6±0.3	4.4±0.4±0.2	4.1±0.4±0.2	4.3±0.1±0.07	4.1±0.3±0.12	4.5±0.3±0.2	4.2±0.4±0.2	4.9±0.4±0.2
	(4.2-4.7),4.8	(4.5-5.6),13.1	(4.1-4.9),8.1	(3.6-4.4),8.7	(4.2-4.5),3.3	(3.8-4.4),6.3	(4.1-4.9)7.5	(3.7-4.6),8.9	(4.4-5.2),7.3
Excretory pore to head end(µm)	174.8±9.6±4.8	179.7±8.2±4.1	175.8±6.7±3.3	171.9±3.5±1.7	171.2±7.5±3.8	162.4±5.9±3.0	168.2±4.04±2.02	167.7±3.7±1.9	182.3±7.9±3.9
	(162.1-184.6),5.5	(170.0-188.0),4.6	(167.0-182.9),3.8	(169.0-176.6),2.0	(160.2-176.7),4.4	(154.1-168.1),4.4	(162.8-172.5),2.4	(163.0-172.0),2.2	(171.4-190.1),4.3
Spicule length(µm)	29.8±2.9±1.5	30.7±2.2±1.1	30.1±1.9±0.9	31.0±1.5±0.8	30.4±2.8±1.4	29.8±2.5±1.3	31.2±2.3±1.2	30.6±1.8±0.9	31.3±3.6±1.8
	(27.0-33.7),9.9	(28.0-33.2),7.1	(28.0-32.0),6.1	(29.0-32.7),4.9	(27.0-33.9),9.3	(27.2-33.2),8.5	(28.0-32.7),7.5	(28.4-32.7),5.9	(27.0-37.0),11.5
Gubernacul um length(µm)	6.1±0.7±0.4	6.4±0.7±0.3	6.2±0.5±0.3	6.1±0.6±0.3	6.2±0.8±0.4	5.4±0.6±0.3	5.5±0.3±0.1	5.3±0.4±0.2	6.2±0.6±0.3
	(5.1-6.9),12.3	(5.4-7.0),10.9	(5.6-6.8),8.2	(5.4-6.8),9.6	(5.1-6.9),12.5	(4.9-6.3),11.2	(5.2-5.9),5.4	(4.8-5.7),7.4	(5.4-6.7),9.4
Testis length(µm)	808.4±130.4±65.2	812.4±125.5±62.8	805.5±118.5±59.3	801.3±116.3±58.1	809.1±130.3±65.1	798.8±121.0±60.5	808.3±116.1±58.1	789.6±102.9±51.4	817.4±113.6±56.8
	(675.8-982.5),16.1	(678.0-975.0)15.4	(674.0-938.8),14.7	(671.0-920.0),14.5	(676.0-982.5),16.1	(676.2-962.0),15.1	(675.2-938.8),14.4	(670.8-891.2),13.0	(672.0-924.2),13.9
a value	38.0±3.9±1.9	38.1±3.6±1.8	38.07±3.7±1.8	38.8±2.9±1.5	38.3±4.1±2.1	38.5±3.7±1.9	39.0±3.6±1.8	38.8±3.8±1.9	38.5±4.0±2.0
	(34.4-42.6),10.4	(34.4-42.4),9.4	(34.5-42.5),9.6	(34.9-42.2),1.7	(34.6-43.0),10.9	(35.0-43.1),9.7	(35.0-43.0),9.3	(34.0-43.0),9.8	(34.2-43.6),10.3
c value	128.5±24.3±12.1	128.1±24.0±12.0	129.0±24.3±12.1	129.7±24.4±12.2	132.3±23.6±11.8	132.5±24.0±12.0	133.5±23.8±11.9	133.5±23.7±11.9	131.4±24.1±12.1
	(95.4-152.4),18.9	(95.2-150.4),18.7	(96.0-152.1),18.8	(96.5-153.1),18.8	(99.2-154.2),17.8	(99.2-155.1),18.1	(99.8-154.8),17.8	(99.8-154.6),17.8	(96.5-152.0),18.4

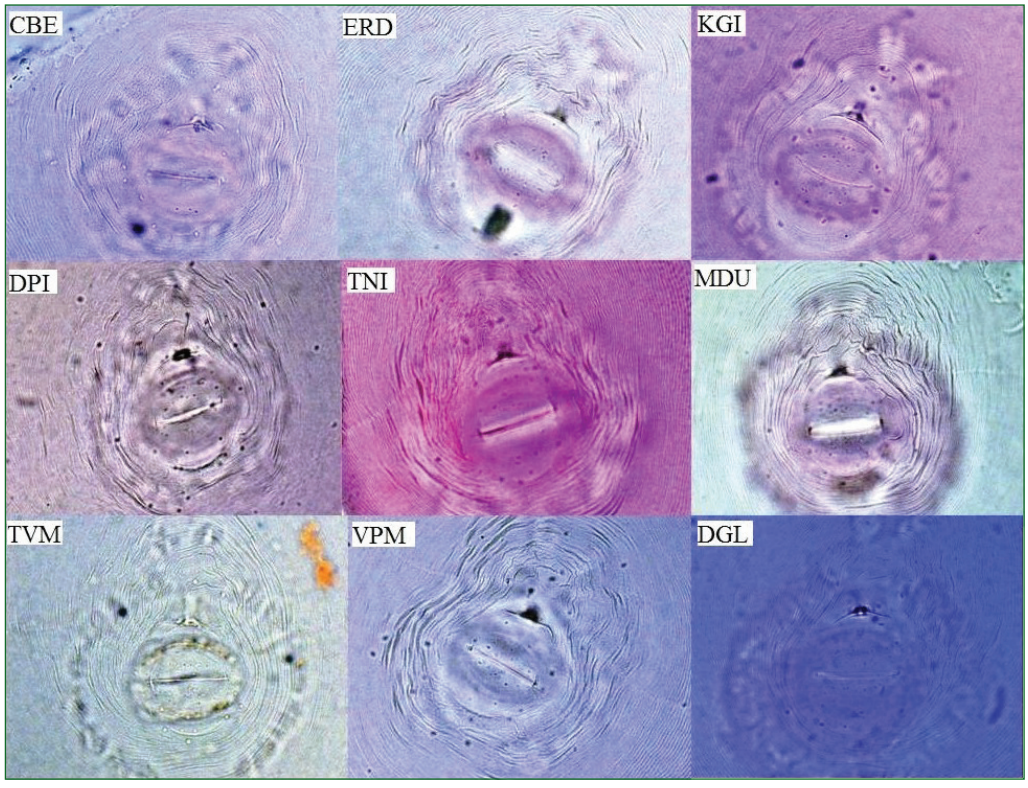

Fig. 6. Micrograph of perineal patterns off emales of. *M.enterolobii* from different guava growing districts in Tamil Nadu.India

had indicated that the association of *M.enterolobii* with guava was highly pathogenic in nature. De Almeida *et al.* (2008) reported that *M.enterolobii* (syn *M.mayaguensis)* is a polyphagous plant parasitic nematode causing severe damage in several plant species in Brazil. During a survey by (Tigano *et al.*, 2011), *M.enterolobii* infestation was observed in guava from all the major guava growing districts of Rio de Janeiro (Brazil) with the symptoms of yellowing, wilting, scorching of leaf margins and leaf drop, yield reduction and plant death within few months.

Morphological identification of *M.enterolobii*

Identification of a pest is the key to any successful pest management approaches. Root knot nematode identified from guava orchards by morphological and molecular characterization as *M. enterolobii* is a recently introduced nematode problem in India Poornima *et al.* (2016). hence, the accurate identification of the species in each of the studies taken up in guava orchards of the state infested with root knot nematode has been given much importance, which will help in containing it and preventing its further movement from the areas of introduction to uninfested areas. This study scanned nine guava growing districts of Tamil Nadu and examined the morphological and morphometric characters of the root knot nematode for confirmation of thespecies.

The taxonomic characters such as body length of females showed a maximum of 741.0 µm and a minimum of 723.1 µm in CBE and ERD population, which fit in the range of the *M. enterolobii* population (541.3-926.3 µm). All the characters recorded for the females were similar to that of the type locality (Yang & Eisenback, 1983). The perineal pattern observed with the populations from the surveyed locations showed few variations. The patterns were oval, with coarse and smooth striae, the dorsal arch being moderately high, to nearly rounded or squared.

Morphological comparison of various characters of mature males from nine locations revealed that body width, tail length and gubernaculum length was greater in ERD population. Stylet length, 'a', 'c' ratio was greater for the population from TVM which was on par with VPM population. Distance from DOGO to the stylet base in males has been regarded as a distinguishing character between *M. enterolobii* and *M. incognita*(De Almeida*et al.,* 2008). In the present populations of *M. enterolobii*, DOGO observed for males ranged from 4.1 to 4.9 µm which was in agreement with the original description (range 3.7 µm to 5.3 µm) (Yang and Eisenback, 1983). Maximum value for DOGO of male was noticed with DGL (4.9 µm) and minimum in MDU (4.1 µm) and DPI (4.1 µm) population. The morphometrics of second stage juveniles from nine locations in Tamil Nadu

Table 3. Morphometrics of different populations of juveniles of *M. enterolobii* [Mean ± SD ± SE (range), CV%]

Character	Coimbatore (CBE)	Erode (ERD)	Krishnagiri (KGI)	Dharmapuri (DPI)	Theni (TNI)	Madurai (MDU)	Thiruvannamalai (TVM)	Villupuram (VPM)	Dindigul (DGL)
Body length (µm)	431.7±9.3±4.7 (424.1-445.2),2.2	435.7±9.0±4.5 (427.0-448.0),2.1	430.3±7.1±3.6 (425.0-440.1),1.6	439.8±7.8±3.9 (429.4-446.7),1.8	431.2±8.4±4.2 (424.1-443.1),1.9	433.3±8.3±4.1 (425.6-444.1),1.9	432.6±7.1±3.6 (427.0-432.6),1.6	422.4±5.8±2.9 (416.4-428.9),1.4	438.4±9.3±4.7 (427.0-449.3),2.1
Body width (µm)	15.0±1.1±0.6 (14.3-16.7),7.6	15.1±1.2±0.6 (14.2-16.9),8.0	14.8±0.8±0.4 (13.6-15.4),5.6	15.3±0.7±0.3 (14.4-15.3),4.4	14.8±1.0±0.5 (14.3-16.2),6.5	14.9±1.0±0.5 (14.2-16.3),6.4	14.8±0.9±0.4 (13.6-15.3),5.6	13.9±0.7±0.3 (13.4-14.9),4.9	15.2±0.8±0.4 (14.1-15.9),5.2
Tail length (µm)	54.4±5.6±2.8 (49.2-60.0),10.4	54.3±5.1±2.6 (48.0-59.0),9.5	53.8±6.4±3.2 (48.4-53.8),11.9	53.9±5.4±2.7 (49.1-53.9),10.0	54.0±5.4±2.7 (48.6-59.2),10.0	53.7±3.7±1.9 (48.6-56.0),6.9	54.2±6.1±3.1 (48.4-62.0),11.4	50.6±2.7±1.4 (48.4-54.3),5.4	54.2±5.0±2.5 (49.4-61.1),9.3
Excretory pore to head end(µm)	90.4±4.7±2.3 (86.2-96.3),5.1	91.0±3.2±1.6 (87.4-94.8),3.5	90.5±3.0±1.5 (88.0-94.9),3.3	91.0±2.7±1.4 (89.6-94.0),3.0	87.0±4.1±2.1 (82.1-92.0),4.7	88.3±1.7±0.8 (86.2-90.3),1.9	88.2±2.7±1.4 (85.2-91.6),3.1	83.7±2.1±1.0 (81.3-86.2),2.5	91.0±3.2±1.6 (87.6-94.6),3.5
Stylet length(µm)	11.3±0.5±0.3 (10.9-12.1),4.8	11.4±0.6±0.3 (10.8-12.2),5.3	11.1±0.5±0.2 (10.7-11.8),4.5	11.4±0.5±0.2 (10.8-12.0),4.4	11.0±0.5±0.2 (10.6-11.7),4.4	11.2±0.5±0.3 (10.7-11.9),4.7	11.1±0.4±0.2 (10.7-11.6),3.5	10.8±0.3±0.2 (10.4-11.2),3.1	11.5±0.5±0.3 (10.9-12.1),4.8
Stylet knob height(µm)	1.5±0.2±0.1 (1.4-1.8),12.4	1.5±0.2±0.1 (1.3-1.7),10.9	1.4±0.3±0.1 (1.2-1.8),18.5	1.6±0.2±0.1 (1.4-1.9),13.5	1.2±0.1±0.1 (1.0-1.3),11.8	1.3±0.2±0.1 (1.1-1.5),14.0	1.3±0.3±0.1 (0.9-1.5),21.7	1.0±0.2±0.1 (0.8-1.3),21.6	1.6±0.2±0.1 (1.4-1.8),11.4
Stylet knob width(µm)	2.6±0.2±0.1 (2.4-2.9),8.3	2.5±0.5±0.2 (2.3-3.1),18.8	2.3±0.2±0.1 (2.1-2.6),9.4	2.5±0.3±0.1 (2.2-2.8),11.8	2.3±0.2±0.1 (2.0-2.5),9.4	2.3±0.3±0.1 (2.0-2.6),11.2	2.2±0.2±0.1± (1.9-2.4),9.8	1.7±0.2±0.1 (1.5-2.0),12.7	2.4±0.5±0.2 (2.1-2.7),10.7
DOGO(µm)	3.2±0.3±0.2 (3.0-3.7),10.5	3.2±0.3±0.2 (2.9-3.6),9.9	3.0±0.4±0.2 (2.7-3.5),11.9	3.0±0.4±0.2 (2.6-3.4),12.1	3.0±0.1±0.1 (2.8-3.1),4.7	2.9±0.2±0.1 (2.7-3.2),7.4	3.0±0.3±0.1 (2.7-3.3),8.6	2.6±0.3±0.2 (2.2-2.9),12.1	3.0±0.3±0.1 (2.7-3.3),8.6
a value	28.7±2.3±1.1 (26.0-31.5),8.0	28.9±2.0±1.0 (27.0-31.7),6.9	29.1±1.5±0.7 (28.0-31.2),5.1	29.0±1.3±0.6 (27.6-30.5),4.5	29.1±2.0±1.0 (27.4-32.0),6.9	29.2±2.3±1.1 (27.0-32.3),7.8	29.2±1.6±0.8 (28.0-31.5),5.5	30.3±1.9±0.9 (28.2-32.5),6.2	28.9±2.0±1.0 (27.1-31.2),6.9
c value	7.9±1.0±0.5 (7.0-9.0),12.1	8.0±0.8±0.4 (7.2-9.1),10.1	8.0±0.8±0.4 (7.4-9.0)10.6	8.1±0.9±0.4 (7.1-9.0),10.6	8.0±1.0±0.5 (7.2-9.1),11.9	8.1±0.8±0.4 (7.4-9.2),10.0	7.9±0.7±0.4 (7.4-8.8),9.4	8.3±0.8±0.4 (7.5-9.1),9.8	8.1±0.8±0.4 (7.2-9.0),10.3

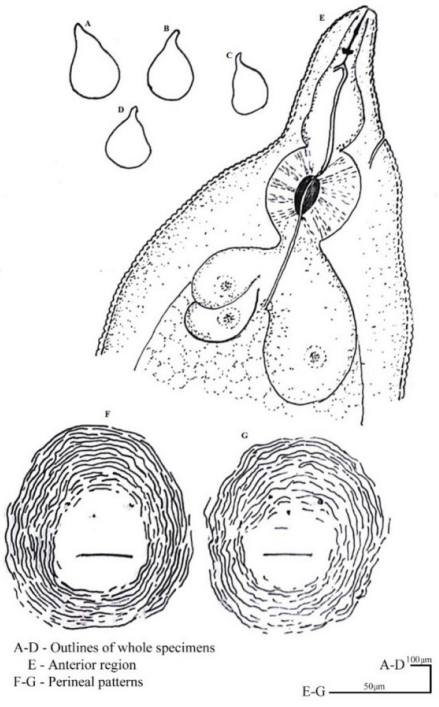

Fig 2. Camera Lucida drawings of female head portion, posterior cuticular pattern and outline of full female of *M. enterolobii*

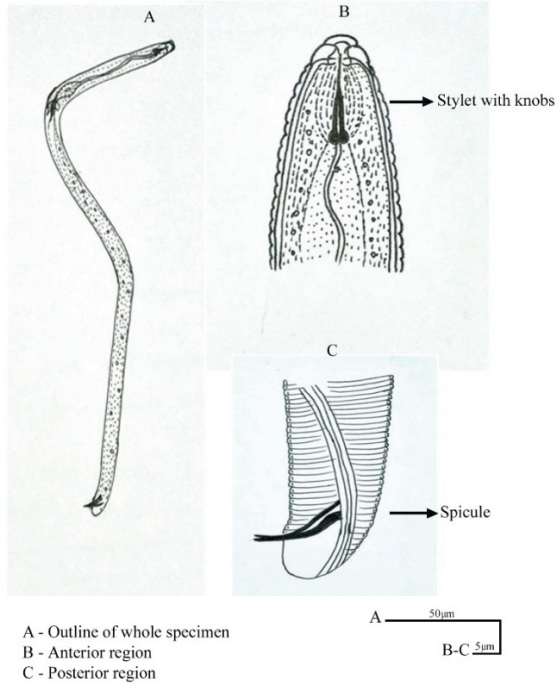

Fig 3. Camera Lucida drawings of males of *M. enterolobii*

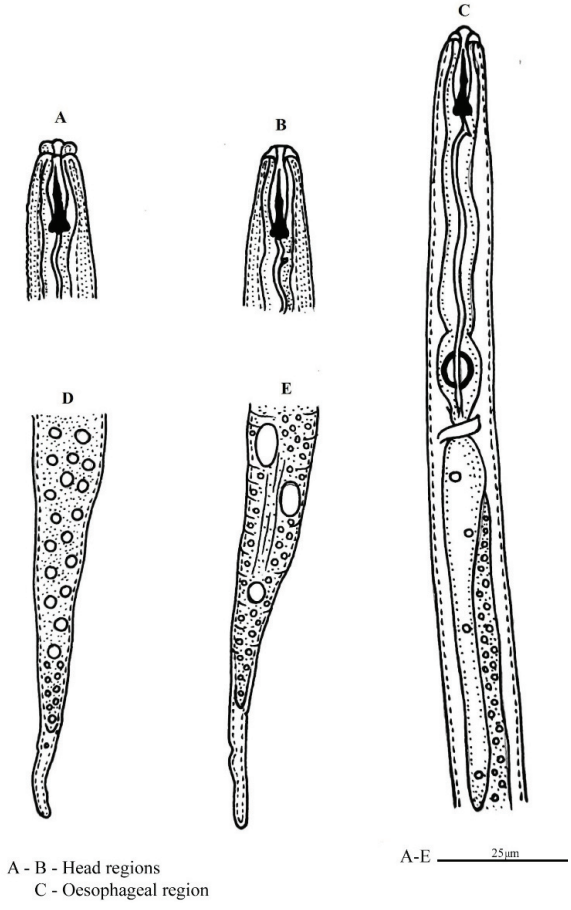

Fig 4. Camera Lucida drawings of 2nd stage juvenilas of *M. enterolobii*

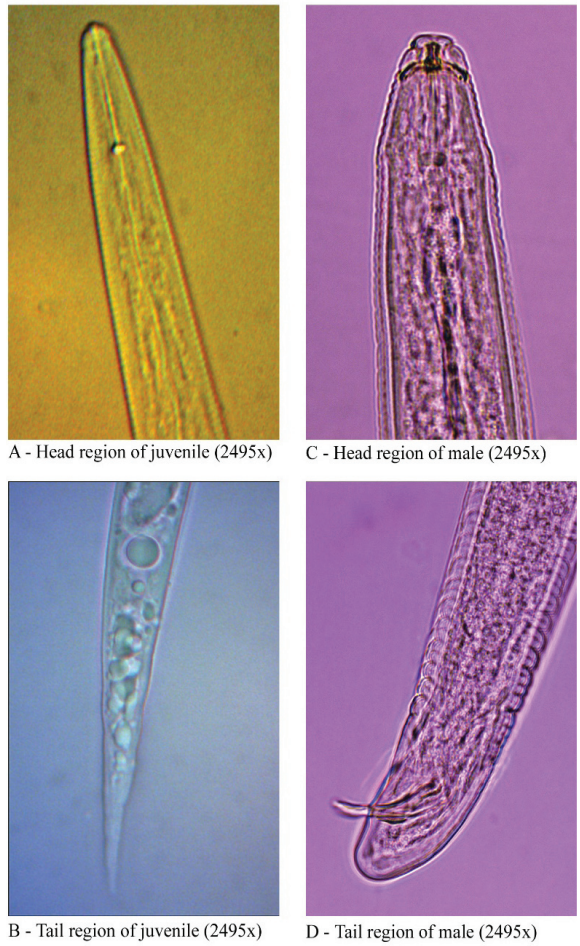

Fig 5. Light microscopic photograph of *M. enterolobii*

Table 4. Morphometrics of perineal pattern in different populations of *M. enterolobii* [Mean ± SD ± SE (range), CV%]

Character (µm)	Coimbatore (CBE)	Erode (ERD)	Krishnagiri (KGI)	Dharmapuri (DPI)	Theni (TNI)	Madurai (MDU)	Thiruvannamalai TVM)	Villupuram (VPM)	Dindigul (DGL)
Length of vulval slit	24.35±1.85±0.92 (22.37-26.02),7.58	23.10±1.69±0.84 (21.20-24.30),7.30	23.96±1.67±0.84 (22.12-25.40),6.99	22.50±1.21±0.61 (21.80-23.90),5.39	23.89±1.18±0.59 (22.60-24.90),4.94	23.50±0.85±0.43 (22.70-24.40),3.62	21.64±0.87±0.44 (20.90-22.60),4.04	20.16±0.93±0.46 (19.40-21.20),4.61	22.64±1.26±0.63 (21.20-23.50),5.5
Anus to vulval slit	20.57±1.41±0.71 (19.15-21.97),6.86	20.16±1.27±0.63 (19.20-21.60),6.28	20.69±1.44±0.72 (19.10-21.90),6.97	19.20±0.36±0.18 (18.80-19.50),1.88	19.62±1.20±0.60 (18.40-20.80),6.12	21.14±1.13±0.56 (20.80-22.80),5.33	20.36±1.12±0.56 (19.40-21.60),5.52	21.17±1.31±0.65 (19.94-22.54),6.18	19.97±1.45±0.73 (18.30-20.98),7.2
Anus to tail terminus	11.67±0.61±0.31 (10.97-12.09),5.27	11.66±0.95±0.48 (10.50-12.20),8.18	10.70±0.75±0.38 (9.97-11.47),7.01	11.71±0.81±0.41 (10.80-12.10),7.29	10.71±0.71±0.36 (10.10-11.50),6.66	9.70±0.76±0.38 (9.30-10.80),7.87	11.20±0.87±0.44 (10.40-12.10),7.80	10.67±0.72±0.6 (10.20-11.50),6.78	11.68±0.97±0.49 (10.60-12.50),8.3
Interphasmid distance	25.64±1.42±0.71 (24.13-26.97),5.55	30.52±2.60±1.30 (28.00-33.20),8.52	31.20±1.69±0.85 (30.60-33.12),5.43	29.30±1.37±0.69 (28.10-30.80),4.69	26.17±1.56±0.78 (24.70-27.80),5.95	29.12±2.76±1.38 (27.20-32.30),9.49	25.91±1.89±0.95 (23.80-27.40),7.30	32.10±2.56±1.28 (29.70-34.80),7.97	27.31±2.44±1.22 (25.80-30.12),8.9

did not differ from the measurements in the original description (Yang & Eisenback, 1983). In juveniles, three characters *viz.*, stylet knobs width, DOGO and tail length were maximum in CBE population. The CBE population averaged 54.4±5.6 µm in tail length and 3.2±0.3 µm in DOGO which were in consistent with the Puerto Rican description of 54.4±3.6 µm tail length and 3.2±0.2 µm DOGO of *M.mayaguensis* (Rammah and Hirschmann, 1988). Morphological characterization and identification use a set of characters includes perineal pattern shape, morphology of male, stylet morphology and morphometrics of male and female and tail as well as the hyaline tail terminus of second stage juveniles. The present study indicated that minor or no significant morphological variability occurred among the populations of *M. enterolobii* collected from various guava growing areas of Tamil Nadu.

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