



Deciphering the Identity of the Root-knot Nematode *Meloidogyne enterolobii* Infecting Guava (*Psidium guajava* L.) Through Morphological and Molecular Tools

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ABSTRACT

Background: Root-knot nematode *Meloidogyne enterolobii* has emerged as a major constraint to guava (*Psidium guajava* L.) cultivation in tropical regions, particularly in association with the guava wilt–nematode disease complex. Accurate identification of the nematode species is essential for understanding its distribution and developing effective management strategies.

Methods: Soil and root samples were collected from guava orchards affected by wilt symptoms in Tamil Nadu. Adult females were dissected from infected roots and examined for perineal pattern morphology, while second-stage juveniles (J2) were analysed for key morphological and morphometric characters. Molecular identification was performed using species-specific primers with amplification of the ITS and COI regions, followed by sequencing and phylogenetic analysis.

Result: The perineal pattern of adult females was oval with a high dorsal arch and absence of lateral lines, characteristic of *M. enterolobii*. J2 exhibited a hemispherical head, slender tail with a rounded terminus and stylet length ranging from 14.5–16.2 µm. Molecular analysis confirmed the identity of *M. enterolobii* and the ITS and COI sequences were deposited in GenBank under accession numbers PX667703–PX667705 and PX658232–PX658234, respectively. Phylogenetic analysis showed close clustering of the isolates with reference *M. enterolobii* sequences.

Key words: COI, Guava, ITS, *Meloidogyne enterolobii*, Molecular identification, Morphological characterization.

INTRODUCTION

Guava (*Psidium guajava* L.) is an economically important fruit crop widely cultivated in tropical and subtropical regions of India (Semwal *et al.*, 2024; Sahu *et al.*, 2025). In recent years, the crop has suffered a severe decline due to the combined effects of root-knot nematode (*Meloidogyne* spp.) infestation and Fusarium-induced wilt, resulting in substantial yield and crop loss (Fernandes Santos *et al.*, 2022; Vishwakarma *et al.*, 2023; Kamboj *et al.*, 2025). Among the major plant-parasitic nematodes, root-knot nematodes (*Meloidogyne* spp.) are highly polyphagous pests that cause severe yield losses in a wide range of economically important crops. Their economic significance and wide host adaptability across fruit crops have been comprehensively reviewed, particularly in pomegranate and other perennial systems (Singh *et al.*, 2019). The annual global crop loss due to root-knot nematode infestation is estimated at approximately 100 billion USD (Jain *et al.*, 2007). The predominant species affecting crops include *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Taylor and Sasser, 1978). Recent reports continue to document the expanding host range and geographical distribution of *Meloidogyne incognita*, including its first record on cucumber from Manipur, India (Sumita and Vivekananda, 2024). In addition to these, *M. enterolobii* has emerged as a particularly destructive species, especially in guava-growing regions of India.

The nematode *M. enterolobii* was first identified and described from the Chinese pacara earpod tree

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(*Enterolobium contortisiliquum*) in 1983 (Yang and Eisenback, 1983). It is regarded as one of the most aggressive root-knot nematodes due to its rapid

reproduction rate, formation of large compound galls and aggressive feeding behavior, which collectively contribute to severe damage in guava plantations (Brito *et al.*, 2004). Formation of root galls results in disruption of nutrient and water uptake, resulting in plant weakening and physiological collapse (Nguyen *et al.*, 2018; Ashokkumar *et al.*, 2023). Because of its high virulence and potential for spread, *M. enterolobii* has been included in the EPPO A2 list of quarantine pests to prevent its introduction and dissemination (Hallmann and Meressa, 2018). In India, the first occurrence of *M. enterolobii* was reported from Ayakudi village in Dindigul district, Tamil Nadu (Ashokkumar and Poornima, 2019).

Accurate identification of *M. enterolobii* is crucial for the development of effective management strategies. However, morphological and morphometric features often overlap with closely related species, making diagnosis difficult (Hunt and Handoo, 2009). Molecular characterization using species-specific markers such as ITS, 28S rDNA and COI mitochondrial regions provides a reliable complement to morphological identification (Dong *et al.*, 2021).

The present study was undertaken to confirm the identity of *M. enterolobii* associated with guava root galls in Tamil Nadu through an integrated approach combining morphological, morphometric and molecular characterization.

MATERIALS AND METHODS

Sample collection and nematode isolation

Guava orchards exhibiting root galling and wilt symptoms were surveyed in Coimbatore, Salem and Dindigul districts of Tamil Nadu. Nematode-infected guava roots were uprooted, collected, placed in polythene bags and transported to the laboratory. Nematodes were extracted using the modified Baermann funnel technique and by dissecting adult females from galled roots.

All experiments were carried out at the SRM College of Agricultural Sciences, SRM Institute of Science and Technology (SRMIST), Chengalpattu, Tamil Nadu, India, from April 2025 to November 2025.

Morphological and morphometric observations

Second-stage juveniles (J2) were killed by gentle heating and fixed in triethanolamine formaldehyde (TAF). Adult females were determined by staining the infected root galls and the perineal patterns were prepared following Taylor and Netscher (1974) and examined under a compound microscope. About 20 females and second-stage juveniles were assessed and their morphometric parameters (body length, stylet length, tail length, hyaline tail terminus) were measured using an ocular micrometer and compared with standard keys (Eisenback and Triantaphyllou, 1991).

Molecular characterization

Genomic DNA was extracted from individual females using the proteinase K lysis method. PCR amplification was performed using species-specific primers for *M. enterolobii* (MeF/MeR) targeting the ITS region and COI primers

(COIF/COIR). PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. Amplicons were sequenced and submitted to GenBank. Sequence similarity was verified using BLASTn and phylogenetic analysis was conducted using MEGA X software with the neighbor-joining method.

RESULTS AND DISCUSSION

Symptoms

The infected plants were found to be stunted in growth with yellowing, browning and bronzing of leaves with marginal necrosis (Fig 1a), defoliation and smaller fruits. The infected plants were uprooted and observed for nematode symptoms. The presence of simple and compound galls in the roots indicated the nematode infestation in guava plants (Fig 1b).

Morphological and morphometric characteristics

Mature females were pear- to globular-shaped with a long, prominent neck and no posterior protuberance, distinguishing them from other root-knot nematode species (Fig 2d). The head region was continuous, comprising the labial disc and medial lips. Annulations were distinct in the posterior region of the body. The stylet conus was slightly curved and tapered distally. The morphometric characteristics of *M. enterolobii* females isolated from guava was closely associated with the original description by Yang and Eisenback (1983), with minor variations (Table 1). The mean body length (752.6 μ m) and width (610.5 μ m) were slightly higher than the original (735.0 μ m and 606.8 μ m), whereas neck length was slightly shorter (212.3 μ m vs. 218.4 μ m). Stylet measurements were similar, with minimal reductions in knob height and width. The excretory pore was positioned slightly further from the head (65.1 μ m vs. 62.9 μ m). Inter-phasmidial and vulval lengths showed small variations, while the vulva-anus distance remained similar. The body ratio ($A = 1.23$) was consistent with the original (1.2) and coefficients of variation (2.45-18.75%) indicated low morphological variability. Nematode egg masses were

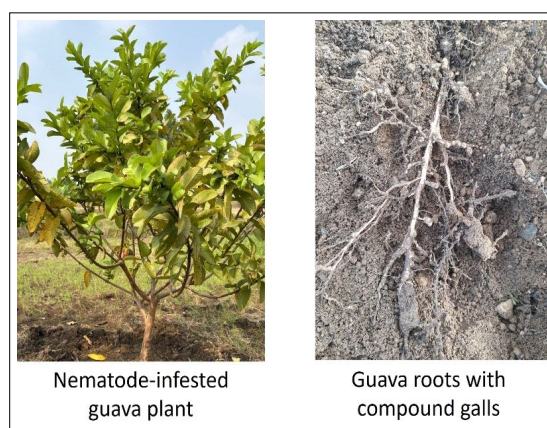


Fig 1: Symptoms of root knot nematode, *M. enterolobii* infection in guava.

gelatinous, irregular to oval in shape, extruded on the root surface and contained numerous oval, thin-shelled eggs. (Fig 2a and 2b).

The perineal pattern of *M. enterolobii* females obtained from guava was predominantly oval, with a moderately high dorsal arch and a rounded to squarish outline in some individuals. Coarse and fine striae were clearly visible, while the perivulval region appeared smooth, lacking striations. Striations extended onto the lateral areas near the vulval tail terminus (Fig 2e). This confirmed the identity of the species as *M. enterolobii* based on its morphological characteristics.

The male was long, translucent white and vermiform with a rounded tail. The head was slightly elevated from the body and the cephalic framework was moderately developed. The stylet was robust with a straight conus, cylindrical shaft and large, rounded knobs detached from the shaft. Spicules were arcuate with a rounded base and

a short, smooth tail (Fig 2f, g). The morphometric traits of *M. enterolobii* males from guava closely resembled those described by Yang and Eisenback (1983), with minor quantitative differences (Table 2). The mean body length (1285.6 μ m) and width (35.4 μ m) were slightly lower than the original description (1599.8 μ m and 42.3 μ m, respectively). Stylet and spicule lengths were comparable, measuring 22.3 μ m and 28.6 μ m against 23.4 μ m and 30.4 μ m in the original, while the gubernaculum length was marginally higher (7.0 μ m vs. 6.2 μ m). The DEGO value (5.6 μ m) and excretory pore position (146.2 μ m) showed slight variations, indicating natural population variability. The tail was shorter (11.2 μ m vs. 12.5 μ m) and the testis length was average with 315 μ m. Ratios A (37.8) and C (118.5) are closely aligned with the original description (37.9 and 131.6).

Infective second-stage juveniles were transparent, slender and tapering at both ends, with a slightly offset

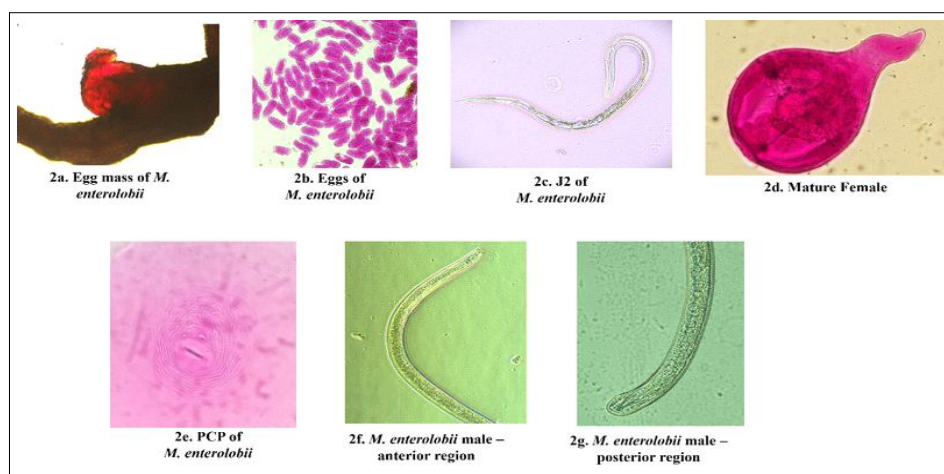


Fig 2: Morphological identification of root knot nematode, *M. enterolobii* in guava.

Table 1: Comparative morphometric characteristics of mature females of *Meloidogyne enterolobii* from guava with the original description (Yang and Eisenback, 1983).

Character	<i>M. enterolobii</i> mature female description Mean $\pm$ SD $\pm$ SE (range)	Original description (Yang and Eisenback, 1983) Mean $\pm$ SD (range)	Difference (Δ)	CV (%) (Present)
Body length (μ m)	752.6 $\pm$ 18.42 $\pm$ 10.63 (720.8-770.4)	735.0 $\pm$ 92.8 (541.3-926.3)	+17.6	2.45
Body width (μ m)	610.5 $\pm$ 29.67 $\pm$ 17.12 (568.0-648.7)	606.8 $\pm$ 120.5 (375.7-809.7)	+3.7	4.86
Neck length (μ m)	212.3 $\pm$ 17.85 $\pm$ 10.31 (192.4-238.5)	218.4 $\pm$ 74.1 (114.3-466.8)	-6.1	8.41
Stylet length (μ m)	15.2 $\pm$ 2.85 $\pm$ 1.64 (12.3-19.1)	15.1 $\pm$ 1.35 (13.2-18.0)	+0.1	18.75
Stylet knob height (μ m)	2.08 $\pm$ 0.27 $\pm$ 0.15 (1.8-2.5)	2.4 $\pm$ 0.26 (1.9-3.1)	-0.32	13.10
Stylet knob width (μ m)	4.81 $\pm$ 0.44 $\pm$ 0.25 (4.1-5.4)	4.9 $\pm$ 0.39 (4.1-5.6)	-0.09	9.15
DOGO (μ m)	4.96 $\pm$ 0.49 $\pm$ 0.28 (4.2-5.6)	4.9 $\pm$ 0.78 (3.7-6.2)	+0.06	9.87
Excretory pore to head end (μ m)	65.1 $\pm$ 6.84 $\pm$ 3.95 (54.0-72.6)	62.9 $\pm$ 10.5 (42.3-80.6)	+2.2	10.51
Inter-phasmidial length (μ m)	30.28 $\pm$ 2.91 $\pm$ 1.68 (26.1-34.8)	30.7 $\pm$ 4.8 (22.2-42.0)	-0.42	9.61
Vulval length (μ m)	26.04 $\pm$ 1.74 $\pm$ 1.00 (24.3-28.8)	28.7 $\pm$ 2.0 (25.3-32.4)	-2.66	6.68
Vulva-anus distance (μ m)	21.96 $\pm$ 1.93 $\pm$ 1.11 (19.0-24.6)	22.2 $\pm$ 1.8 (19.7-26.6)	-0.24	8.79
A (Body length/width ratio)	1.23 $\pm$ 0.05 $\pm$ 0.03 (1.17-1.31)	1.2 $\pm$ 0.2 (0.9-1.9)	+0.03	4.07

head region (Fig 2c). The stylet was fine, with a pointed conus, broader shaft and round knobs. The procropus was distinct, the metacropus was elliptical and the tail was hyaline, thin and contained few fat droplets and the phasmids were indistinct. The morphometric traits of *M. enterolobii* juveniles from guava were closely matched with the original description by Yang and Eisenback (1983), showing only slight variations (Table 3). The present population had a determined mean body length of 427.5 μ m and a width of 14.6 μ m, which is slightly lower than the original values. Tail length (52.4 μ m) and excretory pore position (82.1 μ m) were marginally reduced. Stylet dimensions (12.3 μ m; knobs 1.7 $\times$ 3.0 μ m) and DOGO (3.0 μ m) were comparable to the reference. The body ratios A (29.5) and C (8.1) remained consistent, confirming overall morphological similarity with minor variations induced by host or environment.

Molecular confirmation

PCR amplification produced a distinct band of approximately 720 bp for ITS and 450 bp for COI (Fig 3),

confirming their species identity. The sequences of the isolates was submitted to the GenBank with an Accession Number PX 667703, PX667704 and PX667705 of ITS primer and PX 658232, PX658233 and PX658234 of COI primer. BLAST analysis of the sequences showed 99–100% similarity with *M. enterolobii* reference isolates from China (MN509752), Brazil (ON341109) and India (OK678123). Phylogenetic clustering placed the guava isolate within the *M. enterolobii* clade (Fig 4), clearly separated from *M. incognita* and *M. javanica*.

Morphological identification of *M. enterolobii* is challenging due to similarities with *M. incognita* and *M. javanica*. However, the high dorsal arch and smooth perineal striae observed in this study are consistent with earlier descriptions by Kiewnick *et al.* (2019) and Ashokkumar *et al.* (2021). The morphometric ranges of J2 and females corroborate previous findings on *M. enterolobii* populations from tropical fruit crops. The characterization of root knot nematode species based on morphological features is time consuming and requires highly skilled technical personnel (Blok *et al.*, 2002). Identification of *Meloidogyne*

Table 2: Comparative morphometric characteristics of males of *Meloidogyne enterolobii* from guava with the original description (Yang and Eisenback, 1983).

Character	<i>M. enterolobii</i> Male population Mean $\pm$ SD (range)	Original Description (Yang and Eisenback, 1983) Mean $\pm$ SD (range)
Body length (μ m)	1285.6 $\pm$ 165.8 (910.3-1410.7)	1599.8 $\pm$ 159.9 (1348.6-1913.3)
Males body width (μ m)	35.4 $\pm$ 3.3 (29.2-40.8)	42.3 $\pm$ 3.5 (37.0-48.3)
Tail length (μ m)	11.2 $\pm$ 3.0 (8.0-15.8)	12.5 $\pm$ 2.24 (8.6-20.2)
Stylet length (μ m)	22.3 $\pm$ 1.6 (20.1-26.3)	23.4 $\pm$ 0.96 (21.2-25.5)
Stylet knob height (μ m)	3.2 $\pm$ 0.3 (2.5-3.8)	3.3 $\pm$ 0.33 (2.6-3.9)
Stylet knob width (μ m)	4.9 $\pm$ 0.4 (4.0-6.9)	5.4 $\pm$ 0.34 (4.5-5.8)
DEGO (μ m)	5.6 $\pm$ 1.1 (4.0-6.8)	4.7 $\pm$ 0.4 (3.7-5.3)
Excretory pore to head end (μ m)	146.2 $\pm$ 29.5 (100.4-202.3)	178.2 $\pm$ 11.2 (159.7-206.2)
Spicule length (μ m)	28.6 $\pm$ 3.0 (21.5-34.0)	30.4 $\pm$ 1.2 (27.3-32.1)
Gubernaculum length (μ m)	7.0 $\pm$ 0.7 (5.8-8.6)	6.2 $\pm$ 1.0 (4.8-8.0)
Testis length (μ m)	315.0 $\pm$ 82.1 (240.0-450.0)	-
A (Body length/width ratio)	37.8 $\pm$ 3.6 (32.0-44.0)	37.9 $\pm$ 3.2 (34.1-45.5)
C (Body length/tail length ratio)	118.5 $\pm$ 39.8 (90.0-168.0)	131.6 $\pm$ 24.1 (72.0-173.4)

Table 3: Comparative morphometric characteristics of second stage juveniles of *Meloidogyne enterolobii* from guava with the original description (Yang and Eisenback, 1983).

Character	Guava (Pre mean $\pm$ SD (range)	Original description (Yang and Eisenback, 1983) Mean $\pm$ SD (range)
Body length (μ m)	427.5 $\pm$ 28.9 (380-475)	436.6 $\pm$ 16.6 (405.0-472.9)
Body width (μ m)	14.6 $\pm$ 1.3 (12.8-17.5)	15.3 $\pm$ 0.9 (13.9-17.8)
Tail length (μ m)	52.4 $\pm$ 6.8 (43.5-77.0)	56.4 $\pm$ 4.5 (41.5-63.4)
Excretory pore to head end (μ m)	82.1 $\pm$ 4.6 (74.8-95.0)	91.7 $\pm$ 3.3 (84.0-98.6)
Stylet length (μ m)	12.3 $\pm$ 0.5 (11.5-12.9)	11.7 $\pm$ 0.5 (10.8-13.0)
Stylet knob height (μ m)	1.7 $\pm$ 0.1 (1.5-1.9)	1.6 $\pm$ 0.1 (1.5-1.8)
Stylet knob width (μ m)	3.0 $\pm$ 0.2 (2.5-3.4)	2.9 $\pm$ 0.2 (2.4-3.4)
DOGO (μ m)	3.0 $\pm$ 0.3 (2.5-3.9)	3.4 $\pm$ 0.3 (2.8-4.3)
A (Body length/width ratio)	29.5 $\pm$ 2.9 (25.0-34.8)	28.6 $\pm$ 1.9 (24.0-32.5)
C (Body length/tail length ratio)	8.1 $\pm$ 0.9 (6.3-10.4)	7.8 $\pm$ 0.7 (6.8-10.1)

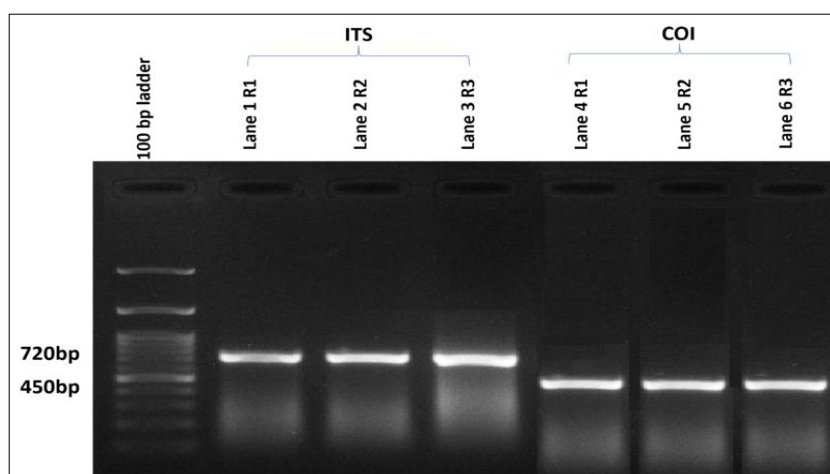


Fig 3: Molecular identification of root knot nematode, *M. enterolobii* in guava using ITS and COI primers.

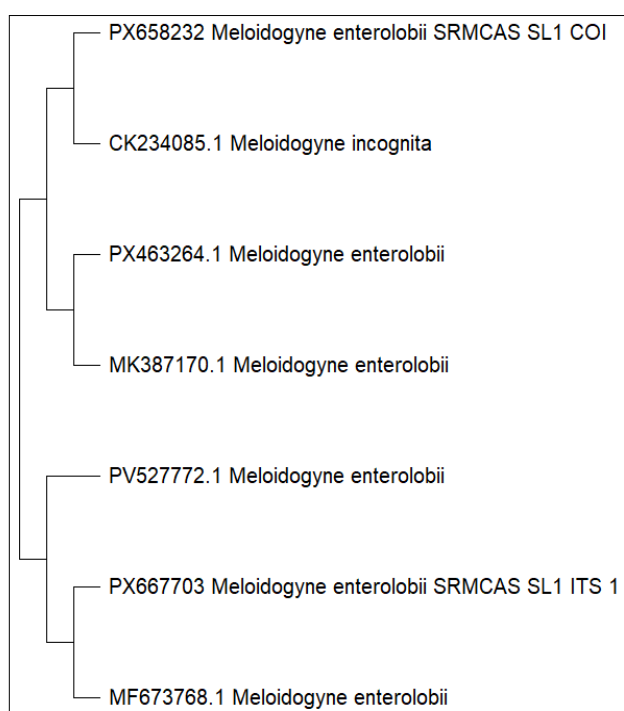


Fig 4: Phylogenetic tree on molecular identification of root knot nematode, *M. enterolobii* in guava using ITS and COI primers.

species is important to design effective management practices such as crop rotation and use of plant resistance (Zijlstra *et al.*, 2000). Studies on varietal susceptibility have demonstrated differential host responses to *Meloidogyne incognita* under controlled conditions, highlighting host resistance as a critical component of nematode management strategies (Ikram *et al.*, 2025). The root knot nematode, *M. enterolobii* was morphologically identified based on infective second stage juveniles, male, female and posterior cuticular pattern.

The bodies of infective second-stage juveniles were long, clear white, vermiform, tapered at both ends and had

a slender tail. The head region was continuous and a little offset from the body and the tail tip was hyaline, very thin and had a few fat droplets. The description derived from the second-stage juveniles of the collected samples was similar to the original descriptions given by Rammah and Hirschmann (1988).

Posterior cuticular pattern of *M. enterolobii* was rounded to oval with a medium to high dorsal arch and a lengthy vulval slit. The posterior cuticular patterns of *M. enterolobii* encountered during the current study showed round to dorsoventrally ovoid, long vulval slit with medium to high dorsal arch, as observed from multiple populations of collected samples and their characters were similar to the original descriptions given by Rammah and Hirschmann (1988) and Yang and Eisenback (1983). However, Brito *et al.* (2004) determined that the perineal patterns of *M. enterolobii* isolates in 70% of the populations studied were round to dorsoventrally ovoid, while the remaining 30% had trapezoidal dorsal arches comparable to those of *M. incognita* in Florida. Brito *et al.* (2004) found that the morphometrics of *M. mayaguensis* females were identical to those reported in the original description in West Africa.

Molecular approaches for identifying *Meloidogyne* species using DNA are being developed by utilizing polymerase chain reaction (PCR), which permits the investigation of a single nematode (Akyazi and Felek, 2013). In the current study, PCR analyses were used to amplify the internal transcribed spacer using ITS and COI primers. Isolates from various guava orchards were identified as *M. enterolobii*. The sequencing results revealed 90-100% similarity with the relevant *M. enterolobi* strains. For *Meloidogyne* spp. ITS regions of nematodes were site-specific and the size of the amplicon was amplified at 620 base pairs. Molecular identification using ITS and COI markers proved conclusive, aligning with global reports highlighting their diagnostic reliability (Dong *et al.*, 2021). The detection of *M. enterolobii* in multiple guava-growing regions indicates its widespread prevalence and potential to exacerbate the guava wilt-nematode complex. The integrative

diagnostic approach employed here and provides a reliable basis for surveillance, quarantine and development of host-resistance screening programs.

CONCLUSION

This study provides the first comprehensive morphological and molecular characterization of *M. enterolobii* infecting guava in Tamil Nadu. The results confirm its identity and distribution and underscore the need for continuous monitoring and development of integrated nematode management strategies.

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Informed consent

This study did not involve human participants or vertebrate animals. All experimental procedures involving nematode samples were conducted in accordance with standard laboratory protocols and institutional guidelines.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. No funding agency or sponsor had any role in the study design, data collection, analysis, interpretation of results, decision to publish, or preparation of the manuscript.

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