International Journal of Environment, Agriculture and Biotechnology
Vol-10, Issue-2; Mar-Apr, 2025
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Morphometric Taxonomy of *Meloidogyne* spp. Infesting Vegetable Crops in Sri Lanka via Female Perineal Pattern Analysis

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Received: 16 Jan 2025; Received in revised form: 18 Feb 2025; Accepted: 25 Feb 2025; Available online: 03 Mar 2025

Abstract— *Meloidogyne spp. are among the most significant plant-parasitic nematodes in agriculture, necessitating precise identification for effective management. This study aimed to identify and taxonomically characterize Meloidogyne spp. infesting vegetable crops in Sri Lanka using female perineal pattern analysis. A total of 54 root and soil samples were collected from 17 agricultural regions and analyzed at the Horticultural Crop Research and Development Institute (HORDI), Sri Lanka. Single egg-mass cultures were established using a nematode-susceptible tomato variety (KWR), and second-stage juveniles (J2) were used for precise inoculation. Perineal patterns of mature females were prepared following standard protocols and examined microscopically. A total of 101 female nematodes were identified, revealing the presence of M. arenaria (33%), M. javanica (30%), M. incognita (25%), and M. hapla (13%). M. arenaria exhibited forked lateral fields and a low dorsal arch, while M. javanica displayed distinct double lateral incisures. M. incognita was characterized by an angularly oval pattern with a high dorsal arch, and M. hapla showed a concentrated punctation between the anus and tail terminus. Host range analysis indicated that M. hapla had a narrow host range, whereas the other species were oligophagous, with M. incognita affecting a broad spectrum of crops, including tomato, spinach, brinjal, and okra. The study confirmed that M. incognita, M. javanica, and M. arenaria are the predominant Meloidogyne spp. in Sri Lanka's vegetable-growing regions, with M. arenaria being the most frequently detected. These findings align with previous research and highlight the necessity of targeted management strategies. The results provide a valuable foundation for developing species-specific nematode control measures in Sri Lanka's agricultural systems.*

Keywords— ***Female perineal pattern, Meloidogyne spp., KWR, Second stage juveniles***

I. INTRODUCTION

Meloidogyne spp. are among the most economically significant plant-parasitic nematodes in agricultural systems worldwide, requiring accurate identification and taxonomic characterization for effective management. Female perineal pattern analysis is a widely used and reliable method, particularly in the absence of molecular techniques, as it provides species-level identification essential for agricultural applications [3,8,12]. This technique involves examining the dorsal esophageal gland orifice and the surrounding perineal region of mature females, where distinct patterns, including the shape and arrangement of

striae and the presence of a dorsal arch, facilitate differentiation among *Meloidogyne* spp. Several studies [2,6,13] have demonstrated the effectiveness of this method for species identification. Given the variations in host preferences, life cycles, and resistance levels among *Meloidogyne* spp., species-specific management strategies are necessary for effective control. Therefore, this study aims to identify and taxonomically characterize *Meloidogyne* spp. present in Sri Lanka using female perineal pattern analysis to facilitate the development of targeted management strategies.

II. METHODOLOGY

2.1 Sample Collection and Nematode Culture Establishment

Fifty-four nematode-infested root samples were collected from 17 Agriculture Instructor (AI) ranges across Sri Lanka, covering various vegetable crops (Table 1.). Sampling was conducted from December 2016 to March 2017 at the Division of Entomology and Nematology, Horticultural Crop Research and

Development Institute (HORDI), Gannoruwa, Peradeniya, Sri Lanka.

Table 1: Details of the root samples collected from vegetables growing fields in seventeen AI rangers in Sri Lanka

Name of the AI range	Crop Type *	Name of the AI range	Crop Type *
Wendaruwa	Tomato	Eadanduwawa	Gotukola
Nugethenne	Carrot		Kankun
Kalunthenne *	Tomato	Galewela	Guava
	Beetroot	Dambagahapitiya	Tomato
Madamahanuwara	Tomato	Ukuwela	Brinjal
Mahawella	Tomato		Okra
Udispaththuwa	Tomato	Warallagama	Wing bean
	Chilli	Malabe	Wing bean
	Spinach	Kibissa	Thumba
Digana *	Knolkhol		
	Kankun		
	Spinach		
	Chinese kale		

* One sample from each location except three tomato samples from Kalunthenna and 3 spinach samples from Digana

Single egg-mass cultures of *Meloidogyne* spp. were established using a nematode-susceptible tomato variety (KWR) under controlled plant house conditions. The potting mixture (86% sand, 7% clay, 4% silt, 3% organic matter) was sterilized at 120°C for six hours. Sterilized clay pots (15 cm) were filled with 4 kg of the mixture, and basal fertilizers (Urea: 2.6 g/pot, TSP: 13.4 g/pot, MOP: 2.6 g/pot) were applied. Eighteen-day-old tomato seedlings were transplanted at a rate of one per pot.

Infested roots were washed, cut into 2–3 cm pieces, and egg masses were isolated and incubated in sterilized distilled water for second-stage juvenile (J2) hatching. One week after transplanting, seedlings were inoculated with J2s by introducing a suspension into four holes around each plant. Ten seedlings per sample were inoculated using J2s from ten separate egg masses to ensure consistency.

1.1. Perineal Pattern Preparation and Microscopy

One month after inoculation, plants were uprooted, and female nematodes were extracted from root galls. Perineal pattern preparation followed according to Hartman and Sasser [7]. Root galls were cut into 1–2 cm sections and placed in sterilized water. Individual females were transferred onto slides with 45% lactic acid drop, ruptured using a scalpel, and body contents were dissolved with forceps. The perineal region was carefully excised, and 10–15 slides per sample were prepared for microscopic examination at 10×100 magnification (Fig.1).

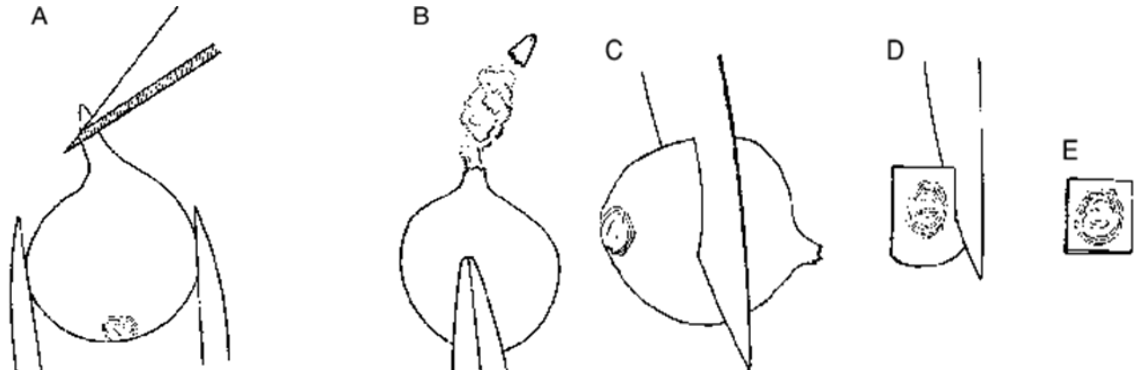


Fig.1: Procedure for microscope preparation of female perineal pattern for identification: A-B. Excised female being raptured and body tissues gently removed, C-E. Cuticle being trimmed from around the perineal pattern

2.2 Species Identification

Meloidogyne spp. were identified based on perineal pattern morphology using descriptions by Eisenback and standard plates from Dr. J. N. Sasser [5,7] (Figure 02, Plate 01). Identification focused on *Meloidogyne incognita, M. javanica, M. arenaria,* and *M. hapla*, considering striae patterns, lateral lines, and dorsal arch features (Table 2).

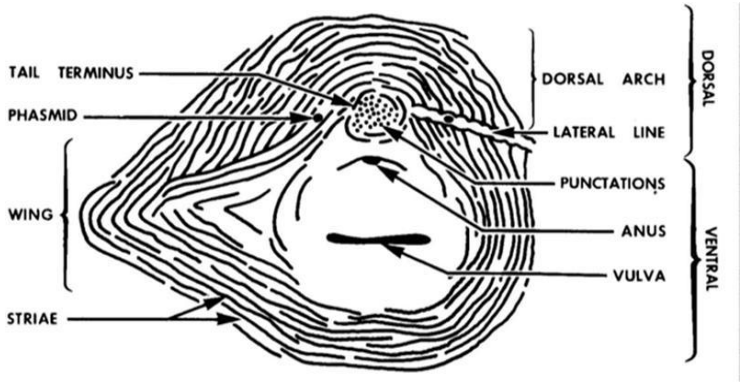
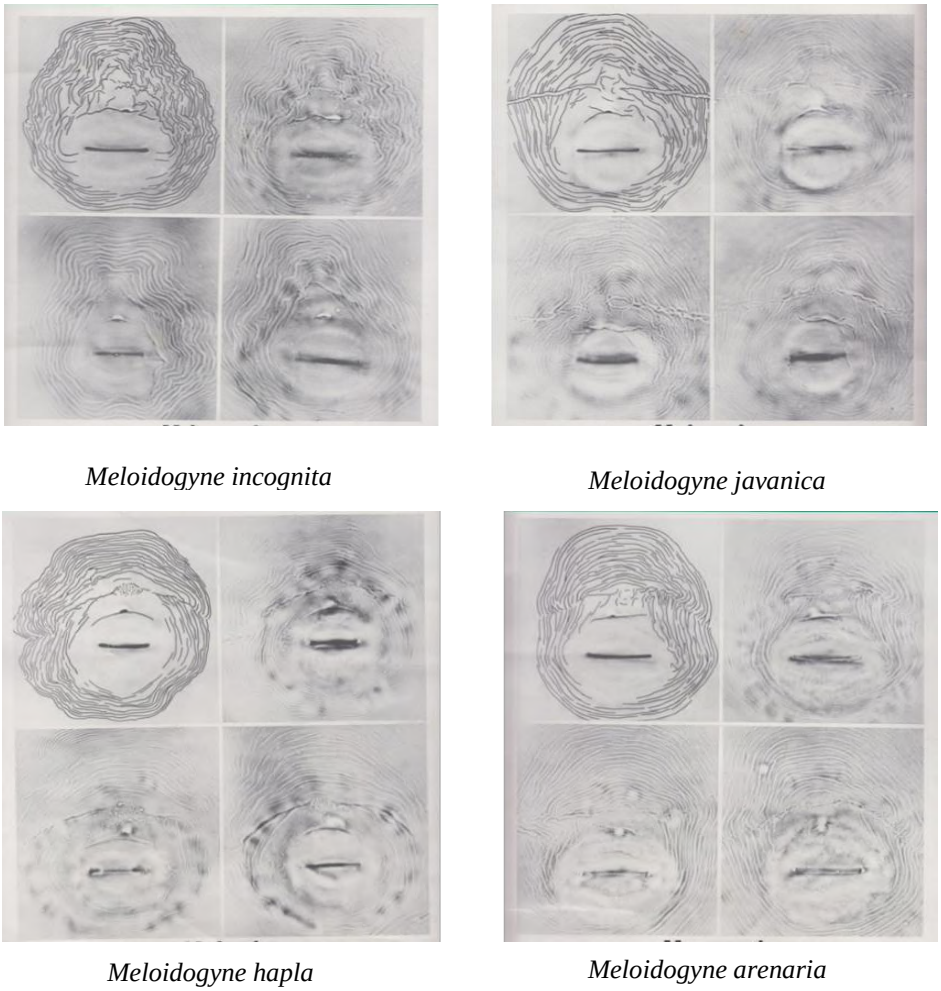


Fig.2: Diagrammatic representation of the perineal pattern of Meloidogyne spp.

Table 2: Comparison of Characteristics of female perineal pattern among the four main Meloidogyne spp.

Characteristic	*M. arenaria*	*M. javanica*	*M. incognita*	*M. hapla*
Striae (patterns of lines)	Smooth and slightly wavy, sometimes forming wings laterally	Smooth and somewhat wavy	Smooth and wavy, sometimes zigzag	Close, smooth, and wavy, sometimes forming wings
Lateral lines/ridges	Forked, irregular lateral fields	Unique, distinct lateral lines present	Absent	Lateral fields marked by irregularities in the striae
Dorsal arch characteristics	Low and indented near lateral fields, forming rounded shoulders	Low or rounded, sometimes with a whorl in tail terminus area	Squares and high, with a whorl around tail terminus	Usually low and rounded, but may be high and squarish



Meloidogyne incognita *Meloidogyne javanica*

Meloidogyne hapla *Meloidogyne arenaria*

Plate.1: Standard perineal pattern images for the most common Meloidogyne spp.

III. RESULTS AND DISCUSSION

Analysis of perineal patterns from 101 female nematode samples identified four *Meloidogyne* species: *M. arenaria* (n=33), *M. javanica* (n=30), *M. incognita* (n=25), and *M. hapla* (n=13). The perineal pattern characteristics observed were consistent with previous studies [1,10].

M. arenaria exhibited a forked lateral field, fractured striae in a winged form, and a low dorsal arch [1,10]. *M. javanica* showed distinct lateral fields with well-defined double incisures [5,10]. *M. incognita* displayed an angularly oval perineal pattern with a high dorsal arch and an Inverted-V shape [10]. *M. hapla* was distinguished by a concentrated punctation between the anus and tail terminus, consistent with previous descriptions [5,10].

Host range analysis (Table 3) confirmed that *M. hapla* had a more restricted host range compared to the other three species, which exhibited an oligophagous nature. The most frequently infested crops included Centella, *Alternanthera sessilis*, Knolkhol, Water Spinach, Spinach, Chinese Kale, Brinjal, Beetroot, Okra, Tomato, Guava, Carrot, Chilli, and Spine gourd.

The study found *M. incognita*, *M. javanica*, and *M. arenaria* to be the dominant *Meloidogyne* spp. in vegetable-growing areas of Sri Lanka, with *M. arenaria* being the most prevalent (32.67%). These findings align with previous reports on *Meloidogyne* spp. distribution in Sri Lanka [15] and globally [10].

Effective management strategies, including resistant cultivars, crop rotation, soil amendments, and strict quarantine measures, are essential to mitigate the impact of root-knot nematodes in vegetable production systems in Sri Lanka.

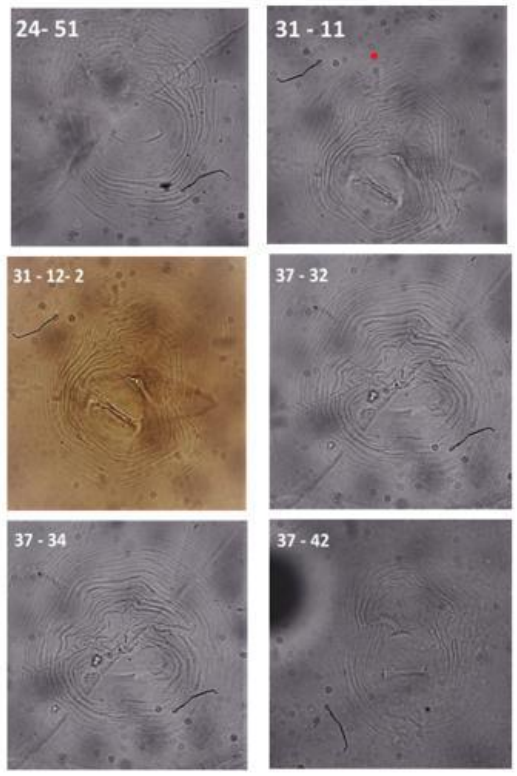


Plate.1: Perineal patterns of Meloidogyne arenaria isolates collected from the Central region of Sri Lanka

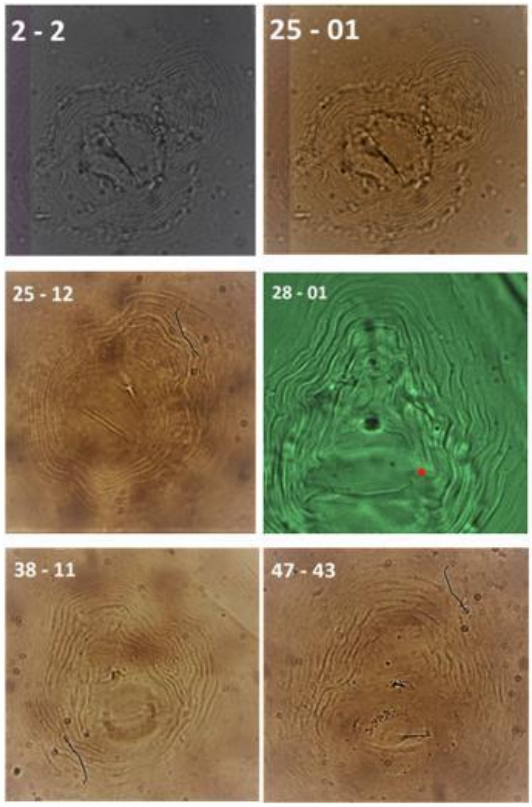


Plate.3: Perineal patterns of Meloidogyne incognita isolates collected from the Central region of Sri Lanka

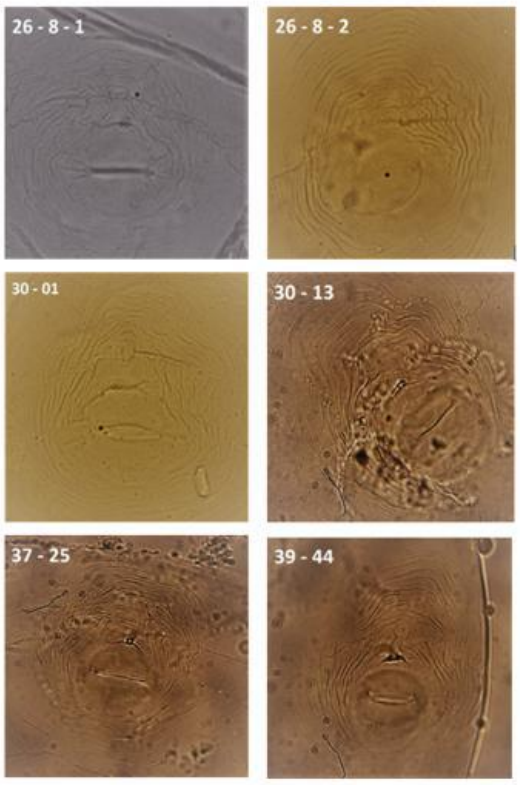


Plate.2: Perineal patterns of Meloidogyne javanica isolates collected from the Central region of Sri Lanka

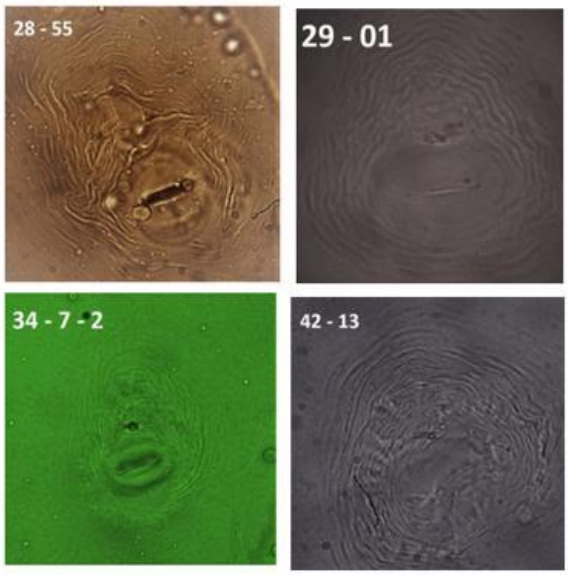


Plate.4: Perineal patterns of Meloidogyne hapla isolates collected from the Central region of Sri Lanka

Table 3: Main crops attacked by the Meloidogyne spp. in Sri Lanka

***Meloidogyne* spp.**	**Host**
Meloidogyne arenaria	Guava (*Psidium guajava*), Gotukola (*Centella asiatica*), Mukunuwenna (*Alternanthera sessilis*), Tomato (*Solanum lycopersicum*), Carrot (*Daucus carota*), Spinach (*Spinacia oleracea*), Wing bean (*Psophocarpus tetragonolobus*), Okra (*Abelmoschus esculentus*)
Meloidogyne javanica	Mukunuwanna, Carrot, Tomato, Brinjal (*Solanum melongena*), Beetroot (*Beta vulgaris*), Gotukola, Guava
Meloidogyne incognita	Gotukola, Mukunuwanna, Knolkhol (*Brassica oleracea*), Water Spinash (*Ipomoea aquatica*), Spinash, Chinese Kale (*Brassica oleracea var. alboglabra*), Brinjal, Beetroot, Okra, Tomato, Spinach, Guava
Meloidogyne hapla	Mukunuwanna, KnolKhol, Chilli (*Capsicum annum*), Spine guard (Thumba) (*Momordica dioica)*

IV. CONCLUSION

Female perennial patterns based on taxonomic characterization identified four common species of *Meloidogyne* spp., namely *M. incognita*, *M. javanica*, *M. Arenaria*, and *M. hapla* on wide range of crops grown in Sri Lanka. The occurrence of each species varied in numbers demonstrating a wide to narrow host range. *M. arenaria* was detected at the highest frequency but *M. incognita* shows a broad host range indicating a high impact on crops. *M. hapla* was detected at low frequency with a narrow host range showing its minimum contribution on crop losses. These findings are useful for decision making on affective management of nematodes.

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