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Research Article

Multivariate morphometric analysis of *Meloidogyne* spp. in Tamil Nadu, India: A PCA-based approach to population differentiation in carrot ecosystems

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Abstract
Root-knot nematodes (*Meloidogyne* spp.) are major agricultural pests threatening carrot (*Daucus carota* L.) cultivation in Tamil Nadu, India, causing significant yield losses. This study employed Principal Component Analysis (PCA) to investigate the morphometric traits of second-stage juveniles (J2) and adult females from eight *Meloidogyne spp*. populations collected from carrot-growing regions across Tamil Nadu. Morphological characterization involved extracting J2 and adult females, preparing whole mounts in glycerin, dissecting female perineal patterns, and recording morphometric measurements using Camera Lucida, phase contrast, and scanning electron microscopy. It revealed variations in key traits, including body and stylet length, with significant differences between nematode populations from hill and plain regions. PCA reduced the high-dimensional data, explaining 93.80% and 93.45% of the total variance in females and juveniles, respectively. Strong positive correlations were observed between body length and median bulb length in juveniles, dorsal gland orifice (DOGO), and body width in females. Negative correlations were also noted for stylet width and DOGO in females. PCA clustering revealed three distinct groups, with juveniles from Shoolagiri and Hosur forming one group and females from the Nilgiris and Kodaikanal clustering together, indicating geographic and environmental influence on morphometric traits. Stylet length emerged as a key distinguishing factor for both juvenile and female populations. These findings clearly explain the morphometric diversity within *Meloidogyne* populations, offering new insights into nematode management strategies in Tamil Nadu's carrot ecosystems. This study also underscores the utility of PCA in streamlining morphometric analysis, enhancing the precision and speed of nematode identification.

Keywords: *Daucus carota* L., *Meloidogyne incognita, Meloidogyne hapla,* Nematode identification, Principal Component Analysis (PCA)

INTRODUCTION

Conventional morphometric analysis is commonly used in nematology to distinguish species and populations based on physical characteristics such as body size, shape, and anatomical measurements. This method typically involves multiple variables, including length, width, and body ratios, which provide important taxonomic clues. However, the complexity of the generated data poses significant challenges. The extensive number of variables makes the analysis labour-intensive, time-consuming, and often difficult to interpret. Additionally, the high-dimensional nature of the data can obscure subtle variations between populations or species, complicating the identification of meaningful patterns (Bogale *et al.,* 2020).

Researchers have adopted advanced statistical techniques like Principal Component Analysis (PCA) to address these challenges. PCA is a powerful multivariate tool that reduces the dimensionality of complex datasets while preserving essential information. By transforming the original variables into new, uncorrelated components (principal components), PCA simplifies the analysis and allows researchers to focus on the most influential variables (Jolliffe *et al.,* 2016). In nematology, Nyaku et al. (2018) successfully applied PCA to distinguish root-knot nematode (*Meloidogyne* spp.) populations in Ghana, demonstrating how this method can mitigate the limitations of conventional morphometrics. Their work underscored PCA's utility in identifying key morphometric traits significantly contributing to variation among nematode populations, enabling faster and more accurate species identification.

Efficient and accurate identification of root-knot nematodes (*Meloidogyne* spp.) is crucial for developing targeted management strategies. In Tamil Nadu, these nematodes threaten carrot cultivation, causing significant yield losses by inducing galls on roots, adversely affecting crop quality and market value (Gowda *et al.,* 2019). Despite their economic importance in carrot ecosystems, studies on morphometric characterization of root-knot nematodes in Tamil Nadu are limited. Wesly *et al.* (2021) confirmed the presence of root-knot nematodes in the carrot ecosystems of both the hills and plains of Tamil Nadu through basic morphological and molecular identification.

This study addresses this gap by employing PCA to analyze the morphometric characteristics of eight different juvenile and female *Meloidogyne* populations from carrot ecosystems in Tamil Nadu. PCA allows us to explore similarities and variations among these populations, providing a clearer understanding of their distinct morphometric traits.

MATERIALS AND METHODS

Before harvest, the survey for nematodes parasitizing carrots was conducted across major carrot-growing districts in Tamil Nadu, including The Nilgiris, Dindigul, Erode, and Krishnagiri (Figure 1). Infestations of root-knot nematodes were identified based on above-ground symptoms such as wilting, stunted growth, leaf chlorosis, and below-ground symptoms like galling, bearding, forking, and stubbing of roots. A total of 36 fields were surveyed, with root-knot nematode infestations observed in eight fields. Soil samples (50 cc) were collected from the rhizosphere region along with feeder roots, pooled into a zip-lock bag after collecting ten subsamples in a zigzag manner and labelled with essential details. Egg masses extracted from infected roots were hatched in water with aeration, and second-stage juveniles (J_2) were inoculated into one-month-old carrots in the net house. J_2 of root-knot nematodes were extracted from soil using the decanting and sieving technique and a modified Baermann's technique (Viglierchio and Schmitt, 1983). Roots were washed in running water to extract nematodes from roots, cut into 1-3 cm pieces, stained with a boiling acid fuchsin or cotton blue and lactophenol solution, and destained overnight. Adult root-knot nematode females were picked from the roots using forceps and a needle under a Binocular stereo-zoom microscope (Bridge and Page,

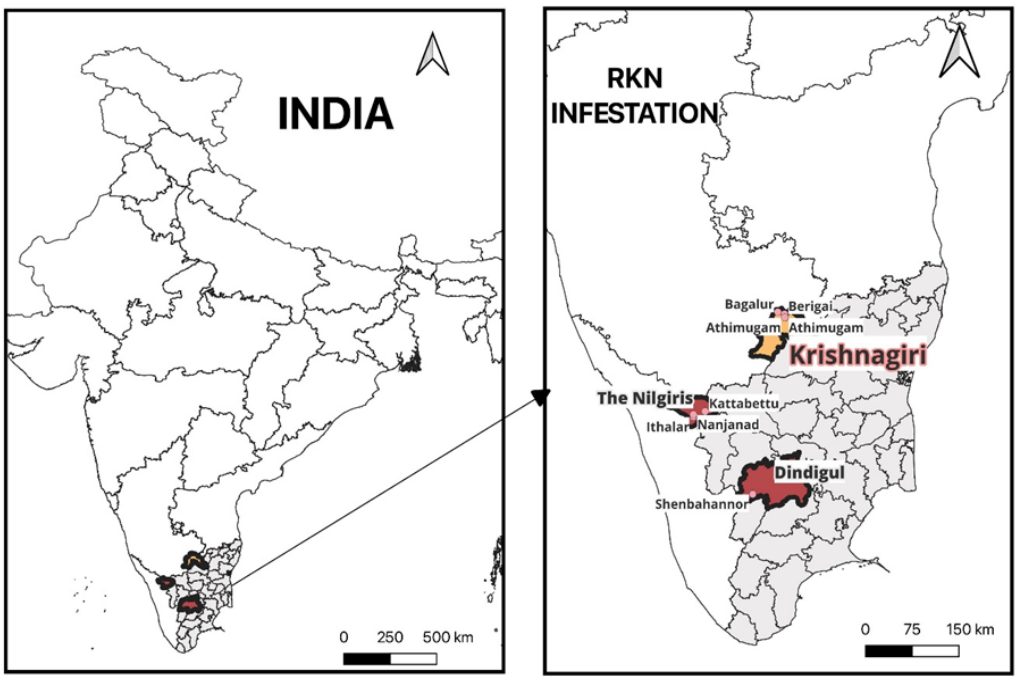

Fig. 1. *Locations of root-knot nematode infested carrot fields in Tamil Nadu*

1982).

Nematode suspensions obtained from the modified Baermann method were killed and fixed by mixing them with an equal volume of a 4% formalin solution (Hallmann *et al*, 2018). The nematodes were transferred into a cavity block containing Seinhorst I solution and placed in a closed desiccator with 95% ethanol for 24 hours. After incubation, the excess ethanol and Seinhorst I solution were removed using a fine pipette, leaving only the nematodes. Subsequently, Seinhorst II solution was added to the partially closed cavity block in the desiccator containing calcium chloride to facilitate the slow evaporation of alcohol. Whole mounts of the nematode specimens were prepared on an anhydrous glycerin medium on glass slides, which were then sealed with nail polish (Cobb, 1918).

To prepare the posterior cuticle pattern, the posterior end of the mature female nematode was sliced using a surgical blade, and the head was set aside. The body tissues were gently brushed away from the inner surface of the cuticle using a nylon bristle. The cuticle was carefully trimmed just above the perineal pattern. The perineal pattern was then transferred to a drop of dehydrated glycerine on a glass slide, covered with a coverslip, and sealed with transparent nail polish (Eisenback et al., 1980).

Specimens were captured using the Liss View application and drawn with a mirror-type Camera Lucida. Morphometric measurements were recorded based on the Camera Lucida drawings and a micrometer. The formulas for morphometric measurements followed the guidelines provided by De Man (1880). Images obtained from the compound microscope were captured using an image analyzer with Liss View 7.0 software (Hallmann *et al.,* 2018). Scanning electron microscope (SEM) images were acquired using a Quanta 250 manufactured by FEI (now Thermo Fisher Scientific), Czech Republic. Phase contrast images were captured with Leica Application Suite version 4.11.0. R software version 4.1.0 was used to study the relationship between morphometric variables through PCA, utilizing a correlation heatmap, monoplot, and biplot (R Core Team, 2023.

RESULTS

Characterization of root-knot nematodes infesting carrots grown in plains

Female:

Sedentary endoparasitic adult females were pear-shaped with a distinct neck projection, pearly white in color, and lacking a posterior protuberance. Stylet knobs were rounded and offset. Total body length ranged from 524 to 729 µm, with a maximum width of 327 to 423 µm. The stylet measured 13 to 17 µm, with rounded, backward-sloping knobs. The spear base width was 3.63 to 4.54 µm, and the dorsal gland orifice (DOGO) was located 2.37 to 3.12 µm behind the spear base. Median bulb dimensions were 36.6 to 47.2 µm in length and 30.8 to 44.1 µm in width. The vulval slit length ranged from 22 to 28 µm, and the vulva-to-anus distance was 21 to 30 µm. The excretory pore was posterior to the stylet knobs (Table 1 and Fig. 2a, 2c).

Posterior cuticular pattern

The posterior cuticular pattern exhibited a prominent dorsal arch with coarse, closely spaced zigzag striae and a distinct whorl at the tail terminus, while lateral fields and punctuations were absent (Fig. 2a, 2c).

Second stage juvenile (J2) The J2 head had a truncated lip, with rounded spear knobs and a lateral field defined by four incisures. The slender, hyaline tail tapered to a rounded tip. Body length ranged from 332 to 422 µm, with 'a', 'b', and 'c' values of 26-34 µm, 4-6.9 µm, and 6.7-9.8 µm, respectively. Tail length was 38-52 µm, and stylet length was 9.9 to 11.7 µm. Median bulb dimensions were 9.8-13.1 µm in length and 5.7-8.9 µm in width (Table 2 and Fig. 2a, 2c).

Characterization of root-knot nematodes infesting hill-grown carrots

Female:

Adult females were pear-shaped with a short, bent neck, pearly white coloration, and no posterior protuberance. Stylet knobs were rounded and offset. Total body length ranged from 439 to 890 µm, with a maximum width of 341 to 526 µm. Stylet length varied from 10.2 to 14.3 µm, and the spear base width was 2 to 3.5 µm. The DOGO was located 4.3 to 6.3 µm from the spear base. Median bulb dimensions ranged from 35.8 to 44.4 µm in length and 29.2 to 36.1 µm in width, while vulval slit length was 21 to 27 µm and vulva-to-anus distance was 22 to 29 µm (Table 1 and Fig. 2b, 2d).

Posterior cuticular pattern

The posterior cuticular pattern was flattened ovoid or roughly circular, featuring very fine striae and subcuticular punctations between the anus and tail terminus. The dorsal arch was low and rounded, with absent lateral ridges and a distinct whorl at the tail (Fig. 2b, 2d).

Second stage juvenile (J2)

The J2 head had a truncate cone without offset from the body, and the lateral field was defined by four incisures. Tail tips varied between subacute and bifid. Body length ranged from 315 to 376 µm, with 'a', 'b', and 'c' values of 22-28.9 µm, 5.9-8.2 µm, and 7-9 µm, respectively. Tail length was 38-51 µm, stylet length ranged from 7.8 to 11.4 µm, and median bulb dimensions were 9.8-13.1 µm in length and 6-10.9 µm in width (Table 2 and Fig. 2b, 2d).

Table 1. Morphometric measurements of females of *Meloidogyne incognita* and *Meloidogyne hapla*

Character	*Meloidogyne incognita* - Female				*Meloidogyne hapla* - Female			
	Chitwood, 1968*	Manoj *et al.*, 2004**	Shoolagiri population [a]	Hosur population [a]	Chitwood, 1968*	Manoj *et al.*, 2001**	Nilgiris population [a]	Kodaikanal population [a]
Total Body length	609 (500-723)	621 (530-712)	636.7±92.1 (524-729)	618.5±60.4 (544-679)	632 (419-845)	635 (420-850)	661.0±178.0 (439-843)	702.4±182.9 (473-890)
Maximum body width	415 (331-520)	425 (350-500)	357.8±25.8 (327-383)	388.4±34.7 (346-423)	436 (311-561)	437.5 (310-565)	437.2±77.4 (341-516)	445.4±79.3 (347-526)
Stylet length	14 (13-16)	14 (13-15)	15.6±1.3 (14-17)	14.1±0.9 (13-15)	11.5 (10-13)	12.5 (11-14)	11.7±1.2 (10.2-13)	12.6±1.6 (10.5-14.3)
Width of spear base	4 (3-5)	4.5 (3-6)	4.0±0.3 (3.63-4.41)	4.2±0.2 (3.91-4.54)	2.5 (2-3)	2.5 (2-3)	2.8±0.6 (2-3.4)	2.9±0.4 (2.4-3.5)
DOGO	3 (2-4)	4 (3-5)	2.6±0.2 (2.49-2.9)	2.8±0.3 (2.37-3.12)	5 (4-6)	6 (5-7)	5.4±0.7 (4.5-6.1)	5.4±0.8 (4.3-6.3)
Length of Median Bulb	46 (37-49)	49.5 (39-60)	42.4±4.6 (36.6-47.2)	40.4±1.9 (38.1-42.3)	37 (31-43)	39 (33-45)	38.8±1.4 (37.1-40.2)	40.5±0.8 (35.8-44.4)
Width of Median Bulb	39 (31-49)	39.5 (33-46)	34.3±2.9 (30.8-37.18)	41.2±2.8 (37.8-44.1)	31.5 (26-37)	33.5 (29-38)	32.1±2.4 (29.2-34.5)	33.5±2.5 (30.5-36.1)
Vulval slit length	23 (20-30)	-	25±2.4 (22-27.5)	25.7±2.1 (23-28)	20 (20-27)	-	23.6±1.7 (21.5-25.3)	24.3±2.6 (21-27)
Vulva to anus distance	21.5 (21-29)	-	25.9±2.4 (21-30)	24.7±2.2 (22-27)	24 (20-26)	-	25.6±2.8 (22-28.5)	26.5±2.4 (23.6-29)

*Original description ** Previous study in Tamil Nadu [a] Present study; All measurements are in the form of mean ± SD (range) and denoted in µm ; n = Number of populations observed = 10; DOGO - Dorsal Oesophageal Gland Opening

Table 2. Morphometric measurements of second stage juveniles of *M. incognita* and *M. hapla*

Character	*Meloidogyne incognita* – Second Stage Juvenile				*Meloidogyne hapla* – Second stage juvenile			
	Chitwood, 1968*	Manoj *et al.*, 2004**	Shoolagiri population [a]	Hosur population [a]	Chitwood, 1968*	Manoj *et al.*, 2001**	Nilgiris population [a]	Kodaikanal population [a]
Body length (L)	371 (300-393)	377.5 (360-395)	385.1±36.4 (340-422)	371.6±33.1 (332-404)	337 (312-355)	354.5 (334-375)	340.8±22.2 (315-362)	355.3±21.7 (330-376)
a value	46.3 (29-33)	32.5 (30-35)	29.3±2.6 (26-32)	30.9±3.1 (27-34)	23.9 (20.1-	29 (26-32)	25.3±1.7 (23.2-27)	25.7±3.1 (22-28.9)
b value	6 (5.6-6.4)	6.3 (5.8-6.8)	5.8±1.0 (4.6- 6.9)	5.3±1.1 (4.0- 6.6)	7 (6-8)	7.5 (6-9)	7±0.9 (5.9-8)	7.3±0.8 (6.2-8.2)
c value	8.7 (8-9.4)	9.2 (8.5-9.9)	8.6±0.7 (7.7-9.4)	8.4±1.3 (6.7-9.8)	7.9 (7.3-10.2)	7.75 (7-8.5)	7.6±0.5 (7-8.2)	8.5±0.5 (7.7-9)
Tail length	46 (38-55)	-	45.7±6.2 (38-52)	45.1±4.8 (39-50)	43 (33-48)	-	45.1±5.8 (38-51)	41.7±6.2 (34-48)
Stylet length	10 (10-12)	10.5 (10-11)	11±0.6 (10.2-11.7)	10.8±0.7 (9.9-11.5)	9.7 (7.9-10.9)	10.7 (9.6-11.8)	9.6±0.8 (8.5-10.5)	9.76±1.5 (7.8-11.4)
Length of Median Bulb	11.3 (10.1-12.9)	-	11.7±1.4 (10-13.1)	11.4±1.3 (9.8-12.8)	-	-	10.6±1.3 (10-13.1)	10.9±1.5 (9.8-12.8)
Width of Median Bulb	7.3 (5.8-8.3)	-	8.3±1.2 (5.7-8.6)	8.7±2.1 (6-8.9)	-	-	7.9±2.0 (6.7-9.6)	8.1±1.6 (6-10.9)

* Original description ** Previous study in Tamil Nadu [a] Present study; All measurements are in the form of mean ± SD (range) and denoted in µm; n = Number of populations observed = 10;a value =Total body length divided by maximum body diameter;b value =Total body length divided by pharyngeal length;C value =Total body length divided by tail length

Principal component analysis (PCA) of morphometric traits

Eigenvalues

Nine eigenvalues were recorded for female nematodes and eight for juveniles, with the highest variability observed in principal components (PC1 and PC2). Cumulative variability for PC1 and PC2 was 93.80% for females and 93.45% for juveniles (Table 5).

Eigenvectors

The eigenvectors indicated positive or negative variable coefficients, most contributing positively to PC1 for both sexes and PC2 for females. However, many factors negatively contributed to PC2 in juveniles, including total body and median bulb length (Table 5).

Table 3. Coefficient of variance of second stage juveniles of *Meloidogyne* spp. from carrot of Tamil Nadu

Character	*Meloidogyne incognita*		*Meloidogyne hapla*	
	Shoolagiri population	Hosur population	Nilgiris population	Kodaikanal population
Body length (L)	9.46	8.92	6.537	6.12
a value	9.14	10.10	6.81	12.02
b value	17.37	21.55	13.10	12.15
c value	9.08	16.34	7.44	6.96
Tail length	13.64	10.70	12.88	14.87
Stylet length	6.27	6.58	9.18	16.28
Length of Median Bulb	12.03	11.85	13.01	14.09
Width of Median Bulb	15.67	24.85	25.49	20.11

* All CV (Coefficient of Variation) values are expressed in per cent; a value =Total body length divided by maximum body diameter; b value =Total body length divided by pharyngeal length; C value =Total body length divided by tail length

Character	*Meloidogyne incognita*		*Meloidogyne hapla*	
	Shoolagiri population	Hosur population	Nilgiris population	Kodaikanal population
Total Body length	14.47	9.77	26.92	26.05
Maximum body width	7.22	8.93	17.7	17.81
Stylet length	8.80	6.71	10.48	13.41
Width of spear base	8.69	6.86	22.22	16.30
DOGO	7.19	12.18	13.27	16.36
Length of Median Bulb	11.05	4.85	3.62	9.57
Width of Median Bulb	8.57	7.00	7.76	7.57
Vulval slit length	9.79	8.53	7.36	11.10
Vulva to anus distance	15.38	9.171	11.21	9.40

* All CV (Coefficient of Variation) values are expressed in per cent;DOGO - Dorsal Oesophageal Gland Opening

Pearson's Correlation

Correlations between variables varied in both populations. In juveniles, strong positive correlations were noted between body length and median bulb length (0.997147), stylet length and median bulb length (0.97943), and body length and stylet length (0.961598). Strong negative correlations were found between 'a' and 'b' values (-0.98203), stylet length and 'b' value (-0.91856), and median bulb width and 'b' value (-0.89003). In females, strong positive correlations were observed between DOGO and body width (0.962466174) and stylet length and median bulb length (0.922647469), while strong negative correlations were seen between stylet width and DOGO (-0.982000049) (Figure 3a, 3b).

Correlations with Principal components

The correlation biplot shows that stylet length and median bulb width for the female population were positively correlated with PC1 and PC2. A strong positive correlation was also observed for stylet length and tail length. DOGO and vulval-anal distance in females had very strong negative correlations with PC1 and PC2, while 'b' and 'c' values were negatively correlated as well. The correlogram further illustrates these correlations. Short vectors for body length and vulval slit length in females and median bulb width in juveniles suggest these components may be better represented in higher dimensions (Figure 3c, 3d).

Biplot clustering

The biplot of *Meloidogyne* spp. juvenile and female populations revealed three major clusters. Juvenile populations from Shoolagiri and Hosur formed a single group (J3), while the other two juvenile populations diversified into separate groups (J1 and J2). Adult populations from The Nilgiris and Kodaikanal clustered together (F1), while the other two female populations were classified into separate groups (F2 and F3) (Figure 3e, 3f).

Coefficient of variation (CV): CV for nine female variables ranged from 3.62 to 26.92. The lowest CV (3.62) was for bulb length in the Nilgiris population, while the highest (26.92) was for total body length. The Hosur population had the least CV range (4.8 to 12.1), and

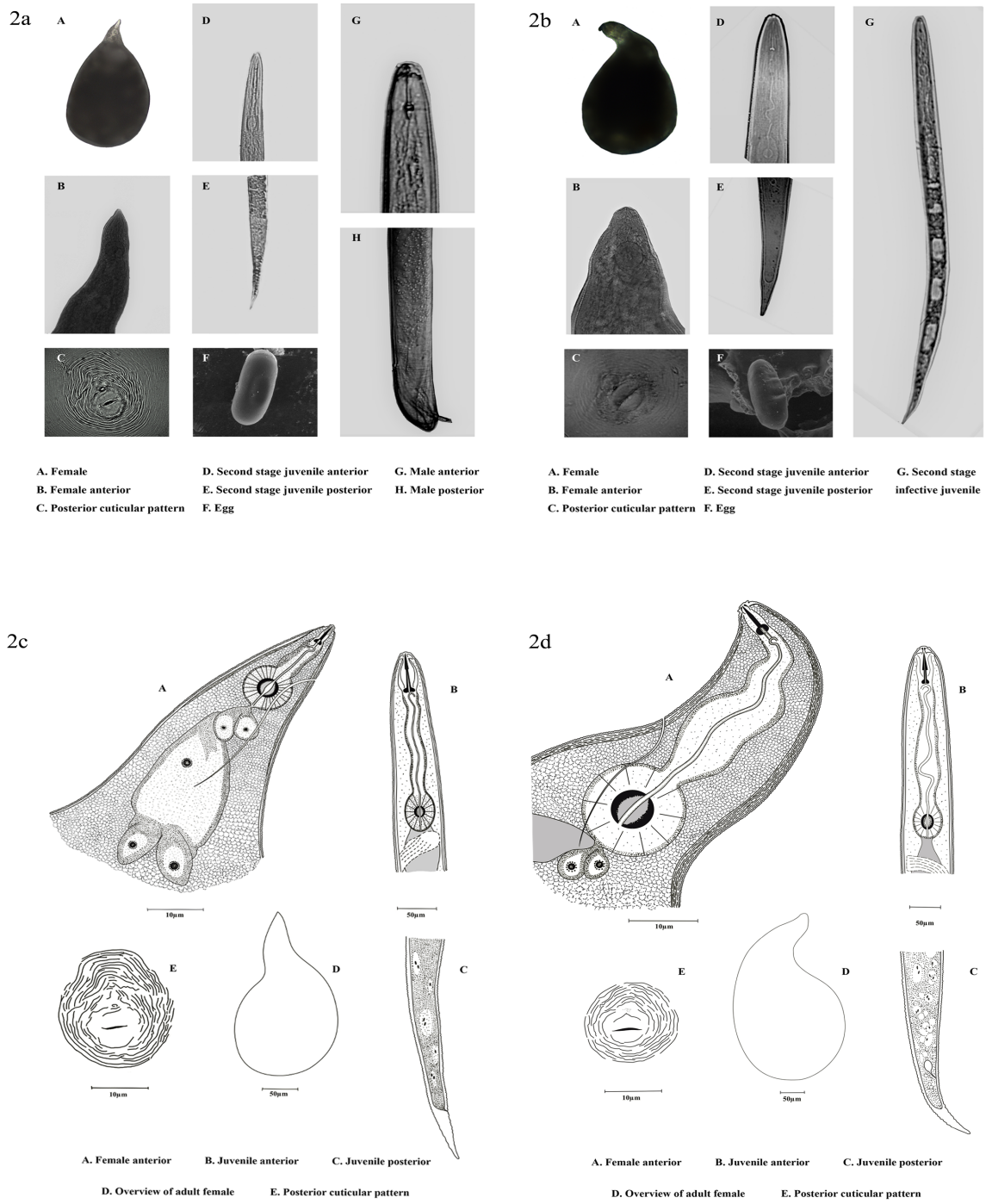

Fig. 2. *a. Microscopic images of Meloidogyne. incognita, b. Microscopic images of M. hapla, c. Camera lucida drawings of M. incognita, d. Camera lucida drawings of M. hapla*

the Nilgiris population had the widest (3.62 to 26.92). Among all variables, body length recorded the greatest CV range (9.7 to 26.9), while median bulb width had a narrower range (7 to 8.5). For the eight variables in second-stage juvenile populations, CV ranged from 6.1 to 25.4. The lowest CV (6.1) was for total body length in Kodaikanal, while the highest (25.4) was for median bulb width in Nilgiris. Shoolagiri showed the least range (6.3 to 17.4), contrasting with Nilgiris' wider range (6.5 to 25.4). Median bulb width had the highest CV range (15.6 to 25.4), while body length had the lowest (6.1 to 9.4) (Table 3, 4).

Standard deviation (SD): Among female populations, Kodaikanal had the highest SD for body length (182.9), while the lowest SD values were recorded for DOGO in Shoolagiri and spear base in Hosur (both at 0.2). For juvenile populations, Shoolagiri exhibited the greatest

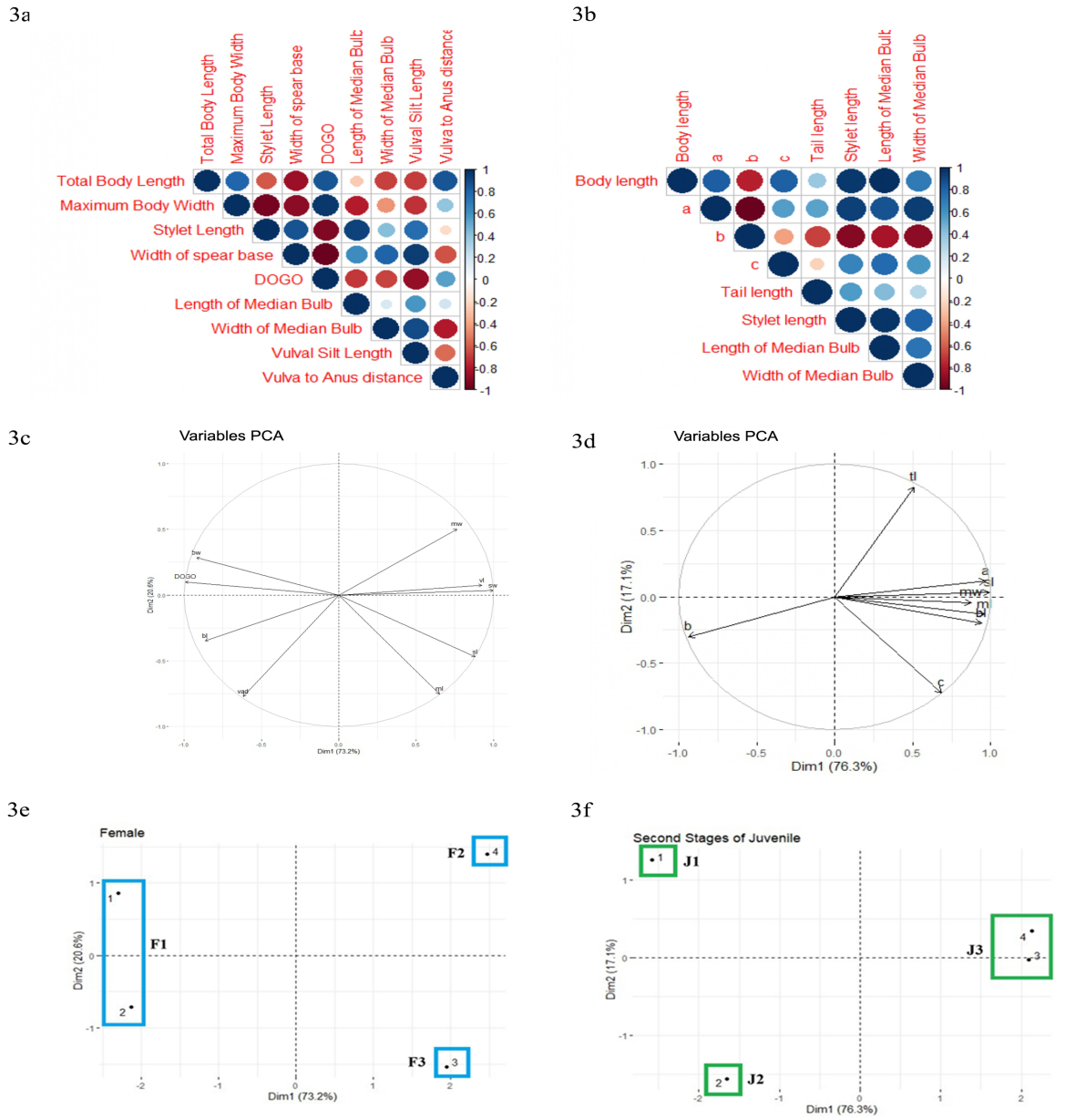

Fig. 3. *a. Pearson's Correlation between morphometric variables of Meloidogyne female, b. Pearson's Correlation between morphometric variables of Meloidogyne J_2, c. Correlation between morphometric variables and PC1 and PC2 of Meloidogyne female, d. Ccorrelation between morphometric variables and PC1 and PC2 of Meloidogyne J_2, e. Biplot for clustering populations of Meloidogyne female population in carrot (F1 - The Nilgiris and Kodaikanal; F2 – Shoolagiri; F3 – Hosur), f. Biplot for clustering populations of Meloidogyne J_2 population in carrot (J1 - The Nilgiris; J2 – Kodaikanal; J3 - Shoolagiri and Hosur)*

SD for body length (36.4), while the lowest SD values (0.5) were for the "c" value in Kodaikanal and Nilgiris (Tables 3, 4).

DISCUSSION

Plant-parasitic nematodes (PPNs) are among the most significant soil-borne pests, inflicting an estimated annual loss of USD 175 billion in global crop yields. Among them, root-knot nematodes (RKNs) (*Meloidogyne* spp.) are among the most economically destructive, causing approximately USD 157 billion yearly losses. This genus encompasses over 100 species, many exhibiting remarkable plasticity, allowing them to adapt to diverse geographic regions and a wide range of host plants (Subbotin *et al.*, 2021). Furthermore, climate change projections by the Intergovernmental Panel on Climate Change (IPCC) suggest that rising temperatures and increased moisture levels may accelerate RKN infection, development, and reproduc-

Table 5. Eigenvectors and Eigenvalues for different populations of *Meloidogyne* spp.

Characters	Female		Second stage juveniles	
	PC1	PC2	PC1	PC2
Body length	-0.337	-0.256	0.381	-0.171
Maximum body width	-0.358	0.209	-	-
Stylet length	0.343	-0.346	0.401	0.029
Width of spear base	0.389	0.026	-	-
DOGO	-0.386	0.073	-	-
Length of Median Bulb	0.253	-0.552	0.389	-0.114
Width of Median Bulb	0.297	0.369	0.354	-0.038
Vulval slit length	0.361	0.055	-	-
Vulva to anus distance	-0.240	-0.567	-	-
a value	-	-	0.392	0.102
b value	-	-	-0.380	-0.257
c value	-	-	0.277	-0.618
Tail length	-	-	0.206	0.705
Eigenvalue	6.59	1.85	6.10	1.37
Variance (%)	73.23	20.56	76.31	17.13
Cumulative variability (%)	73.23	93.80	76.31	93.45

DOGO - Dorsal Oesophageal Gland Opening;a value =Total body length divided by maximum body diameter;b value =Total body length divided by pharyngeal length.C value =Total body length divided by tail length;

tion, leading to their prevalence and distribution shifts. Given their substantial impact on agriculture, accurate species identification is critical for implementing effective and sustainable management strategies (Vashisth *et al.,* 2024). Morphological identification remains a valuable, cost-effective approach, though its accuracy is contingent upon the number of diagnostic traits assessed and the availability of sufficient specimens (Rusinque *et al.,* 2023).

Historically, the identification of root-knot nematode species was primarily based on the perineal pattern of adult females. Over time, phenetic approaches, such as de Man's ratios and body measurements of second-stage juveniles and adults, became widely used for species identification (Sneath and Sokal, 1973). In the present study, based on the morphometric data and comparison with the original descriptions by Whitehead (1968) and previous records from Tamil Nadu (Dehury, 2001), the study identified the presence of *M. incognita* and *M. hapla* in both hilly and plain regions of Tamil Nadu. The morphometric ranges of the present populations showed considerable overlap with those described in earlier studies. However, some parameters exceeded the ranges provided in the original descriptions, such as body length and stylet length in *M. incognita* females and several key features in *M. hapla* juveniles and females. Conversely, certain measurements were lower than the original descriptions, such as the stylet length and specific ratios in *M. hapla* juveniles *and M. incognita* juveniles.

Moderate variations in vulval slit length were noted in *M. incognita* populations, similar to the findings of Kaur and Attri (2013) and Sowmya *et al.* (2018). Other studies, such as those by Ganguly *et al.* (2000), reported significant body length variation in *M. hapla* populations from Himachal Pradesh, while Brito *et al.* (2004) noted substantial variability in female body length but minimal variation in the 'a' value and vulval slit length. These variations in nematode morphometrics may be attributed to environmental factors such as altitude, soil composition, temperature, nutrition, pH, and host and crop growth stages (Norton, 1979). Factors like fixation posture can also affect the measurements of specific anatomical structures, such as the median bulb. The coefficient of variation (CV) in our study ranged from 3-27%, which is higher than the 0-10% CV reported by Nyaku *et al. (*2018) but similar to the 4-26% CV observed by Sowmya *et al.* (2018) in *Meloidogyne* populations in Tamil Nadu. This suggests that CV can be an important metric for distinguishing populations when using PCA.

The present study used PCA to assess the similarities and differences among juveniles and females of four *Meloidogyne* populations. PCA is a powerful multivariate tool for identifying correlations between multiple quantitative variables and detecting linear relationships. This method is particularly useful for distinguishing be-

tween closely related populations (Neha, 2015). In present study, PCA revealed a strong positive correlation between body length and median bulb length in juvenile populations, while in female populations, DOGO and body width showed the strongest positive correlation. Conversely, stylet width and DOGO displayed a strong negative correlation in females, and a similar negative relationship was observed between the 'a' and 'b' values in juveniles. These findings contrast Nyaku *et al.* (2018), who reported strong positive correlations between body length and the 'a' value in *M. incognita* juveniles, and the lowest negative correlation between the 'c' value and greatest body width.This might be due to the fact that different *Meloidogyne* populations may exhibit distinct morphological traits due to genetic variation, adaptation to different hosts, or environmental influences.

The PCA results grouped the four populations into three distinct clusters. Juvenile populations from Shoolagiri and Hosur formed one group, while female populations from Kodaikanal and the Nilgiris were clustered into another group. This indicates significant morphometric variation between females from plain areas and juveniles from hilly regions, suggesting that these three clusters could reflect geographic and environmental diversity. Similarly, Nyaku *et al.* (2016) clustered nine populations of reniform nematodes into three major groups. Stylet length emerged as a key variable in distinguishing both juvenile and female populations, showing the highest correlation with PC1. Tail length in juveniles and metacorpus width in females were strongly correlated with PC2, indicating that these traits are critical for differentiating between populations. Stylet and tail length have also been recognized as important distinguishing variables in previous studies, including those by Nyaku *et al.* (2018) and Eisenback *et al.* (1980), who noted the importance of stylet length in distinguishing *M. hapla* races.

Conclusion

In conclusion, PCA enabled us to examine the linear relationships among morphometric traits and assess the relative importance of nine quantitative variables in females and eight in juveniles. The findings have highlighted the significance of stylet length and other key traits in capturing morphological variations among *Meloidogyne* populations, particularly within Tamil Nadu's diverse carrot ecosystems. This approach enhances the present understanding of population differentiation and streamlines future studies, making morphometric analysis more efficient and less time-consuming.

Conflict of Interest

The authors state that they have no conflicts of interest.

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