

## Morphological and Morphometrical Characterization of *Meloidogyne incognita* from Different Host Plants in Four Districts of Punjab, India

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**Abstract:** The population of *M. incognita*, the root knot nematode (RKN) was found infesting five different host plants (okra, banana, sunflower, bottle gourd, and brinjal) out of 24 examined from four districts of Punjab, India (Gurdaspur, Ludhiana, Patiala, and Hoshiarpur). Morphological and morphometrical characterization indicated that in the case of mature female, the characters of body length and width, neck length, ratio 'a', anus to tail terminus (ATT), interphasmid distance (IPD), and perineal pattern were recorded as stable characters. These taxonomic characters can be reliable for identification. All characters of second-stage juvenile (J2) such as body length, stylet length, head to median bulb length (H-MB), distance from median bulb to excretory pore (MB-EP), tail length, anal body width (ABW), and ratios C and C' were highly variable. Analysis of interpopulation morphometric characters of mature female of *M. incognita*, namely, body length, width, and ratio 'a' were moderately variable characters (CV 0.26% to 20%) and stylet length, neck length, length of median bulb (LMB), and width of median bulb (WMB) were highly variable (CV 1.0% to 36.1%). In the perineal pattern, the two characters ATT and IPD were moderately variable (CV 8.8% to 17.6%) and two characters, anus to vulval slit (AVS) and length of vulval slit (LVS), were highly variable (CV 2.1% to 40.5%). In J2, body length, stylet length, H-MB, MB-EP, ABW, tail length, ratios C, and C' were highly variable characters (CV > 12%).

**Key words:** Female, juveniles, morphology, morphometrical, *M. incognita*, perineal pattern.

Crop plants are of great importance for a country, and when these plants suffer from diseases they cause serious losses and adversely affect the agricultural economy of a country (Hafeez, 1986). The root knot nematode (RKNs) reduce the yield of the world's 40 major cash crops by an average of 12.3% (Sasser, 1987). In total, more than 97 *Meloidogyne* species have been identified to date (Hunt and Handoo, 2009) and only four species, namely, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood are widely distributed throughout the agricultural regions of the world. In India, much scientific information has been generated on various aspects of RKNs (Dasgupta and Gaur, 1986), but so far there has been hardly any information pertaining to morphological and morphometric features of Indian populations of RKNs. Therefore, the objective of this study was aimed at the intraspecific morphological diversity of *M. incognita* populations in the locality of Punjab in order to assess the stability of various taxonomic characters of this species.

### MATERIALS AND METHODS

Soil (250 gm) along with feeder roots were put in polythene bags and tied with rubber band to check evaporation. Supporting data regarding name of the host, locality, date of collection, etc., was tagged to the bag. The samples were then brought to the laboratory for further processing. Nematodes were killed and fixed in one operation (Seinhorst, 1966) with SN solution (Seinhorst Method). The cavity block containing

nematodes was kept in dessicator for 12 hr and then placed in an oven at 37°C to evaporate the ethanol. The nematodes left in pure glycerine were picked and mounted. For female, galled portions of root were selected and fixed in acid fuchsin (Eisenback and Triantophyllu, 1981). The adult females of *Meloidogyne* spp. were removed from the root tissue by teasing apart with the help of fine forceps and were collected in a cavity block having warm lactophenol. One female specimen was placed in a drop of lactophenol on a glass slide. The posterior end of the female having vulva and anus was cut with a sharp blade. The inner tissue was carefully removed with a nylon bristle and the perineal pattern was transferred in a drop of glycerol on a clean glass slide and cover slip was applied and sealed with the mixture of distyrene, plasticizer, and xylene (DPX). Ten specimens from a population were identified and examined. The measurements of length of median bulb (LMB), width of median bulb (WMB), width of neck, neck length, distance from head to median bulb (H-MB), length of vulval slit (LVS), distance from anus to vulval slit (AVS), anus to tail terminus area (ATT), and interphasmid distance (IPD) were taken of perineal pattern. Length, width, ratios C and C', distance from head to median bulb (H-MB), anal body width (ABW), and tail length for second-stage juvenile (J2) were made under a ×40 microscope with calibrated oculomicrometer. The arithmetic mean, standard error of mean (SEM), standard deviation (SD), and coefficient of variance (CV) for each measurement were computed. Based upon CV values, the characters were rated as least variable, moderately variable, and highly variable, using scale < 12%, 12% to 20%, and > 20% for female, respectively, and < 8%, 8% to 12%, and > 12% for J2, respectively (Sahoo and Ganguly, 2000). The obtained data was compared with the earlier descriptions of this species (Chitwood, 1949; Whitehead, 1968; Jepson, 1987).

Received for publication December 11, 2012.

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This paper was edited by Zafar A. Handoo.

## RESULTS

The following results on morphological and morphometric data of the mature females, perineal pattern, and J2 of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood in all four districts of Punjab populations.

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

*Measurements*: Female: length = 530 to 812  $\mu\text{m}$ ; width = 510 to 692  $\mu\text{m}$ ; a = 1.03 to 1.43  $\mu\text{m}$ ; stylet length = 16 to 27  $\mu\text{m}$ ; neck length = 176 to 280  $\mu\text{m}$ ; LMB = 20 to 42  $\mu\text{m}$ ; and WMB = 20 to 42  $\mu\text{m}$ .

*Perineal pattern*: LVS = 16.5 to 26  $\mu\text{m}$ ; AVS = 16 to 29  $\mu\text{m}$ ; ATT = 12 to 18  $\mu\text{m}$ ; and IPD = 17 to 26  $\mu\text{m}$ .

*Second-stage juveniles (J2)*: L = 200 to 380  $\mu\text{m}$ ; stylet = 11 to 25  $\mu\text{m}$ ; H-MB = 35 to 68  $\mu\text{m}$ ; MB-EP = 18 to 33  $\mu\text{m}$ ; tail length = 15 to 60  $\mu\text{m}$ ; C = 4.5 to 17  $\mu\text{m}$ ; ABW = 8 to 17  $\mu\text{m}$ ; and C' = 1 to 6.1  $\mu\text{m}$ .

*Host plants*: Okra, brinjal, banana, turnip, and sunflower.

*Locality*: All four districts, i.e., Gurdaspur (GSP), Patiala (PTA), Hoshiarpur (HSP), and Ludhiana (LDH).

The following are morphological characters of four populations of *M. incognita* in Punjab.

## A. Mature female:

Body shape and size: large-sized, 530 to 812  $\mu\text{m}$  in length, pear-shaped body, no posterior protuberance, and 510 to 692  $\mu\text{m}$  wide. Neck protrudes anteriorly 176 to 280  $\mu\text{m}$ . Stylet 16 to 27  $\mu\text{m}$  in length with stylet cone having anterior half distinctly curved dorsally. Stylet knobs set off rounded to transversely elongate indented anteriorly. Esophagus has a large muscular bulb with conspicuous valve plate. Median bulb 20 to 42  $\mu\text{m}$  in diam. is located in the middle of neck.

## B. Perineal pattern:

*PPI*: Oval to rounded 89 to 99  $\mu\text{m}$  in length and 85 to 99.2  $\mu\text{m}$  in width. Typically high with dorsal arch. Anus anteriorly located 16 to 29  $\mu\text{m}$  distance from vulval slit (AVS) and anus located 12 to 18  $\mu\text{m}$  distance from tail terminus (ATT). Lateral field weakly demarcated by breaks and forked striae. Striae distinct and wavy. Dorsal striae smooth, closely placed wavy to zigzag range from 50 to 99  $\mu\text{m}$  in length and 67 to 99  $\mu\text{m}$  in width. Phasmids distinct with 17 to 26  $\mu\text{m}$  distance between them (IPD). *PP 2*: Arch high, wavy striae. Lateral fields weakly demarcated. The pattern near its edge is fine and evenly spaced. Tail terminus is smooth, 13 to 16  $\mu\text{m}$  in size with a pair of small phasmids, 16 to 12  $\mu\text{m}$  distance between them (IPD). Variants very similar to patterns of *M. incognita acrita* (Race-1).

## C. Second-stage juveniles (J2):

Length 200 to 380  $\mu\text{m}$ , labial region not offset, labial disc elevated. Lateral lips usually absent. Stylet 11 to 25  $\mu\text{m}$  in length, basal knobs offset, rounded to transversely elongated. Hemizonid anterior or adjacent to excretory pore. Tail 15 to 60  $\mu\text{m}$  in length, tip rounded,

anal body width 8 to 17  $\mu\text{m}$ . Lateral lines four with incisures. Phasmids subterminal, dot-like near cloacal aperture.

The following are morphometric characters of four populations of *M. incognita* in Punjab.

## A. Mature female:

The range for mean values of body length and width in four populations were 683.6 to 765  $\mu\text{m}$  and 565.3 to 601  $\mu\text{m}$ , respectively. The maximum mean value was in GSP population. These characters were rated as moderately variable with maximum variability in HSP and PTA populations, which was up to 19.4% for length and 17.5% for width, respectively.

The character of neck length was moderately variable (CV 0.79% to 13.8%). LDH population had smallest neck (177  $\mu\text{m}$ ). The maximum mean value for neck length (260  $\mu\text{m}$ ) was observed in PTA population.

Stylet length showed large differences among four populations in their mean values, which ranged from 16.5 to 20.7  $\mu\text{m}$ . These were rated as highly variable characters (CV 3% to 26.3%). The size of median bulb, 29.6 to 37  $\mu\text{m}$  in length (LMB) and 30 to 37  $\mu\text{m}$  in width (WMB), were maximum in LDH population whereas LMB was minimum in GSP population. Therefore, the size of median bulb is also ranked as highly variable character in all four populations. The ratio 'a' (BL/BW) was moderately variable (CV 3.6% to 17.1%) mean value minimum in LDH population (1.15  $\mu\text{m}$ ) and maximum in GSP population (1.27  $\mu\text{m}$ ) (Table 1).

## B. Perineal pattern:

The LVS (18.5  $\mu\text{m}$ ) and AVS (19  $\mu\text{m}$ ) were minimum in HSP population and the IPD (20.6  $\mu\text{m}$ ) was minimum in PTA population. Maximum mean values for LVS (23.5  $\mu\text{m}$ ) was in LDH population and for AVS (23  $\mu\text{m}$ ) in PTA population, while HSP population exhibited maximum mean values for IPD (22.3  $\mu\text{m}$ ). The ATT varied in four populations with mean values ranging from 13 to 16.5  $\mu\text{m}$ . The coefficient of variability for the four characters of perineal pattern varied from population to population. Two characters, LVS and AVS, were rated as highly variable (CV 4.3% to 20.4%; 19.2% to 32.2%, respectively) whereas two other characters, ATT and IPD, were rated as moderately variable (CV 6.4% to 17.2%; 11.2% to 19.2%, respectively) (Table 2).

## C. Second-stage juveniles (J2):

The average body length of J2 was 241 to 350.6  $\mu\text{m}$ , with maximum body length recorded in PTA population (350.6  $\mu\text{m}$ ). Unlike the case of female, ratios C and C' were highly variable in J2 (CV 21% to 49.4%; 0.09% to 55.9%, respectively).

The stylet length of 14 to 19.6  $\mu\text{m}$  in four populations showed high variability (CV 5.8% to 38.6%). The H-MB and MB-EP were maximum in HSP population with mean values 62.3 and 27.4  $\mu\text{m}$ , respectively.

TABLE 1. Morphometric characters of mature female in four populations of *M. incognita* [Mean  $\pm$  SD  $\pm$  SE; (range); C.V.%], n = 5.

Characters	Gurdaspur (GSP)	Patiala (PTA)	Hoshiarpur (HSP)	Ludhiana (LDH)	CR
Length ( $\mu\text{m}$ )	765 $\pm$ 14.5 $\pm$ 4.8 (750-779) 1.8	713.3 $\pm$ 111.7 $\pm$ 37.2 (592-812) 15.6	683.6 $\pm$ 133 $\pm$ 44.3 (530-762) 19.4	704.5 $\pm$ 77 $\pm$ 38.5 (650-759) 10.5	MV
Width ( $\mu\text{m}$ )	593.6 $\pm$ 41.2 $\pm$ 13.2 (561-640) 6.9	575.6 $\pm$ 101.2 $\pm$ 33.6 (510-692) 17.5	565.3 $\pm$ 80.7 $\pm$ 26.9 (510-658) 14.2	601 $\pm$ 30.4 $\pm$ 15.2 (580-623) 5.0	MV
Stylet length ( $\mu\text{m}$ )	20.7 $\pm$ 5.4 $\pm$ 1.8 (17-27) 26.3	17.1 $\pm$ 2 $\pm$ .67 (16-19.5) 11.8	16.5 $\pm$ .5 $\pm$ .16 (16-17) 3.0	19.5 $\pm$ 2.1 $\pm$ 0.1 (18-21) 10.8	HV
Neck length ( $\mu\text{m}$ )	248.6 $\pm$ 34.4 $\pm$ 11.4 (210-276) 13.8	260 $\pm$ 28 $\pm$ 9.3 (228-280) 10.7	244 $\pm$ 17.7 $\pm$ 5.9 (230-264) 7.2	177 $\pm$ 1.4 $\pm$ 0.7 (176-178) .79	MV
LMB ( $\mu\text{m}$ )	29.6 $\pm$ 8.5 $\pm$ 2.8 (20-36) 28.6	31.6 $\pm$ 9 $\pm$ 3 (22-40) 28.6	30.2 $\pm$ 9.5 $\pm$ 3.1 (21-40) 31.4	37 $\pm$ 7 $\pm$ 3.5 (32-42) 19.1	HV
WMB ( $\mu\text{m}$ )	30 $\pm$ 8.7 $\pm$ 2.9 (20-36) 29	32.3 $\pm$ 10 $\pm$ 3.3 (22-42) 30.9	30.5 $\pm$ 9 $\pm$ 3 (29.7-40) 29.5	37 $\pm$ 7 $\pm$ 3.5 (32-42) 17.1	HV
A	1.27 $\pm$ .08 $\pm$ .02 (1.21-1.37) 6.5	1.24 $\pm$ .13 $\pm$ .04 (1.16-1.40) 10.8	1.20 $\pm$ 0.02 $\pm$ .06 (1.03-1.43) 17.1	1.15 $\pm$ 0.04 $\pm$ .02 (1.12-1.18) 3.6	MV

Character ranking (CR): LV = least variable (<12%); MV = moderately variable (12%-20%); HV = highly variable (>20%). LMB = length of median bulb, WMB = width of median bulb (WMB).

The coefficient of variability was rated as highly variable (CV H-MB, 8.8% to 14.5%; MB-EP, 15.4% to 35.9%).

In general, tail length was smaller (16.7  $\mu\text{m}$ ) in GSP population than the other three population. The longest tail length (43.6  $\mu\text{m}$ ) was in PTA population. The ABW was smallest in PTA population (11.3  $\mu\text{m}$ ) and greatest in GSP population (16.1  $\mu\text{m}$ ). Among the tail characters, tail length, ABW, and ratios C and C' were rated as highly variable (Table 3).

The following are morphometric characters of *M. incognita* as found in each of the five different host plants in Punjab.

#### A. Mature female:

The range for mean values of body length and width in five different host plants were 621 to 754.5  $\mu\text{m}$  and 545 to 627.5  $\mu\text{m}$ , respectively; The maximum mean values were in okra. These characters were rated as moderately variable with minimum variability in sunflower, which was 6.6% and 9% for length and width, respectively.

The character of neck length was highly variable (CV > 20%). Sunflower had the smallest neck (199  $\mu\text{m}$ ). The maximum mean values for neck length (262  $\mu\text{m}$ ) was observed in okra with CV value 20%.

Stylet length showed much differences among the five host plants in their mean values, which ranked from

16.2 to 25.5  $\mu\text{m}$ . These were rated as highly variable characters (CV > 20%).

The size of median bulb 41 to 24.7  $\mu\text{m}$  LMB and 24.7 to 41  $\mu\text{m}$  WMB was maximum in sunflower. LMB was minimum in banana (24.7  $\mu\text{m}$ ). The size of median bulb is also ranked as highly variable in all specimen from different host plants.

The ratio of 'a' (BL/BW) 1.14 to 1.28 was moderately variable (CV 0.26% to 18.6%), with minimum mean value in sunflower (1.14  $\mu\text{m}$ ) and maximum in okra (1.28  $\mu\text{m}$ ) (Table 4).

#### B. Perineal pattern:

The LVS (18.4  $\mu\text{m}$ ) and IPD (15.6  $\mu\text{m}$ ) were minimum in *M. incognita* from bottle gourd and the AVS was minimum in okra (16.7 to 18  $\mu\text{m}$ ). Maximum mean values for LVS (23.5  $\mu\text{m}$ ) and ATT (16  $\mu\text{m}$ ) were in sunflower. Banana also exhibited maximum mean values for IPD (23.9  $\mu\text{m}$ ) and AVS (23.5  $\mu\text{m}$ ). The ATT varied in different host plants with mean values ranging from 13.5 to 16  $\mu\text{m}$ . The coefficient of variability for the four characters of perineal pattern varied in specimens from different host plants. LVS and AVS were highly variable (CV > 20%) LVS and IPD were rated as moderately variable (CV 12% to 20%) (Table 5).

TABLE 2. Morphometric characters of perineal pattern in four populations of *M. incognita* [Mean  $\pm$  SD  $\pm$  SE; (range); C.V.%], n = 5.

Characters	Gurdaspur (GSP)	Patiala (PTA)	Hoshiarpur (HSP)	Ludhiana (LDH)	CR
LVS ( $\mu\text{m}$ )	23 $\pm$ 1.0 $\pm$ 0.3 (22-24) 4.3	20.3 $\pm$ 4.1 $\pm$ 1.3 (17-25) 20.4	18.5 $\pm$ 3 $\pm$ 1.0 (16.5-22) 16.4	23.5 $\pm$ 3.5 $\pm$ 1.7 (21-26) 15	HV
AVS ( $\mu\text{m}$ )	21.1 $\pm$ 6.8 $\pm$ 2.2 (16.5-29) 32.2	23 $\pm$ 4.5 $\pm$ 1.5 (18-27) 19.9	19 $\pm$ 4.3 $\pm$ 1.4 (16-24) 22.9	22 $\pm$ 4.2 $\pm$ 2.1 (19-25) 19.2	HV
ATT ( $\mu\text{m}$ )	13 $\pm$ 1.1 $\pm$ 0.3 (12-14.2) 8.4	14.6 $\pm$ 2.5 $\pm$ 0.8 (12-17) 17.2	16.1 $\pm$ 1.0 $\pm$ 0.34 (15-17) 6.4	16.5 $\pm$ 2.1 $\pm$ 1 (15-18) 12.8	MV
IPD ( $\mu\text{m}$ )	21.9 $\pm$ 3.5 $\pm$ 1.1 (19.8-26) 16	20.6 $\pm$ 3.5 $\pm$ 1.1 (17-24) 16.9	22.3 $\pm$ 2.5 $\pm$ 0.83 (20-25) 11.2	22 $\pm$ 4.2 $\pm$ 2.1 (19-25) 19.2	MV

Character ranking (CR): LV = least variable (<12%); MV = moderately variable (12%-20%); HV = highly variable (>20%). ATT = anus to tail terminus, AVS = anus to vulval slit, IPD = interphasmid distance, LVS = length of vulval slit.

TABLE 3. Morphometric characters of second-stage juveniles (J2) in four populations of *M. incognita* [Mean ± SD ± SE; (range), C.V.%], n = 5.

Characters	Gurdaspur (GSP)	Patiala (PTA)	Hoshiarpur (HSP)	Ludhiana (LDH)	CR
Length (µm)	241 ± 36.3 ± 12.1 (220-283) 15	350.6 ± 30 ± 10 (320-380) 8.5	270.6 ± 78 ± 26.1 (200-380) 28.9	261.5 ± 40 ± 20.1 (233-290) 15.4	HV
Stylet length (µm)	16.6 ± 3 ± 1 (14-20) 18.3	17 ± 1 ± 0.3 (16-18) 5.8	19.6 ± 7.5 ± 2.5 (11-25) 38.6	14 ± 4.2 ± 2.1 (11-17) 30.2	HV
H-MB (µm)	41.2 ± 5.6 ± 1.8 (35-46) 13.6	52 ± 7.5 ± 2.5 (45-60) 14.5	62.3 ± 8.1 ± 2.7 (53-68) 13	48 ± 4.2 ± 2.1 (45-51) 8.8	HV
MB-EP (µm)	23.6 ± 8 ± 2.6 (19-33) 34.1	23.3 ± 8.3 ± 2.7 (18-33) 35.9	27.4 ± 4.2 ± 1.4 (22.5-30) 15.4	24.5 ± 4.9 ± 2.4 (21-28) 20.1	HV
Tail length (µm)	16.7 ± 1.6 ± 0.53 (15-18.2) 9.6	43.6 ± 15 ± 5 (28-58) 43.4	27.3 ± 1.1 ± 0.38 (26-28) 4.2	42.5 ± 24.7 ± 12 (25-60) 58.2	HV
ABW (µm)	16.1 ± 1.0 ± .34 (15-17) 6.4	11.3 ± 4.9 ± 1.6 (8-17) 43.5	13.4 ± .49 ± 0.16 (13.1-14) 4.2	12 ± 2.8 ± 1.4 (10-14) 23.5	HV
C	14 ± 2.9 ± 0.98 (10.8-16.6) 21	8.8 ± 3.5 ± 1.1 (5.5-12.5) 39.8	9 ± 4.5 ± 1.4 (4.6-13.5) 49.4	4.9 ± 6.1 ± 3 (11-17) 28	HV
C'	1.03 ± .05 ± 0.01 (1-1.1) 5.5	4.5 ± 2.5 ± 0.84 (1.64-5.6) 55.9	3.3 ± 2.4 ± 0.8 (1.9-6.1) 4	3.8 ± 3 ± 1.5 (1.7-6) 0.09	HV

Character ranking (CR): LV = least variable (< 8%); MV = moderately variable (8%-12%); HV = highly variable (> 12%). ABW = anal body width, H-MB = head to median bulb length, MB-EP = median bulb to excretory pore.

C. Second-stage juveniles (J2):

The average body length of J2 was 226 to 331.5 µm with maximum body length recorded in *M. incognita* from brinjal (331.5 µm). Unlike female, ratios C and C' were highly variable in J2.

The stylet length (13.5 to 22.5 µm) in five different host plants showed high variability with (CV > 12%). The H-MB and MB-EP were maximum in okra (64.3 and 31.4 µm, respectively). The coefficient of variability H-MB and MB-EP ranked as highly variable (CV < 12%).

In general, tail length was smallest in okra (22.1 µm) and longest in banana (44 µm). The ABW was smallest in banana (10.7 µm) and greatest in bottle gourd (16 µm). Among the tail characters, tail length, ABW, and ratios C and C' were rated as highly variable (Table 6).

DISCUSSION

This study examined the morphological and morphometric characterization of *M. incognita* populations

in four districts of Punjab. After comparing the mature female and perineal pattern of different populations, it was found that body width, LMB, WMB, LVS, and ATT were maximum in LDH population. GSP population had maximum mean values for body length, stylet, and ratio 'a'. For neck length, AVS maximum mean values were recorded in PTA population. IPD was recorded maximum in HSP population (Tables 1 and 2).

Interpopulation comparison of morphometric characters of J2 found that the GSP population to be the smallest of all populations. All other populations shared almost similar mean values for body length. Besides body length, H-MB, tail length, and ratio C were also minimum in GSP population. For the two characters of body length and tail length, maximum mean values were observed in PTA population. For the four characters of stylet, H-MB, MB-EP, and ratio C, the HSP population exhibited maximum mean values (Table 3).

On the basis of different levels of CV in four populations, the characters of mature female body length,

TABLE 4. Morphometric characters of mature female of *M. incognita* in five different host plants in Punjab [Mean ± SD ± SE; (range); CV%], n = 5.

Characters	Okra	Banana	Sunflower	Brinjal	Bottle gourd	CR
Length (µm)	754.5 ± 6.3 ± 3.1 (750-759) 8.4	774 ± 83.9 ± 20.8 (623-812) 11.1	621 ± 41 ± 20 (592-650) 6.6	648 ± 166.8 ± 83.4 (530-766) 20	696.6 ± 5.5 ± 2.3 (653-726) 3.9	MV
Width (µm)	627.5 ± 74.8 ± 18.7 (520-692) 11.9	594.5 ± 94 ± 47.3 (528661) 155	545 ± 49.4 ± 24.7 (510-580) 9	545 ± 49.4 ± 24.7 (510-580) 9.1	594.4 ± 5.5 ± 2.1 (561-620) 4.3	MV
Stylet length(µm)	21.7 ± 7.4 ± 3.5 (16.5-27) 34.1	16.2 ± 1.7 ± 0.42 (14-18) 10.2	20.2 ± 1.0 ± 0.53 (19.5-21) 5.2	21.5 ± 7.7 ± 3.8 (16-27) 36.1	25.5 ± .22 ± 1.1 (20-24) 4.2	HV
Neck length (µm)	262 ± 2.8 ± 1.4 (260-264) 1.0	243 ± 47.2 ± 11.8 (230-280) 19.4	199 ± 41 ± 20.5 (176-228) 20	220 ± 14.1 ± 7 (210-230) 6.4	220 ± 2.8 ± 1.4 (198-234) 6.3	HV
LMB (µm)	34.9 ± 7.2 ± 3.6 (29.7-40) 20.9	24.7 ± 7.5 ± 1.8 (20-36) 30.5	41 ± 1.4 ± 0.07 (40-42) 3.4	36 ± 5.6 ± 2.8 (32-40) 15.6	34.2 ± 1.1 ± 0.03 (30-42) 5.5	HV
WMB (µm)	34.9 ± 7.2 ± 3.6 (29.8-40) 20.6	24.7 ± 7.5 ± 1.8 (20-36) 30.5	41.5 ± .70 ± 0.35 (41-42) 1.7	35.5 ± 6.3 ± 1.7 (31-40) 17.9	34.3 ± 1.2 ± 0.05 (30-42) 5.5	HV
a	1.28 ± .21 ± 0.03 (1.13-1.43) 5	1.18 ± .02 ± 0.006 (1.15-1.21) 0.26	1.14 ± .02 ± 0.01 (1.12-1.16) 2.4	1.17 ± .20 ± 0.07 (1.03-1.32) 18.6	1.17 ± .14 ± 0.7 (1.11-1.29) 5.9	MV

Character ranking (CR): LV = least variable (< 12%); MV = moderately variable (12%-20%); HV = highly variable (> 20%). LMB = length of median bulb, WMB = width of median bulb (WMB).

TABLE 5. Morphometric characters of perineal pattern of *M. incognita* in five different host plants in Punjab. [Mean  $\pm$  SD  $\pm$  SE; (range); CV%], n = 5.

Characters	Okra	Banana	Sunflower	Brinjal	Bottle gourd	CR
LVS ( $\mu\text{m}$ )	19.8 $\pm$ 4.6 $\pm$ 2.3 (16.5-23.1) 23.5	21.5 $\pm$ 3.3 $\pm$ 0.82 (17-25) 15.4	23.5 $\pm$ 3.5 $\pm$ 1.7 (21-26) 15	20.5 $\pm$ 4.9 $\pm$ 2.4 (17-24) 24	18.4 $\pm$ .6 $\pm$ 0.3 (16-20) 16.5	HV
AVS ( $\mu\text{m}$ )	16.7 $\pm$ .35 $\pm$ 0.17 (16.5-17) 2.1	23.5 $\pm$ 3.8 $\pm$ 0.86 (18-27) 16.4	17 $\pm$ 1.4 $\pm$ 0.7 (16-18) 8.3	22.5 $\pm$ 9.1 $\pm$ 4.4 (16-29) 40.5	16.8 $\pm$ .26 $\pm$ 0.13 (16-19) 7.7	HV
ATT ( $\mu\text{m}$ )	15.3 $\pm$ 1.62 $\pm$ 0.81 (14.2-16.5) 10.5	15.4 $\pm$ 2.0 $\pm$ 0.5 (13-17) 13.2	16 $\pm$ 2.8 $\pm$ 1.4 (14-18) 17.6	13.5 $\pm$ 2.1 $\pm$ 1.0 (12-15) 15.7	14.8 $\pm$ .26 $\pm$ 0.13 (14-16) 8.8	MV
IPD ( $\mu\text{m}$ )	18 $\pm$ 1.9 $\pm$ 0.98 (17-19.8) 4	23.9 $\pm$ 2.3 $\pm$ 0.58 (20.6-26) 9.7	18.4 $\pm$ 1.4 $\pm$ 0.7 (17-19) 7.8	20.9 $\pm$ 1.5 $\pm$ 0.7 (17.8-22) 7.4	15.6 $\pm$ .52 $\pm$ 2.4 (12-19) 16.6	MV

Character ranking (CR): LV = least variable, (<12%); MV = moderately variable (12%-20%); HV = highly variable (>20%). ATT = anus to tail terminus, AVS = anus to vulval slit, IPD = interphasmid distance, LVS = length of vulval slit.

width, neck length, and ratio 'a' were moderately variable characters (CV 0.79% to 19.4%), whereas stylet length, LMB, and WMB were highly variable (CV 3.0% to 30.9%). In perineal pattern, LVS and AVS were highly variable, whereas ATT and IPD were moderately variable (CV-6.4% to 17.2%, 11.2% to 19.2%, respectively). In J2, body length width, stylet, H-MB, MB-EP, tail length, ratios C and C', and ABW were not stable characters exhibiting high variability (CV > 20%) (Table 4).

Comparing the morphometric characters of four populations with the type description of *M. incognita* by Kofoed and White (1919) showed that these populations shared most of the characters excepting for slightly larger size of female, stylet, and smaller length of the J2 in Indian populations. The present populations of *M. incognita* were in conformity with the morphological characters of type species. The differences recorded were considered as intraspecific variations in *M. incognita*.

Comparing the morphological characters of mature female and its perineal pattern with five different host plants in Punjab it was found that mean value for body length, AVS, and IPD were maximum in banana. For body width, neck length, and ratio 'a', maximum mean values were recorded in okra. Maximum mean value

of stylet length was found in bottle gourd; and LMB, WMB, LVS, and ATT were recorded maximum in sunflower (Tables 5 and 6). In the present study, the gross range of female stylet length was between 16 and 27  $\mu\text{m}$  with mean value 19  $\mu\text{m}$ , which was exceptionally longer than earlier reported populations (Jepson, 1983; Chitwood, 1949; Hunt and Handoo, 2009). According to Jepson (1983), the stylet length structure is likely to be adapted to the feeding habit of the nematode and, in *M. incognita* and its other species, those with perennial hosts have considerably longer stylets than the species on nonperennial hosts.

Interpopulation comparison of morphometric characters of J2 of *M. incognita* with five different host plants showed that the maximum mean values were recorded in body length and ratio 'C' in brinjal whereas minimum mean values for body length was found in okra. Tail length of J2 of *M. incognita* had maximum mean value of 44  $\mu\text{m}$  in banana. Stylet length was recorded maximum in okra and minimum in sunflower. H-MB and MB-EP also showed maximum mean value in okra. For the two characters of body length and ratio C, maximum mean values were found in brinjal. ABW showed maximum mean value in bottle gourd (Table 7.).

TABLE 6. Morphometric characters of second-stage juveniles (J2) in *M. incognita* of five different host plants in Punjab [Mean  $\pm$  SD  $\pm$  SE; (range); C.V.%], n = 5.

Characters	Okra	Banana	Sunflower	Brinjal	Bottle gourd	CR
L ( $\mu\text{m}$ )	226 $\pm$ 8.4 $\pm$ 4.2 (220-232) 3.7	280 $\pm$ 84.2 $\pm$ 21.2 (200-380) 30.3	292.5 $\pm$ 84.1 $\pm$ 42 (233-352) 28.7	331.5 $\pm$ 68.5 $\pm$ 34 (232-380) 20.7	330 $\pm$ 6.8 $\pm$ 3.4 (280-370) 10.3	HV
Stylet length ( $\mu\text{m}$ )	22.5 $\pm$ 3.5 $\pm$ 1.7 (20-25) 15.6	15.5 $\pm$ 3.1 $\pm$ 1.1 (11-18) 20	13.5 $\pm$ 3.5 $\pm$ 1.7 (11-16) 26.1	19.5 $\pm$ 7.7 $\pm$ 3.8 (14-25) 39.8	16 $\pm$ .48 $\pm$ 0.24 (13-19) 15.2	HV
H-MB ( $\mu\text{m}$ )	64.3 $\pm$ 2.3 $\pm$ 1.1 (62.7-66) 3.6	41 $\pm$ 8.4 $\pm$ 2.1 (33-51) 20.6	48 $\pm$ 4.2 $\pm$ 2.1 (45-51) 8.8	57 $\pm$ 15.5 $\pm$ 7.7 (46-68) 27.2	43.2 $\pm$ .72 $\pm$ 0.36 (39-47) 8.4	HV
MB-EP ( $\mu\text{m}$ )	31.4 $\pm$ 2.3 $\pm$ 1.4 (29-33) 7.4	19.2 $\pm$ 1.2 $\pm$ 0.3 (18-21) 6.5	30.5 $\pm$ 3.5 $\pm$ 1.7 (28-33) 11.5	24 $\pm$ 8.4 $\pm$ 4.2 (18-30) 35.3	31 $\pm$ .42 $\pm$ 0.21 (28-33) 6.8	HV
ABW ( $\mu\text{m}$ )	14.8 $\pm$ 2.3 $\pm$ 1.1 (13.2-16.5) 15.7	10.7 $\pm$ 1.2 $\pm$ 0.3 (8-15) 27.7	15.6 $\pm$ 2.1 $\pm$ 1.0 (14-17) 13.6	15.5 $\pm$ 2.1 $\pm$ 1.0 (9-13) 25.6	16 $\pm$ .24 $\pm$ 0.12 (14-17) 7.6	HV
Tail length ( $\mu\text{m}$ )	22.1 $\pm$ 5.5 $\pm$ 2.7 (18.2-26) 24.9	44 $\pm$ 20.7 $\pm$ 5.1 (15-16) 47	26.5 $\pm$ 2.1 $\pm$ 1.0 (25-28) 36.9	22.5 $\pm$ 7.7 $\pm$ 3.8 (17-25) 26.9	26.6 $\pm$ .26 $\pm$ 0.13 (25-28) 5	HV
C	9.8 $\pm$ 1.3 $\pm$ 0.6 (8.9-10.8) 13.6	7.7 $\pm$ 4.6 $\pm$ 1.1 (4.6-14.6) 59.5	10.9 $\pm$ 2.2 $\pm$ 1.0 (9.3-12.5) 20.7	15.6 $\pm$ 2.1 $\pm$ 1.0 (1.7-2.5) 14.5	11.2 $\pm$ .46 $\pm$ 0.23 (8.9-13.6) 10.7	HV
C'	1.53 $\pm$ .60 $\pm$ .30 (1.1-1.96) 39	4.8 $\pm$ 2.5 $\pm$ 0.6 (1-6) 53.1	1.67 $\pm$ .04 $\pm$ .021 (1.64-1.70) 2.5	1.5 $\pm$ .70 $\pm$ .35 (1-2.0) 47.1	3.5 $\pm$ .20 $\pm$ .10 (2.2-4.8) 8.0	HV

Character ranking (CR): LV = least variable (<8%); MV = moderately variable (8%-12%); HV = highly variable (>12%). ABW = anal body width, H-MB = head to median bulb length, MB-EP = median bulb to excretory pore.

TABLE 7. Comparison of the gross range of morphometric data recorded for four populations of *M. incognita* with Chitwood, 1949; Jepson, 1983; and Hunt & Handoo, 2009.

Characters	Chitwood, 1949		Jepson, 1983		Hunt & Handoo, 2009	Gross range in four populations
	<i>M. incognita</i>	<i>M. incognita acrita</i>	<i>M. incognita acrita</i>	<i>M. incognita wartellei</i>	<i>M. incognita</i>	
Females						
Length (µm)	510-690	440-670	–	–	–	530-812 (717)
Width (µm)	300-430	364-545	–	–	–	510-692 (597)
Stylet (µm)	15-16	16	20 (15.4 ± 0.15)	25 (16.5 ± 0.12)	15-16	16-27 (19)
LMB (µm)	–	–	–	–	–	20-42 (33)
WMB (µm)	–	–	–	–	–	20-42 (33)
Neck length (µm)	–	–	–	–	–	176-280 (233)
A	–	–	–	–	–	1.03-1.43 (1.3)
Perineal pattern						
LVS (µm)	–	–	–	–	–	16.5-26 (22)
AVS (µm)	–	–	–	–	–	16-29 (22)
ATT (µm)	–	–	–	–	–	12-18 (15)
IPD (µm)	–	–	–	–	–	17-26 (22)
Second-stage juveniles (J2)						
Length (µm)	360-393	345-396	–	–	–	200-380 (281)
C	–	–	–	–	–	4.6-16.6 (10)
Stylet (µm)	–	10-11	–	–	–	11-25 (17)
C'	–	–	–	–	–	1-6 (4)
H-MB (µm)	–	–	–	–	–	35-68 (51)
MB-EP (µm)	–	–	–	–	–	18-33 (25)
ABW (µm)	–	–	–	–	–	8-17 (14)

ABW = anal body width, ATT = anus to tail terminus, AVS = anus to vulval slit, IPD = interphasmid distance, LMB = length of median bulb, LVS = length of vulval slit, H-MB = head to median bulb length, MB-EP = median bulb to excretory pore, WMB = width of median bulb.

On the basis of different levels of CV in *M. incognita* from five different host plants, the characters of mature female, body length, width, and ratio 'a' were moderately variable characters (CV 0.26% to 20%) and stylet length, neck length, LMB, and WMB were high variability (CV 1.0% to 36.1%). In perineal pattern, the two characters of ATT and IPD were moderately variable (CV 8.8% to 17.6%) and the two characters of AVS and LVS were highly variable (CV 2.1% to 40.5%). In J2, body length, stylet length, H-MB, MB-EP, ABW, tail length, and ratios C, and C' were highly variable characters (CV > 12%).

One subspecies *M. incognita acrita* was found in okra. On the basis of perineal pattern this subspecies was identified. According to literature there is good taxonomic character for identification.

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