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Characterization of *Meloidogyne indica* (Nematoda: Meloidogynidae) Parasitizing Neem in India, with a Molecular Phylogeny of the Species

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Abstract

Neem is a perennial plant of family Meliaceae grown very commonly in India. During a survey in Rajasthan, India; a population of root-knot nematode was found in association with tender neem plants causing yellowing, stunting, and heavy root galling. Inspection of the perineal pattern morphology of the adult females, extracted from the galled roots, primarily led to identification of the species as *Meloidogyne indica*. Further, detailed morphological and morphometric illustrations of second-stage juveniles, males and females were carried out by light compound and scanning electron microscopy. Gross morphology and measurements were found consistent with the original description of *M. indica* infecting citrus by Whitehead (1968). The neem population was found to infect and reproduce on citrus. Additionally, evolutionary relationship was deduced by Maximum likelihood method using ITS rRNA, D2D3 expansion segment of 28S rRNA and mitochondrial COI sequences. Phylogenetic analyses based on these sequences showed sufficient divergence of *M. indica* to be differentiated as a unique species under the genus *Meloidogyne*.

Key words
COI; D2D3; ITS; *Meloidogyne indica*; Neem.

Neem or margosa or Indian lilac (*Azadirachta indica* A. Juss), a perennial plant of family Meliaceae, is well known for containing bioactive compounds against several insects and plant-parasitic nematodes (Kraus, 1995). Azadirachtin, the main chemical component responsible for the toxic effect has nematicidal potential with respect to reduced fecundity and hatching of various plant-parasitic nematodes (Alam, 1993). The final population density of root-knot nematodes (RKN) has been found to decline with the application of neem seed kernel extract as a soil drench, and use of neem cake as a soil amendment etc. (Riga and Lazarovits, 2001; Kumar and Khanna, 2006). In spite of the widespread use of neem-based botanicals in plant protection, it is still not free from disease and pest attack. In the Indian subcontinent, it is known to be infested by different insects and fungi (Beeson, 1953; Tewari, 1992; Cornezo, 1999), but any nematode species attacking neem is largely unknown to date.

RKN (*Meloidogyne* spp.) are the most economically important group of obligate sedentary plant-parasitic species distributed worldwide encompassing more than 3,000 plant hosts (Jones et al., 2013). Genus *Meloidogyne* is known to include more than 90 nominal species to date (Karssen, 2002; Karssen and Moens, 2006). The use of DNA-based diagnostics in combination with morphological and morphometric analyses aid in authoritative identification of the RKN species and also help in resolving phylogenetic relationships (Blok and Powers, 2009). Molecular markers that have proved useful in these studies include mitochondrial gene cytochrome c oxidase subunit I (COI) (Blok et al., 2002; Hebert et al., 2004; Xu et al., 2004), the small subunit 18S rRNA gene (De Ley et al., 2002; Tenente et al., 2004; Tigano et al., 2005), D2D3 expansion segment of the large subu-

nit 28S rRNA (Chen et al., 2003; Palomares-Ruis et al., 2007) and ribosomal internal transcribed spacer (ITS) 1 and 2 (Powers and Harris, 1993; Powers et al., 1997). All these markers have proven useful for identification of *Meloidogyne* spp. (Castillo et al., 2003; Tigano et al., 2005; Powers et al., 2005).

During a survey in Jaipur district of Rajasthan, India a species of *Meloidogyne* was found in association with tender neem plants. The nematode species was initially considered to be *M. indica* (Whitehead, 1968) based on the perineal pattern morphology, when compared with the original species description (Whitehead, 1968). Accordingly, detailed morphological and morphometric illustrations were carried out for expanded and robust characterization of the species. Additionally information on molecular markers, viz., ITS rDNA, and D2D3 expansion segment of 28S rRNA gene and mitochondrial COI were also generated for the species that were not presented in its earlier description.

Materials and methods

Nematode population

Tender neem plants showing the symptoms of stunting and yellowing were collected along with the rhizosphere soil from Jaipur district, Rajasthan, India; located at N26°55′10″ and E75°47′16″. The soil type was found to be sandy to sandy loam with medium phosphorus and medium to high potassium content. Average annual rainfall in the area ranges from 500 to 600 mm (Anonymous, 1995). For diagnosis and identification purposes, females were directly dissected out of the galled roots for preparing perineal patterns and genomic DNA extraction. Eggmasses were hand-picked from the infected neem roots and were used for hatching the second-stage infective juveniles. Both second-stage juveniles (J2s) and males were collected by the modified Baermann's technique (Whitehead and Hemming, 1965).

Morphological studies

For conducting taxonomic studies, the J2s and males were processed by the glycerol–ethanol method following standard procedures (Seinhorst, 1959). The morphological characters used for taxonomic identification were employed as described by Karssen (2002) and Carneiro et al. (2014). Morphometric illustrations and measurements (in μm) were made with a camera lucida and an ocular micrometer within an Olympus BX50 compound microscope. The posterior portion with cuticular markings surrounding the vulva and anus of stained-fixed females was cut using a fine-pointed scalpel to obtain the perineal pattern. The inner tissue was carefully removed using flexible bristle, trimmed and transferred to a drop of anhydrous glycerol on a clean glass slide (Southey, 1986) to study the perineal pattern morphology. Photomicrographs of perineal pattern, females, males, and J2s were taken with a Zeiss Axiocam M2m compound microscope equipped with differential interference contrast optics.

Scanning electron microscopy (SEM) was done using a JEOL (UK) JSM 6700 FEG scanning electron microscope equipped with a GATAN (UK) Alto 2500 Cryo unit. The instrument was prepared by cooling with liquid nitrogen and the temperature in the microscope chamber was maintained at –160°C throughout sample examination. The nematode specimens were pipetted onto a 595-grade Whatman™ filter paper attached to a cryo stub with OCT mountant (Sakura Finetek, Europe NL) and frozen by plunging into pre-slushed liquid nitrogen. The sample was then transferred to the GATAN cryo chamber stage under vacuum and etched to remove contaminating ice by increasing the temperature to –95°C for 1 min. Once the temperature of the stage had returned to –160°C, the sample was coated with Au/Pd alloy for 1 min and transferred to the SEM chamber and mounted on the microscope stage for examination at –160°C. Images were recorded using the JEOL on board system and software.

Molecular characterization

For molecular analysis, single adult female was lysed in worm lysis buffer (Subbotin et al., 2000) and the molecular markers, viz., ITS, D2D3, and COI were PCR amplified using the primers listed in Table 1. The reaction condition for amplification of the ITS gene was followed as described in Subbotin et al. (2001, 2006). For D2D3 and COI genes, the thermal cycling program was used as described in Ye et al. (2007). Amplified products were separated by electrophoresis on 1% agarose gel and purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). The gel purified PCR products were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and transformed into *E. coli* DH5α competent cells (New England Biolabs). Recombinant plasmids were isolated from the positive clones QIAGEN Plasmid Miniprep kit (Qiagen, Valencia, CA, USA) and sequenced. The raw sequences were checked for quality using MEGA6 (Tamura et al., 2013), aligned, and a consensus sequence was generated for each gene using Bioedit software (Tom Hall; Ibis Biosciences, Carlsbad, CA).

The obtained ITS, D2D3, and COI sequences were compared with the available sequences in the NCBI GenBank database using BLAST search. Corresponding published sequences (of *Meloidogyne* spp.) for each

Table 1. List of primers used for polymerase chain reaction amplification in this study.

Primer name	Gene	Sequence	References
V5367	*ITS*	5′-TTGATTACGTCCCTGCCCTTT-3′	Vrain et al. (1992)
26S	*ITS*	5′-TTTCACTCGCCGTTACTAAGG-3′	Vrain et al. (1992)
D2A	*LSU*	5′-ACAAGTACCGTGAGGGAAAGTTG-3′	Castillo et al. (2003)
D3B	*LSU*	5′-TCGGAAGGAACCAGCTACTA-3′	Castillo et al. (2003)
JB3	*COI*	5′-TTTTTTGGGCATCCTGAGGTTTAT-3′	Bowles et al. (1992)
JB5	*COI*	5′-AGCACCTAAACTTAAAACATAATGAAAATG-3′	Derycke et al. (2005)

gene were retrieved (Table 2) and phylogenetic analyses were carried out using MEGA6 software (Tamura et al., 2013). The sequences were aligned using ClustalW and evolutionary history was deduced by the maximum likelihood (ML) method with selection of appropriate model using Modeltest (Posada and Crandall, 1998). The phylograms were bootstrapped 1,000 times (Felsenstein, 1985) to assess the degree of support for the phylogenetic branching as indicated in the consensus tree. *Pratylenchus vulnus* was used as out-group.

The sequences were deposited in the NCBI GenBank with the accessions KC311146, MF680038, and MF662179 for ITS, D2D3, and COI, respectively.

Inoculation of neem population on citrus

Inoculation assay was conducted in a soil system to test whether the nematode species infecting neem can parasitize and multiply on citrus. One-month-old citrus seedlings were planted in plastic pouches (height: 6

Table 2. List of GenBank accession numbers used in phylogenetic analyses.

Species	ITS rRNA	D2D3 28S rRNA	COI mtDNA
Meloidogyne arabicida	**	KF993624	**
Meloidogyne africana	**	**	KY433441
Meloidogyne arenaria	AF387092	JX987332	JX683705
Meloidogyne artiellia	KC545880	AY150369	KU517173
Meloidogyne baetica	AY150366	AY150367	**
Meloidogyne camelliae	JX912885	KF542869	KM887148
Meloidogyne chitwoodi	AY281852	AF435802	KU517168
Meloidogyne christiei	KR082319	KR082317	**
Meloidogyne dunensis	EF612711	EF612712	**
Meloidogyne duytsi	**	**	KU517177
Meloidogyne enterolobii	KM046989	KJ146862	KT936633
Meloidogyne ethiopica	KF482366	KF482372	**
Meloidogyne exigua	**	AF435795	**
Meloidogyne fallax	AY281853	KC241969	KU517182
Meloidogyne graminicola	KM111531	KJ728847	KY250093
Meloidogyne graminis	JN157866	JN019326	**
Meloidogyne hapla	EU908052	DQ145641	JX683719
Meloidogyne haplanaria	**	**	KU174206
Meloidogyne hispanica	EU443613	EU443607	JX683712

Meloidogyne ichinohei	**	EF029862	KY433448
Meloidogyne incognita	KJ739707	JX100425	JX683696
Meloidogyne indica	KC311146	MF680038	MF662179
Meloidogyne inornata	KF482368	KF482374	**
Meloidogyne izalcoensis	**	KF993621	**
Meloidogyne javanica	KJ739709	KC953092	JX683711
Meloidogyne konaensis	**	AF435797	**
Meloidogyne lopezi	**	KF993616	**
Meloidogyne luci	KF482365	KF482371	**
Meloidogyne mali	JX978228	KF880398	KU517175
Meloidogyne marylandi	JN157854	JN019333	
Meloidogyne minor	KC241953	JN628436	KU517178
Meloidogyne naasi	KJ934132	KC241979	KU517170
Meloidogyne panyuensis	**	**	**
Meloidogyne paranaensis	**	AF435799	**
Meloidogyne silvestris	EU570216	EU570214	**
Meloidogyne spartelensis	KP896294	KP895293	KP997301
Meloidogyne thailandica	AY858795	EU364890	**
Meloidogyne trifoliophila	JX465593	AF435801	**
Pratylenchus vulnus	FJ713011	EU130885	KX349427

** Not found in NCBI database.

inch, diameter: 4 inch) filled with sterilized soil, and inoculated with 2 J2s/g soil. Freshly hatched J2s from the eggmasses, collected from neem roots, were used for inoculation. The experiment was conducted with five replicates.

Results

Plant symptoms and characterization of species

The infected neem seedlings showed the symptoms of yellowing of leaves coupled with stunted growth of the immature plants. Examination of the infected plants revealed the presence of heavy galling on the roots located mostly along the tap root axis with few lateral branches (Fig. 1A–C). For characterization and identification of the species, morphology of adult females, males and J2s was studied (Fig. 1D–K) including SEM photomicrographs (Fig. 2) with minute detailing.

The nematode species infecting neem was found to infect the citrus plants. The citrus roots showed the presence of galls with formation of eggmasses when observed at 35 days post inoculation (Fig. 3). The females, dissected out from the citrus roots, showed similar perineal pattern morphology with that of the neem population (data not shown).

General morphology

Female

Females are completely enclosed within root tissue. The body appeared translucent white, pyroid shaped, and variable in size. Neck prominent and short, bent at various angles. Body annuli smaller in anterior neck region. *En face* view with six prominent lips and stylet opening pore-like. Stylet small and strong, knobs well developed and ovoid. Position of Dorsal oesophageal gland orifice (DGO) is 2.90 ± 0.39 µm from stylet knob. Median bulb well developed, almost rounded. Vulva nearly terminal. The perineal cuticular pattern was rounded with low and rounded dorsal arch and devoid of lateral lines or any forking. Typically it is formed of very fine, closely spaced striae of the cuticle, forming a distinct tail whorl. Phasmid and anus were distinct. Commonly egg sac occurs outside the root.

Male

The body is vermiform, tapering anteriorly. Body annuli large and distinct. In lateral view, labial cap high and rounded. Lip annuli two behind the head cap. Labial disc rounded. Cephalic framework moderately

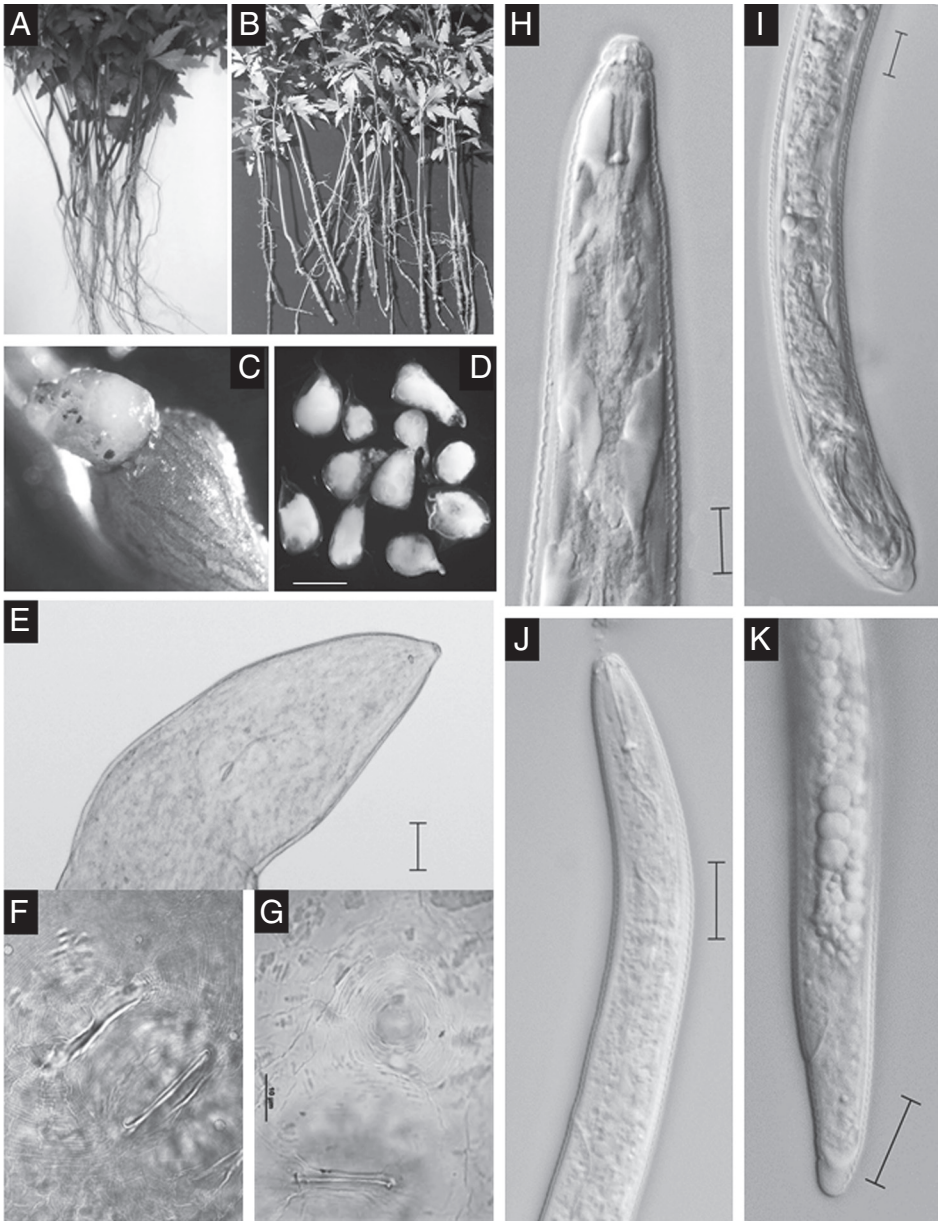

Figure 1: Plant symptoms (A–C) and morphology (D–K) of *Meloidogyne indica* infecting neem. A, Healthy neem seedlings; B, Infected neem seedlings devoid of lateral roots; C, Root gall with eggmass; D, Adult females; E, Anterior region of adult female; F and G, Perineal pattern morphology; H, Anterior region of male; I, Male tail; J, Anterior end of second-stage juvenile (J2); K, Second-stage juvenile tail (scale bar = D: 550 µm; E: 100 µm; H,I: 10 µm; J,K: 20 µm).

developed. Stylet robust and thin with cone pointed, shaft cylindrical and knobs directed posteriorly. DGO 3.11 ± 0.17 µm from the stylet knob. Amphids very distinct. Lateral field with four lateral lines. Testes out-stretched. Tail is short conoid with narrow terminus and smooth end. Spicule arcuate, gubernaculum distinct.

Second-stage juvenile

The body is slender, vermiform, and finely annulated. Lip region not set off from the body. Cephalic framework very weakly developed, amphids distinct. Stylet relatively long, delicate with pointed conus and rounded knobs. DGO 2.86 ± 0.23 µm posterior to stylet knob.

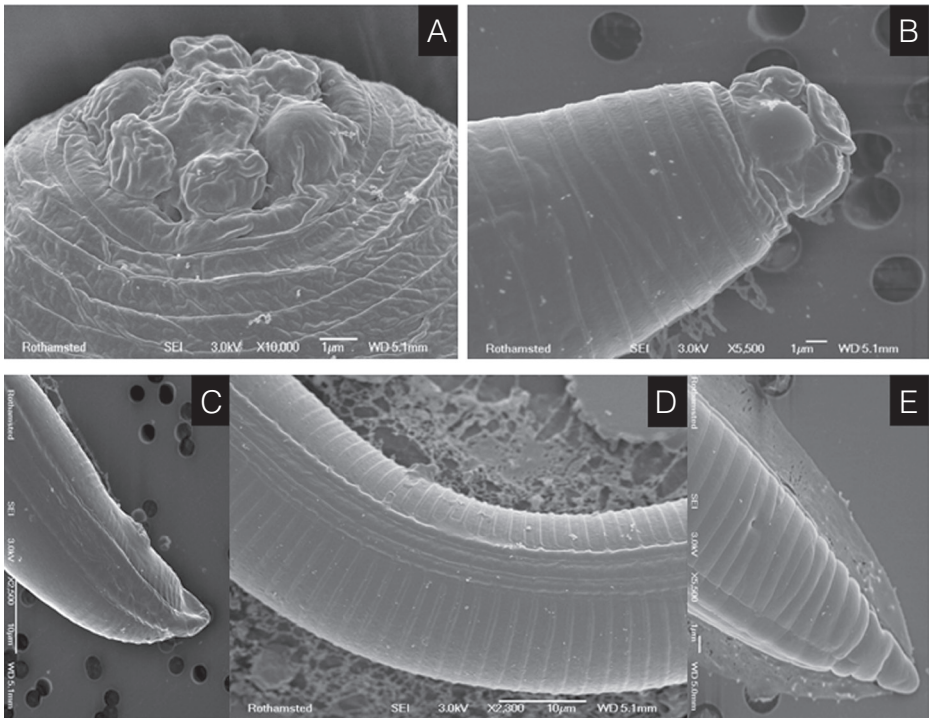

Figure 2: Scanning electron microscopy photomicrographs of *Meloidogyne indica*. A, Female lip region; B, Male lip region; C, Male tail; D, Lateral field with lateral lines; E, Second-stage juvenile tail (scale bar in µm).

Metacorpus oval to rounded. Oesophageal gland lobe overlapping intestine ventrally. Excretory pore distinct, at the level of isthmus. Lateral field with four incisures running almost entire length of body and ending near hyaline tail terminus. Tail length short with very small hyaline portion, terminal blunt, and unstriated.

The morphometric measurements of females, males, and J2s for the present population of RKN species are presented in Table 3.

Differential diagnosis of the RKN species infecting neem showed maximum similarity with *M. indica*, originally described by Whitehead (1968) from New Delhi, India infecting *Citrus aurantifolia*. Minor variations were observed in the general morphology and morphometrics. The measurements originally taken by Whitehead (1968) for description of *M. indica* are also presented in Table 3 for comparison.

Molecular characterization

Phylogenetic analysis of the ITS rRNA and D2D3 fragment of 28S rRNA sequence revealed that the sequences were highly divergent from other *Meloidogyne* species described to date. Phylogenetic relationship as inferred from the ML method using ITS rRNA sequences (Fig. 4) showed that the species holds a basal position in the tree without clustering with other described species of *Meloidogyne*. The closest sequence-related neighbors were

Figure 3: *Citrus* roots showing development of galls and eggmasses upon inoculation with the neem population of *Meloidogyne indica*. A, *Citrus* plant inoculated with neem population of *M. indica*; B, Healthy roots of citrus; C, Infected roots of citrus with galls and eggmasses.

Table 3. Morphometrics for *Meloidogyne indica* infecting neem and citrus. All linear measurements are in micrometer and in the form of mean ± SD.

	Neem population			Citrus population (After Whitehead, 1968)	
Character	**J2**	**Male**	**Female**	**J2**	**Female**
n	20	15	30	25	8
L	484 ± 31.5 (430–520)	1253 ± 80 (1180–1380)	653 ± 92.2 (450–790)	414 ± 4.5 (381–448)	–
Body width	18 ± 1.5 (16.77–21.15)	28 ± 4 (24.55–34.66)	408 ± 75 (325–550)	–	–
A	26.68 ± 1.9 (24.20–29.87)	44.89 ± 3.5 (39.81–48.06)	1.60 ± 0.3 (1.38–2.10)	–	–
Stylet length	13.8 ± 0.1 (13.57–14.21)	16.3 ± 0.4 (15.90–17.08)	13.7 ± 0.4 (13.32–14.18)	12 ± 0.9 (10–14)	14 (12–16)
DGO	2.8 ± 0.2 (2.45–3.25)	3.1 ± 0.1 (2.92–3.30)	2.9 ± 0.3 (2.49–3.67)	–	3 (2–4)
Head-metacorpus	50 ± 2.3 (46.45–53.02)	73 ± 4.1 (68.70–78.14)	–	–	–
Head-oesophageal gland	138 ± 4.8 (129.97–144.65)	–	–	–	–
b′	3.5 ± 0.1 (3.10–3.65)	–	–	–	–
c	26.2 ± 1.2 (24.15–27.65)	–	–	24.9 ± 1.36 (21.2–31)	–
c′	1.6 ± 0.1 (1.52–1.91)	–	–	1.57 ± 0.012 (1.06–1.78)	–
Tail length	18 ± 0.6 (17.50–19.50)	–	–	16.8 ± 1.88 (13–20.1)	–
Anal body width	11.1 ± 1.0 (9.85–12.45)	–	–	–	–
Spicule	–	26 ± 0.6 (25.90–27.50)	–	–	–

found to be *M. panyuensis*, *M. camelliae*, *M. artiellia*, *Meloidogyne mali* and *M. baetica* with high bootstrap support. The ML tree based on the D2D3 expansion segment of the 28S rRNA gene (Fig. 5) showed that the species branched separately being close to the out-group taxon *P. vulnus*. Phylogenetic analysis of the mitochondrial COI sequence (Fig. 6) showed that the species branched out separately without clustering with any *Meloidogyne* species, having sequence similarity to *M. artiellia*. The dataset gave similar results across all tree reconstruction algorithms. Thus substantial sequence divergence for all the three genes tested distinguishes the species from other studied RKN.

Discussion

The present investigation includes identification and characterization of the RKN species parasitizing neem as *M. indica* (Whitehead, 1968) from Rajasthan, India. *Meloidogyne indica* was first described by Whitehead (1968) infecting *Citrus aurantifolia* from New Delhi, India. Whitehead (1968) described the species based on a limited number of specimens; and adequate morphometric data or any molecular phylogenetic analyses were neither presented in the original description nor any other publication to date. Hence, the present study provides additional and useful information on morphology, mophometrics, and molecular

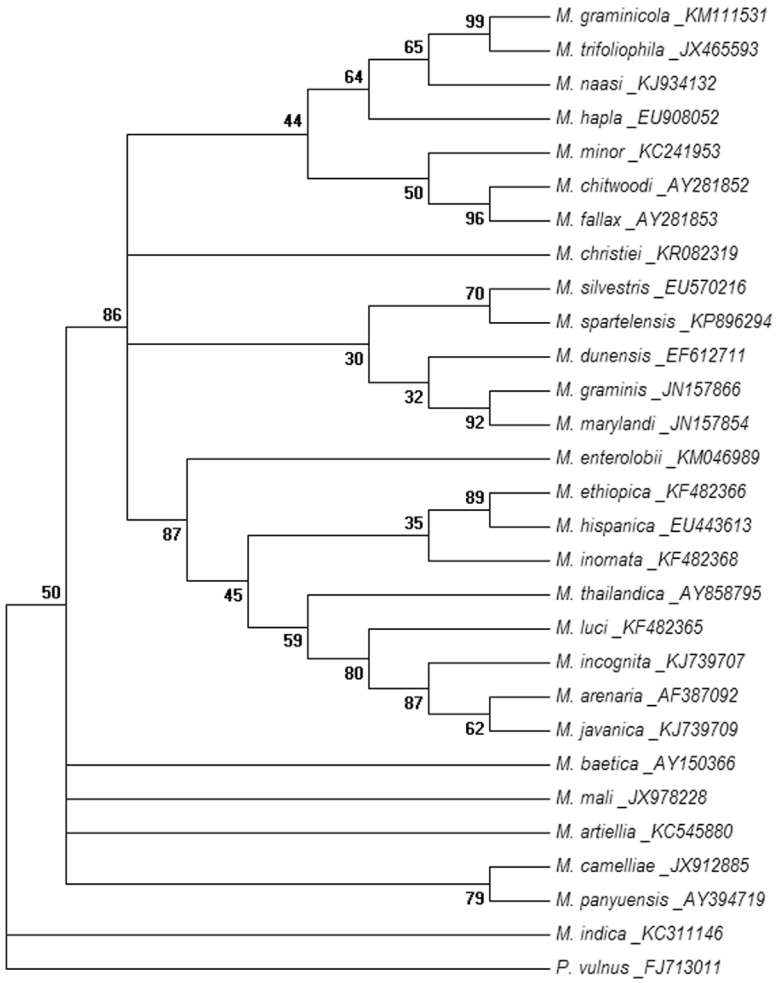

Figure 4: Evolutionary relationship of *Meloidogyne indica* using ITS rRNA sequence. The evolutionary history was inferred by using the maximum likelihood method based on Kimura 2-parameter model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in less than 30% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value (–1869.0466). A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 1.0929]). The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 170 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

phylogeny of the species in detail. The morphometric illustrations are consistent with the description of Whitehead (1968) with slight deviations. The body length (483.70 ± 31.52 µm vs. 414 ± 4.5 µm) and tail length (18.41 ± 0.67 µm vs. 16.8 ± 1.88 µm) of the J2s of the neem population were found to be relatively larger than that of the citrus population. However, these differences may be considered as intra-specific variation for the species. Additional measurements for J2s include length of DGO; lip region-metacorpus length; length of glandular overlapping; *a*- and *b′* ratio that were not presented in the earlier description. The J2s showed distinctiveness in the gross morphology of anterior and posterior ends, tail shape, and tail length typical for the species. The perineal pattern is characteristic for the species as described earlier.

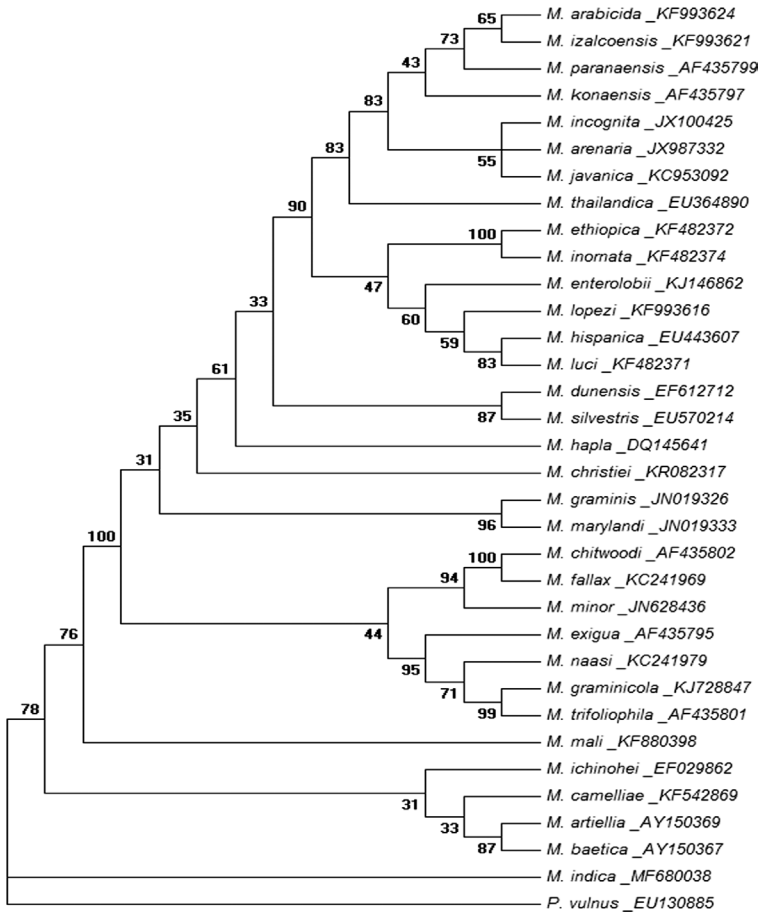

Figure 5: Evolutionary relationship of *Meloidogyne indica* using D2D3 expansion segment of 28S rRNA sequence. The evolutionary history was inferred by using the maximum likelihood method based on General Time Reversible model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in less than 30% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log likelihood value (–4112.4125). A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 0.4693]). The analysis involved 34 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 525 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Morphometrics of males were not studied earlier and therefore the data generated in terms of body length, body width, stylet length, length of DGO, spicule length, and a- ratio could not be compared. Additionally, supplementary morphology has also been generated for males in this study. The scanning electron microscope photomicrographs showed minute morphological details of males, females, and J2s.

For molecular characterization, three molecular markers, viz., ITS rRNA, D2D3 expansion segment of 28S rRNA, and mitochondrial COI genes were PCR amplified and sequenced. Independent ML phylogenetic analyses for each of the three marker sequences of *M. indica* revealed sufficient divergence of this species demonstrating its novelty as a unique species under the genus *Meloidogyne*. Within evolutionary trees *M. indica* was a basal taxon relative to other *Meloidogyne* species when *Pratylenchus* was an outgroup. This can probably establish the primitiveness of the species among the other RKN as they are presum-

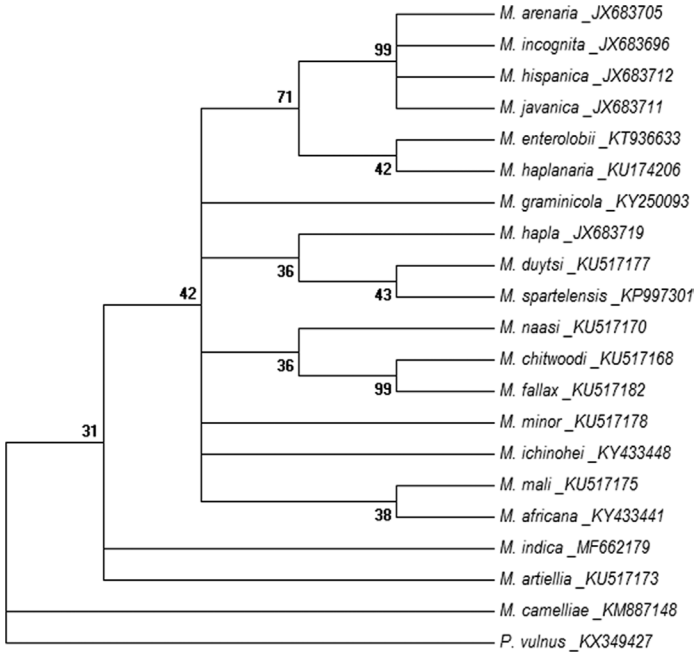

Figure 6: Evolutionary relationship of *Meloidogyne indica* using mitochondrial COI sequences. The evolutionary history was inferred by using the maximum likelihood method based on General Time Reversible model. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the analyzed taxa. Branches corresponding to partitions reproduced in less than 30% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value (–2471.1920). A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories [+G, parameter = 0.5728]). The analysis involved 21 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 309 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

ably thought to arise from a *Pratylenchus*-like ancestor (Quist et al., 2015). Whitehead's grouping of *Meloidogyne* spp. based on the tail shape of J2s and the number of head annuli of males, reflected the similarity of *M. indica* with *M. artiellia* in gross morphology (Whitehead, 1968). The similarity between these two species has also been validated in the present phylogenetic analyses based on ribosomal and mitochondrial genes.

So far *M. indica* has been documented from different species of citrus and *Bt*-cotton (Whitehead, 1968; Franklin, 1978; Patel et al., 1999; Vovlas and Inserra, 2000; Davis and Venette, 2004; Khan et al., 2017). In the present study the species was found to infect neem plants demonstrating the ability of the species to parasitize perennial hosts. The cross-inoculation of the neem population of *M. indica* to citrus plants revealed that it could successfully reproduce on citrus. Most *Meloidogyne* spp. are polyphagous and highly adapted towards their hosts. The ability of *M. indica* to infect neem may be a result of its co-evolution allowing the nematode to withstand the toxic compounds present in the neem plant. Detoxification of plant toxins is reported amongst insects where they can successfully use some enzymes for their own benefit (Dowd et al., 1983; Mello and Silva-Filho, 2002; Jeschke et al., 2016). Digestive enzymes in insects, viz., alpha-amylases, esterases and glutathione S-transferase were found to detoxify plant defence chemicals (Senthil-Nathan, 2013) and similar mechanism may be involved in the parasitism of *M. indica* on neem. One such example is *Meloidogyne incognita* infecting and multiplying on some *Brassica* spp., plants with nematicidal potential (Shivakumara et al., 2016).

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