

# Comprehensive Phylogenetic Analysis of Root-knot Nematodes Predicts Emerging Virulent Species

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**Abstract:** Among the root-knot nematodes three *Meloidogyne* species namely *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* are emerging as an important pest of many cultivated plants, and recognized as the most economically destructive plant parasitic nematodes species of all over the world. Although other root-knot nematodes may virulent for plant but limited information is available. Thus, a comprehensive bioinformatics analysis including sequence acquisition, multiple sequence alignment and the phylogenetic tree construction for well-known *Meloidogyne* species was employed to predict the emerging virulent species. About eighty seven (87) 18S rRNA sequences of three damaging *Meloidogyne* species (*M. javanica*, *M. arenaria* and *M. incognita*) were retrieved from NCBI database, and allowed to construct phylogenetic trees using both NJ and ME methods of Molecular Evolution Genetic Analysis (MEGA) tools. Phylogeny analysis revealed that *M. enterolobii\_1*, *M. sp.\_Mi\_c3a*, *M. sp.\_Mj\_c1a* and *M. sp.\_Mj\_c3a* are genetically as well as evolutionally related to existing well recognized virulent nematodes. Moreover, evolutionally emerging strains of existing virulent species of *M. javanica*, *M. arenaria* and *M. incognita* along with the predicted virulence nematodes could become a great challenge to agriculture. The study could initiate the further analysis for novel insights in the pathogenesis of emerging virulence species of *Meloidogyne* that must be needed for future crop management strategies.

**Keywords:** *Meloidogyne*, Virulence, Phylogenetic Analysis, Plant Parasite, 18S rRNA

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## 1. Introduction

Root-knot nematodes (RKN) are belong to *Meloidogyne* genus, are microscopic obligate endo-parasites that live in soil. RKN feed on the roots of a wide range of plant species [1-3] and consider as an important pathogen of numerous plants including food crops, and known as the most economically destructive genus of plant parasitic nematodes in the world [4, 5]. RKN produce galls on roots that eventually lead to reduced water uptake to shoots that causes yield loss. The severity of yield loss can range from minimal to total depending on the infesting RKN species and crop variety, season, soil type and use of crop rotation [6-9].

Distribution of *Meloidogyne* species is depends on the ability of these obligate root parasites to become established during overwinter in a geographic area. Among the reported 90 *Meloidogyne* species, *M. incognita*, *M. javanica*, and *M. arenaria* are most damaging [10-12].

Two genomic regions that have been used regularly to characterize species among the nematode taxa are the ribosomal RNA array and the mitochondrial genome. Remarkably, the ribosomal RNA (rRNA) genes and their intervening sequences are the best characterized genes or gene regions in Nematoda [13-15]. Typically, the rRNA array consists of three ribosomal genes including 18S, 5.8S, and 28S. Recent findings suggest that 14 *Meloidogyne* species in

a phylogenetic comparison using nearly complete 18S rDNA sequence and reported that within the genus there was about 10% divergence in pairwise comparisons among species, corresponding to considerable diversity for a relatively conserved gene [16-20]. Recent findings suggested that the sequences of a 18S rRNA can also distinguish between species in a genus [21-23]. Thus, polymorphic sites in the 18S gene can be exploited to diagnose individual species as well as can give insights of the inter- and intra-species

relationship. In addition, molecular diagnosis by using 18S rRNA sequence will help to identify and address unforeseen emerging parasites threat to agricultural crop [24, 25]. Although there is no such data is available for *Meloidogyne spp.* Thus, the present study aims to identify the noxious *Meloidogyne spp.* based on the comparison with three damaging *Meloidogyne* species (*M. javanica*, *M. arenaria* and *M. incognita*) using 18S rRNA through comparing conserve regions and phylogenetic tree construction.

**Table 1.** *Meloidogyne* species and the area they showed prevalence in crops.

Species Name	Gene Bank ID of strains	Location (Retrieved from NCBI)	Affected crop
<i>Meloidogyne arenaria</i>	AY438555.1	China	Tomato, potato, olive [11-14]
	AY438554.1	China	
	AF387092.1	USA	
	LC030350.1	Japan	
	LC030351.1	Japan	
	U96301.1	USA	
	LC030356.1	Japan	
	LC030355.1	Japan	
	LC030352.1	Japan	
	LC030354.1	Japan	
<i>Meloidogyne javanica</i>	LC030353.1	Japan	Brinjal, Peanut, potato, olive [11-14]
	AY438555.1	China	
	KJ739710.1	India	
	KC953091.1	China	
	AF387094.1	USA	
	U96305.1	USA	
	AY829374.1	Spain	
	AY829375.1	Malta	
	KJ739709.1	India	
	KC464469.1	AY438556.1 China	
<i>Meloidogyne incognita</i>	JQ405212.1	China	Tomato, pepper, okra, watermelon, cantaloupe, onion, pumpkin, squash, sweet potato, sweet corn, carrot, eggplant, olive, bean and pea [15, 11, 14]
	KJ641591.1	China	
	KJ739708.1	KJ451618.1 China	
	KJ739707.1	India	
	AF516723.1	India	
	KC342236.1	India	
	KJ451617.1	Australia	
	KP179226.1	India	
	U96304.1	India	
	KP179229.1	India	
<i>Meloidogyne incognita</i>	KP179224.1	USA	Tomato, pepper, okra, watermelon, cantaloupe, onion, pumpkin, squash, sweet potato, sweet corn, carrot, eggplant, olive, bean and pea [15, 11, 14]
	KR265163.1	India	
	KP751205.1	India	
	LC030367.1	India	
	LC030366.1	India	
	KP751203.1	Japan	
	KP179223.1	Japan	
	KP179225.1	India	
	KP751204.1	India	
	LC030364.1	India	
<i>Meloidogyne incognita</i>	KP233824.1	India	bean, corn, cucumber, potato, spinach, and tobacco (Maleita et al. 2012) [16]
	KJ913700.1	Japan	
	LC030363.1	India	
	KP233823.1	India	
	FJ534516.1	Japan	
	KC594036.1	India	
	KR265162.1	Malaysia	
	JX885741.1, JX885742.1	China	
	KF482363.1	Brazil	
	FJ768939.1	China	
Others	JX465575.1	New Zealand	Vegetables, flowers and fruits (NCBI)
	JX465577.1	New Zealand	Cucumber (NCBI)
	JX465578.1	New Zealand	Tamarillo (NCBI)

Species Name	Gene Bank ID of strains	Location (Retrieved from NCBI)	Affected crop
	JX465572.1	New Zealand	Tamarillo (NCBI)
	JX465571.1	New Zealand	Tamarillo (NCBI)
	JX465576.1	New Zealand	Tamarillo (NCBI)
	JX465574.1	New Zealand	Tamarillo (NCBI)
	JX465573.1	New Zealand	Tamarillo (NCBI)
	JX465569.1	New Zealand	Tamarillo (NCBI)
	JX465570.1	New Zealand	Kiwifruit (NCBI)
	KF482368.1	Brazil	Kiwifruit (NCBI)
	KF418368.1	China	Vegetables, flowers and fruits (NCBI)
	KF482366.1	Brazil	Vegetables (NCBI)
	JX465565.1	New Zealand	Vegetables, flowers and fruits (NCBI)
	JX465566.1	New Zealand	Tamarillo (NCBI)
	JX465564.1	New Zealand	Tamarillo (NCBI)
	JX465567.1	New Zealand	Tamarillo (NCBI)
	JX465568.1	New Zealand	Tamarillo (NCBI)
	JX465562.1	New Zealand	Kiwifruit (NCBI)
	JX465561.1	New Zealand	Tamarillo (NCBI)
	JF309157.1	Costa Rica	Kiwifruit (NCBI)
	JF309154.1	Costa Rica	Guava (NCBI)
	JF309155.1	Costa Rica	Malpighia sp. (NCBI)
	JF309156.1	Costa Rica	Guava (NCBI)
	JX024149.1	China	Guava (NCBI)
	KT354573.1	China	Euphorbia tirucalli (NCBI)
	KJ146863.1	China	Banana (NCBI)
	KT354575.1	China	Carrot (NCBI)
	KT354565.1	China	Banana (NCBI)
	KP411227.1	Taiwan	Banana (NCBI)
	KF418370.1	China	Guava (NCBI)
	KF418369.1	China	Vegetables (NCBI)
	JQ082448.1	China	Vegetables (NCBI) Carrot [17]

## 2. Methods and Materials

### 2.1. Sequence Retrieval

18S rRNA sequences of three major *Meloidogyne* species (*M. javanica*, *M. arenaria*, and *M. incognita*) were retrieved from the National Centre for Biotechnology Information database (NCBI) (<http://www.ncbi.nlm.nih.gov>). Obtained sequences were blasted in the NCBI database (using nucleotide blast query) that revealed total 87 sequences of different *Meloidogyne* species, which were further used for phylogenetic analysis (Table 1).

### 2.2. Multiple Sequence Alignment

All the retrieved 87 sequences of 18S rRNA were aligned using Clustal omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>); which can align virtually any number of protein sequences quickly and that delivers accurate alignments [26-28]. Clustal omega 18S rRNA sequence alignment of three major *Meloidogyne* species (*M. javanica*, *M. arenaria*, and *M. incognita*) and predicted emerging species were also used for the analysis of conserve regions.

### 2.3. Phylogenetic Tree Construction

First, 18S rRNA sequences of these 87 *Meloidogyne* species were aligned with ClustalW using the default parameters for gap opening and gap extension penalties. The software “Molecular Evolution Genetic Analysis (MEGA)”,

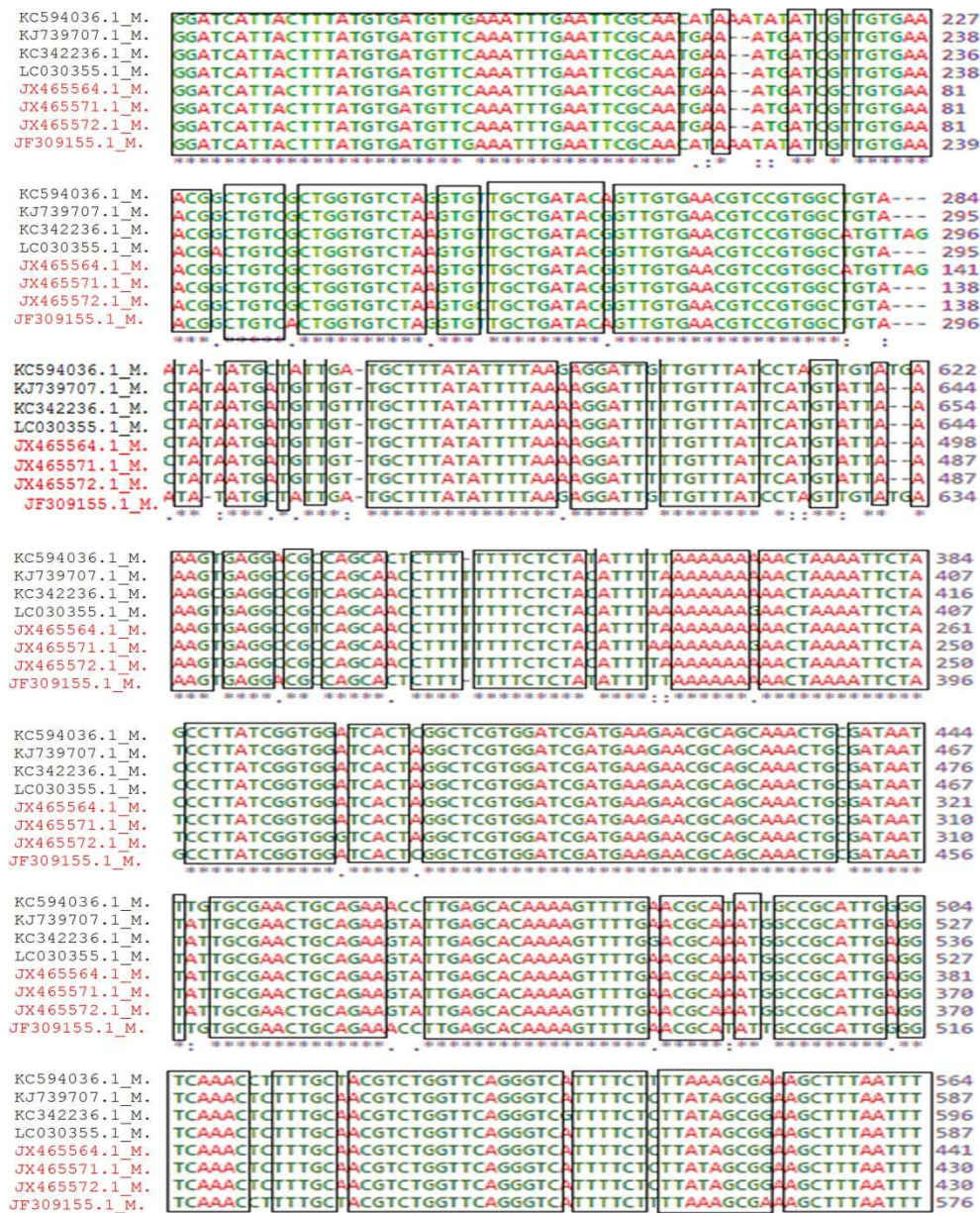
version 6 [29] was used for phylogenetic analysis. “Neighbour Joining (NJ)” and “Minimum Evolution (ME)” methods were used to construct two phylogenetic tree. Evolutionary distances between species were computed using the “Maximum Composite Likelihood” method [30] and the units of the number of base substitutions per site. Total 1000 replicates of bootstrapping were calculated in this purpose.

## 3. Results and Discussion

Multiple Sequence Alignment obtained from Clustal omega revealed that maximum similarity of *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* was found with *M. enterolobii\_1*, *M. sp.\_Mi\_c3a*, *M. sp.\_Mj\_c1a* and *M. sp.\_Mj\_c3a* strains (Figure 1). The phylogenetic trees of 18S rDNA sequences from 87 *Meloidogyne* species that were constructed using both NJ (Figure 2) and ME (Figure 3) methods were grouped into 7 major clades. Clade- I of Minimum Evolution Phylogenetic Tree (MEPT) differentiated in to 2 sub-clades; the first sub-clade was consist of eight nematodes species including *M. incognita\_1* (Figure 2) and *M. incognita* [31-33], are evolutionally closely related with other *Meloidogyne spp.* denoted by accession number JF309155.1, FJ534516.1, JF309158.1, JF309154.1, KF418368.1, JF309157.1, JF309156.1 respectively. In case of sub-clade-2, nematode species denoted with the accession number KF482366.1, KF482368.1, JX885742.1 were found to distantly related with the virulent *M. incognita\_1*. Interestingly, Clade-I of Neighbor Joining Phylogenetic Tree (NJPT) also shared the same result. In other hand, Clade-II constructed by both MEPT and NJPT comprised of different strains of *M.*

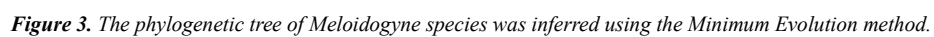
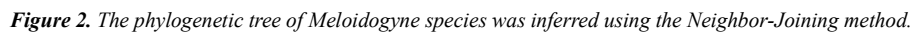
*enterolobii* represented by the accession number KF418370.1, JX024149.1, KT354573.1, KP411227.1, KJ146863.1, KF418369.1, KT354565.1, JQ082448.1, KT354575.1 respectively. Evolutionary tree of Clade-II revealed that these *M. Enterolobii* strains are derived from the same ancestor as of *M. incognita*. Among the four strains (LC030363.1, LC030364.1, LC030367.1, LC030366.1) of *M. incognita* derived from the clade-III; LC030363.1, LC030364.1 were closely related and ancestral to two closely related strains (LC030366.1, LC030367.1). A harmonious genetic relationship was also obtained from the clade-III of NJPT. Clade-IV of NJPT showed that two strains of *M. arenaria* (LC030353.1, LC030356.1) and one strain of *M. incognita* (KP179225.1) were distantly related to each other. In addition, Clade-IV also showed that *Meloidogyne* sp Mh\_c4b (JX465568.1) and *Meloidogyne* sp\_Mi\_c2 b (JX465567.1) are closely related. Clade-IV of

MEPT revealed that *M. arenaria\_1* (LC030353.1) was ancestral to LC030356.1, KP179225.1, JX465568.1, JX465567.1. Clade-V of both MEPT and NJPT comprised with seventeen different *Meloidogyne* spp., were further differentiated into two sub-clades. Sub-clade-1 comprised of eleven distantly related *Meloidogyne* spp. (JX465564.1, KC342236.1, JX465565.1, JX465566.1, JX465576.1, JX465574.1, JX465578.1, JX465570.1, JX465569.1, KP179223.1 and KP179229.1), and Sub-clade-2 comprised of six closely related *Meloidogyne* spp. (JX885741.1, LC030354.1, LC030352.1, JX465561.1, JX465562.1 and KF482363.1). Interestingly, both the MEPT and MJPT analysis revealed that most virulent strains of the world *M. javanica* (KC953091.1, U96305.1), *M. arenaria* (U96301.1, LC030350.1, AY438554.1, AF387092.1, LC030351.1, LC030355.1) and *M. incognita* (JQ405212.1, KJ641591.1, AY438556.1, KC464469.1,



**Figure 1.** Alignment result showing conserved region that are identical in both highly virulent (*M. arenaria* and *M. incognita*) nematodes and some other nematodes species (*M. enterolobii\_1*, *M. sp\_Mi\_c3a*, *M. sp\_Mj\_c1a*, *M. sp\_Mj\_c3a*).

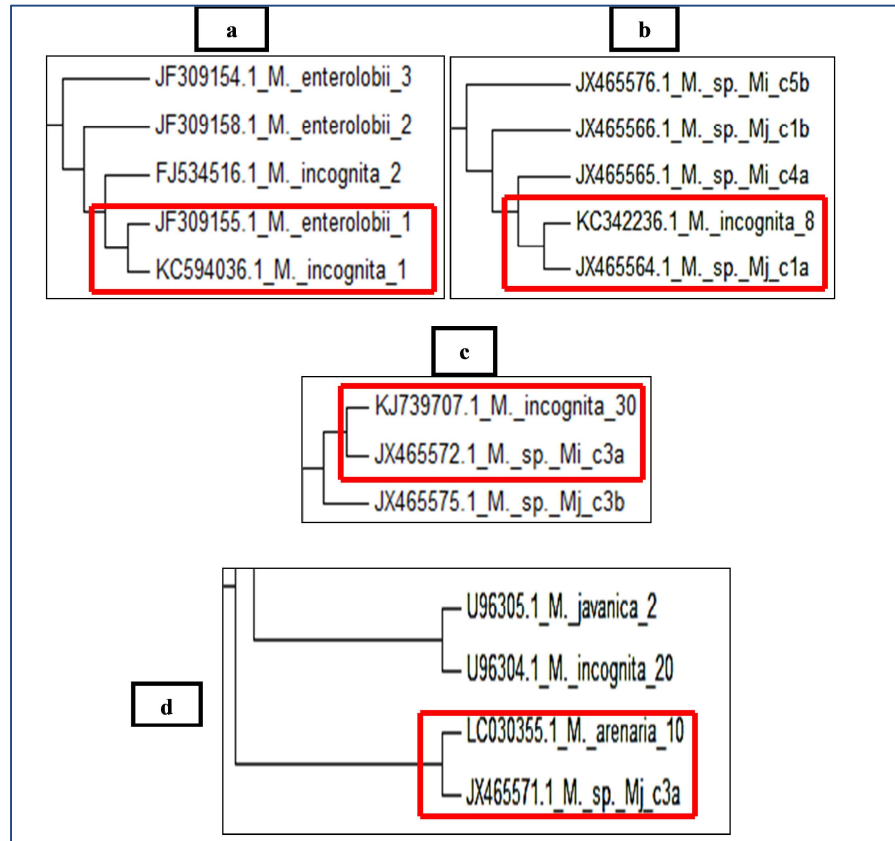




KP751205.1, KJ913700.1, KP233824.1, KP233823.1, AF516723.1, U96304.1) were clustered in Clade-VI.

Previous study supported the close relationships between the three major ameiotic species; *M. arenaria*, *M. javanica* and *M. incognita*. Major clade-VII differentiated into two sub-clades in both the case of MEPT and NJPT. Sub-clade-1 represented the seven closely related strains of *M. javanica* (KJ739709.1) and *M. incognita* (KR265162.1, KP751204.1,

KP751203.1, KP179224.1, KP179226.1, KJ451617.1) and sub-clade-2 stand for eleven different nematodes of *M. Javanica* (AY438555.1, KJ739710.1, AF387094.1, AY823974.1, AY823975.1) and *M. Incognita* (KJ739708.1, KJ451618.1, KR265163.1, KJ739707.1) except (JX465575.1, JX465572.1) [8].



**Figure 4.** *M. enterolobii\_1*, *M. sp\_Mj\_c1a*, *M. sp\_Mi\_c3a* were sharing the same clade with different strains of *M. incognita* (tagged by a, b, c respectively) and *M. sp\_Mj\_c3a* shared the same clade with *M. arenaria* (tagged by d) in both Neighbor-Joining method and Evolution method.

Phylogenetic tree showed that *M. hispanica* is distantly related with *M. incognita*, *M. arenaria*, *M. Enterolobii* and some other root-knot nematode species [34]. All parasitic nematodes were originally evolved from free living nematodes [35-38]. The adoption of parasitism in nematodes probably required either the adaptation of genes present in their free-living ancestors or horizontal gene transfer from bacteria and/or fungus in their environment [39-41]. Thus, there is a good chance that the existing non-virulent species of *Meloidogyne* can become virulent due to single or integrated aforementioned factors. Therefore, evolutionally emerging strains of existing virulent species *M. javanica*, *M. arenaria* and *M. incognita* along with the potential nematodes *M. enterolobii\_1*, *M. sp\_Mi\_c3a*, *M. sp\_Mj\_c1a* that shared the same clade with *M. incognita* and *M. sp\_Mj\_c3a* are evolutionally closely related with *M. arenaria* (Figure 4) could become a great challenge for world agriculture.

## 4. Conclusion

Nematodes, the most widespread organisms on Earth are

capable of colonizing any ecosystem, including extreme environments, such as deserts, hot spring waters, arctic lands and polar seas and are also making impact in agriculture. The unseen enemy of world agriculture rapidly becomes more virulent and in a little while can be responsible for world food crisis. Immediately measures should be taken against the newly emerging strains and potential nematodes species. The study presenting some of such potential nematodes (*M. enterolobii\_1*, *M. sp\_Mi\_c3a*, *M. sp\_Mj\_c1a*, *M. sp\_Mj\_c3a*) based on the genetic as well as evolutionary relationship with existing well recognized virulent nematodes *M. javanica*, *M. arenaria* and *M. incognita*. Further study is needed to more clearly understand the molecular relationship among them that will be beneficial to take more develop control measures.

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