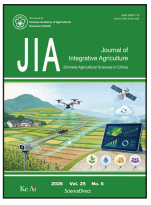
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RESEARCH ARTICLE

The occurrence and genetic diversity of vegetable root-knot nematodes in Xinjiang Uygur Autonomous Region

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Highlights

- The investigation was carried out to explore the occurrence, distribution, and genetic variation of root-knot nematodes (RKNs) infecting vegetables in Xinjiang, China.
- The infected vegetable roots were found to harbor *Meloidogyne incognita* and *M. hapla*, with *M. incognita* being the most prevalent species.
- Genetic diversity analysis indicated that the gene flow of *M. incognita* in Xinjiang originates primarily from nematode populations in southeastern provinces of China.

Abstract

Root-knot nematodes (RKNs) are the most economically damaging plant-parasitic nematodes globally. Xinjiang, encompassing one-sixth of China's landmass, currently lacks comprehensive data regarding the occurrence, distribution, and genetic variation of RKNs infecting vegetables within its borders. Hence, identifying RKNs species and genetic diversity is crucial for devising comprehensive management strategies. Between 2021 and 2023, we present a survey of 130 samples, collected from 86 counties across 14 cities in Xinjiang, China, aiming to comprehensively understand the occurrence, distribution, damage, and species of vegetable RKNs. The results indicated that 57 out of 130 samples collected from the regions of Hami, Tulufan, Ili, Bayingol, Hotan, Aksu, Kashgar, and Kizilsu in Xinjiang were infected by RKNs, suggesting an expansion of RKN disease in the vegetable-producing regions of Xinjiang. The infected vegetable roots were found to harbor *Meloidogyne incognita* and *M. hapla*, with *M. incognita* being the most prevalent species. A phylogenetic analysis of the COI region revealed significant evolutionary divergence between *M. incognita* populations from Xinjiang and those from southeastern provinces. Haplotype analysis of the *COI* gene revealed that *M. incognita* populations are categorized into three major lineages: Asia, Europe, and a combined lineage encompassing both America and Africa. Notable gene flow patterns were observed among *M. incognita* populations, with significant migrations from Europe and America to Asia, specifically from the southeastern China towards Xinjiang. This study's findings indicate a consistent increase in the detrimental effects of vegetables production caused by RKNs in Xinjiang. Implementing effective prevention and control measures is crucial to mitigate the spread of RKNs.

Keywords: root-knot nematode, vegetables, occurrence, genetic diversity, Xinjiang

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1. Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are economically the most damaging plant-parasitic nematodes, causing global economic loss estimated to more than US$170 billion annually (Abad *et al*. 2008). RKNs caused yield loss of 30% and economic losses of US$500 million on vegetable in China (Jin *et al*. 2022). It can parasitize a wide range of plants including grain crops, vegetables, ornamental plants and weeds, especially in the subtropical and tropical regions of world (Brito *et al*. 2018; Desaeger and Watson 2019; Rusinque *et al*. 2022). High nematode densities in soil can induce yellowing in crops, stunt growth, and decrease bioactive constituents in leaves and roots subsequently causing significant yield losses (Naz *et al*. 2021). Additionally, RKN has the ability to establish disease complexes with other soil-borne pathogens, such as *Fusarium* and *Pythium*, thereby limiting plant growth and increasing yield losses (Nnamdi *et al*. 2022; Long *et al*. 2023).

The genus *Meloidogyne* includes over 100 distinct species. Notably, *M*. *incognita*, *M*. *arenaria*, *M*. *hapla*, and *M*. *javanica* have generally been identified as the most abundant and harmful root-knot nematodes (Elling 2013; Jones 2013; Ghaderi and Karssen 2020). In China, *M*. *incognita* is the dominant species among *M*. *incognita*, *M*. *arenaria*, *M*. *hapla*, *M*. *javanica*, and *M*. *enterolobii* that have been found to infect vegetable crops in various provinces (Jin *et al*. 2022). The first recorded instance of RKN infection in chili peppers in Xinjiang was in 1991, which was attributed to *M*. *hapla* (Quan and Guo 1997). Further studies have identified additional cases in Hami City, China (Du *et al*. 2009) and greenhouses in Aksu, Tulufan, Ili, Bayingol, Hotan, and Kizilsu (Li *et al*. 2015). Despite the increasing severity of RKN infestations in Xinjiang's facility agriculture areas, recent patterns of occurrence, damage, and species remain unclear. Therefore, conducting a comprehensive survey on the incidence and distribution of vegetable root-knot nematodes in Xinjiang, which encompasses one-sixth of China's landmass, is crucial.

Accurate identification of RKN species is becoming increasingly critical for formulating effective management practices and control strategies (El-Sagheer 2021). Traditionally, RKN identification relied on morphological, morphometric, isozyme phenotyping, and differential host detection methods (Kiewnick *et al*. 2013; Braun-Kiewnick *et al*. 2016). However, these approaches often run into difficulties and can be somewhat subjective due to interspecific and intraspecific variations, environmental conditions, and host-related differences (Tigano *et al*. 2010; Baidoo *et al*. 2017). These methods also have limitations when it comes to identifying new RKN species (Marquez and Hajihassani 2023). Molecular identification methods are gaining prominence due to their rapidity, sensitivity, and effectiveness at any life stage (Ye *et al*. 2019). Therefore, combining molecular biology with morphology could enhance the accuracy of nematode identification.

To date, there has been growing interest in examining the genetic diversity and dispersal patterns of *Meloidogyne* nematode populations. Shao *et al*. (2020) revealed genetic diversity among *M*. *enterolobii* infecting mulberry trees, utilizing mitochondrial DNA-cytochrome oxidase subunit (*COI*) gene analysis. Liu *et al*. (2023) characterized the population structure of *M*. *graminicola* and reported its northward dispersal in China, particularly from Guangdong and Hainan provinces. Nevertheless, limited research exists on the population structure and genetic diversity of RKN infecting vegetables in China. Hence, gaining insight into genetic disparities among RKN infecting vegetables could offer valuable insights for future investigations into the genetic diversity of plant-parasitic nematodes.

Although Xinjiang accounts for one-sixth of China's landmass, there is a paucity of information regarding the distribution, incidence, and genetic diversity of root-knot nematode species that infect vegetables in this region. Furthermore, previous studies on *Meloidogyne* species in Xinjiang necessitate updating and clarification. Between 2021 and 2023, we conducted an investigation into the prevalence of RKNs infecting vegetables in Xinjiang. The objective of this study was to determine the occurrence and species of vegetable RKNs. Furthermore, we aim to gain insight into the genetic diversity both within and among RKN populations in China. Our findings will play a crucial role in the implementation of future effective and integrated nematode management.

2. Materials and methods

2.1. Sample collection

A survey of the primary vegetable cultivation fields was undertaken in Xinjiang, spanning the period from 2021 to 2023. A total of 130 root samples were investigated from 86 counties in 14 cities (Appendices A and B), including 12 species of vegetables and weeds. Roots exhibiting gall symptoms were excised, packaged in plastic bags along with soil samples, transported to the laboratory and stored at 4°C. Nematode damage was assessed by rating root galling on the 0–5 scale of Taylor and Sasser (1978).

2.2. Morphological identification

The diseased roots displaying characteristic symptoms of root-knot nematode infestation were washed, and subsequently, 20 females originating from different collection areas were delicately selected using a microscope (SZX16, Olympus, Japan). The slides of perineal patterns were performed in accordance with the Hartman and Sasser's (1985) description. Moreover, male nematodes and second-stage juveniles (J2s) were thermally killed in 4% formalin, then infiltrated with glycerin, and finally mounted on permanent microscope slides, following the methods described by Golden (1990) and Hooper (1986). Morphological characterization, measurements, and photography were conducted using a microscope (BX53, Olympus, Japan) equipped with a DP80 digital camera. The morphological identification of the species was conducted in accordance with the guidelines described by Eisenback *et al*. (1985).

2.3. Molecular identification

Genomic DNA extraction from single female was followed the

protocol of Peng *et al*. (2012). Universal primers rDNA1 and rDNA2 (Appendix C) were used to amplify the rDNA-internal transcribed spacer (rDNA-ITS) region (Vrain *et al*. 1992), and the primers JB3 and JB5 (Appendix C) were employed to amplify the *COI* gene (Derycke *et al*. 2010). Species-specific detection was carried out using the primers MI-F and MI-R (Appendix C) specific to *M*. *incognita* (Meng *et al*. 2004), and MH-F and MH-R (Appendix C) specific to *M*. *hapla* (Feng *et al*. 2008). The amplification process occurred in a final volume of 50 μL, comprising 2 μL template DNA, 1 μL each of 10 μmol L^{-1} forward and reverse primers, 25 μL 2× Taq PCR Master Mix (Vazyme, Beijing, China), and 21 μL ddH_2O. PCR amplification was conducted using following program: 95°C for 3 min, followed by 35 cycles of 95°C for 15 s, 56°C for 15 s and 72°C for 15 s and a final extension of 72°C for 5 min. The PCR products were separated on a 1.5% agarose gel and visualized and photographed under UV light. The PCR amplicons were purified and subjected to sequencing services provided by Beijing Liuhe BGI Co., Ltd., China. The sequenced sequences were subjected to BLAST analysis on the NCBI website.

2.4. Phylogenetic analysis

A total of 151 ITS-rDNA and 388 *COI* sequences were downloaded from GenBank for 7 major RKN species including *M*. *incognita*, *M*. *javanica* populations, *M*. *arenaria*, *M*. *hispanica*, *M*. *enterolobii*, *M*. graminicola and *M*. *hapla* (Appendix D). These were combined with the obtained sequences from the 57 Xinjiang RKN populations to construct phylogenetic relationships. Furthermore, to comprehend the genetic disparities among *M*. *incognita* populations in Xinjiang and those throughout China, a phylogenetic analysis was performed comparing the ITS-rDNA and *COI* sequences of 52 *M*. *incognita* populations in Xinjiang from this study with 77 ITS-rDNA and 9 *COI* sequences available on GenBank from various Chinese provinces, respectively. To elucidate the genetic affinity between Chinese and foreign populations of *M*. *incognita*, a phylogenetic tree was constructed utilizing 51 published *M*. *incognita COI* sequences and integrated with an additional 52 sequences obtained in this study. Extract the sequences using PhyloSuite v1.2.2 (Zhang *et al*. 2020), and then align the sequence in batches using MAFFT v7.313 (Katoh and Standley 2013). The optimized data was used by Model Finder (Kalyaanamoorthy *et al*. 2017) to optimize model selection. Use the GTR model to perform MrBayes inference on ITS-rDNA and *COI* sequences in the MrBayes plugin of PhyloSuite. MrBayes with default settings and 5×10^6 coupled MCMC generations run. The phylogenetic tree and posterior probabilities were visualized using FigTree v1.4.2.

2.5. Genetic polymorphism analysis

Compare the sequencing results with the SeqMan program of Cluster X 1.83 and DNAStar 6.0. Following the methodology described by Librado and Rozas (2009), the DnaSP software was utilized to compute various genetic parameters including the percentage of variation in the COI sequences, nucleotide diversity (π), haplotype diversity (Hd), nucleotide disproportionality (Dxy), fixed index (*F*st), standard genetic distance (Gst), and average nucleotide variation (*K*). Using the mitochondrial specific gene formula, *F*st=1/(1+2Nm), the gene flow (Nm) between populations was determined according to Takahata and Palumbi (1985). Tajima D and Fs neutral tests were conducted on DNA fragments using DnaSP 6.0 (Tajima 1989), analyzing both population-level and individual-level genetic diversity.

2.6. Analysis of haplotype and genetic structure

The base content and polymorphic sites were analyzed using MEGA 7.0 (Librado and Rozas 2009). Variation sites between sequences are calculated using the Kimura-2-Parameter (K2P) model. The haplotype phylogenetic analysis employed the identical methodology outlined previously. The haplotype sequences of the *COI* gene were arranged to assess phylogenetic network relationships *via* POPART. The statistical analysis method described by Bandelt and Forster (1999) utilizes the Bayesian clustering program Structure v2.3.4 (Pritchard *et al*. 2000) to deduce population structure. Five independent analyses were performed, ranging from *K*=1 to *K*=10, with each iteration utilizing 5 million MCMC samples. Using the *K*-value optimal approach and the maximum posterior probability method (Pritchard *et al*. 2000), CLUMPAK (Kopelman *et al*. 2015) was utilized to determine the optimal population size (*K*-value) for further calculations.

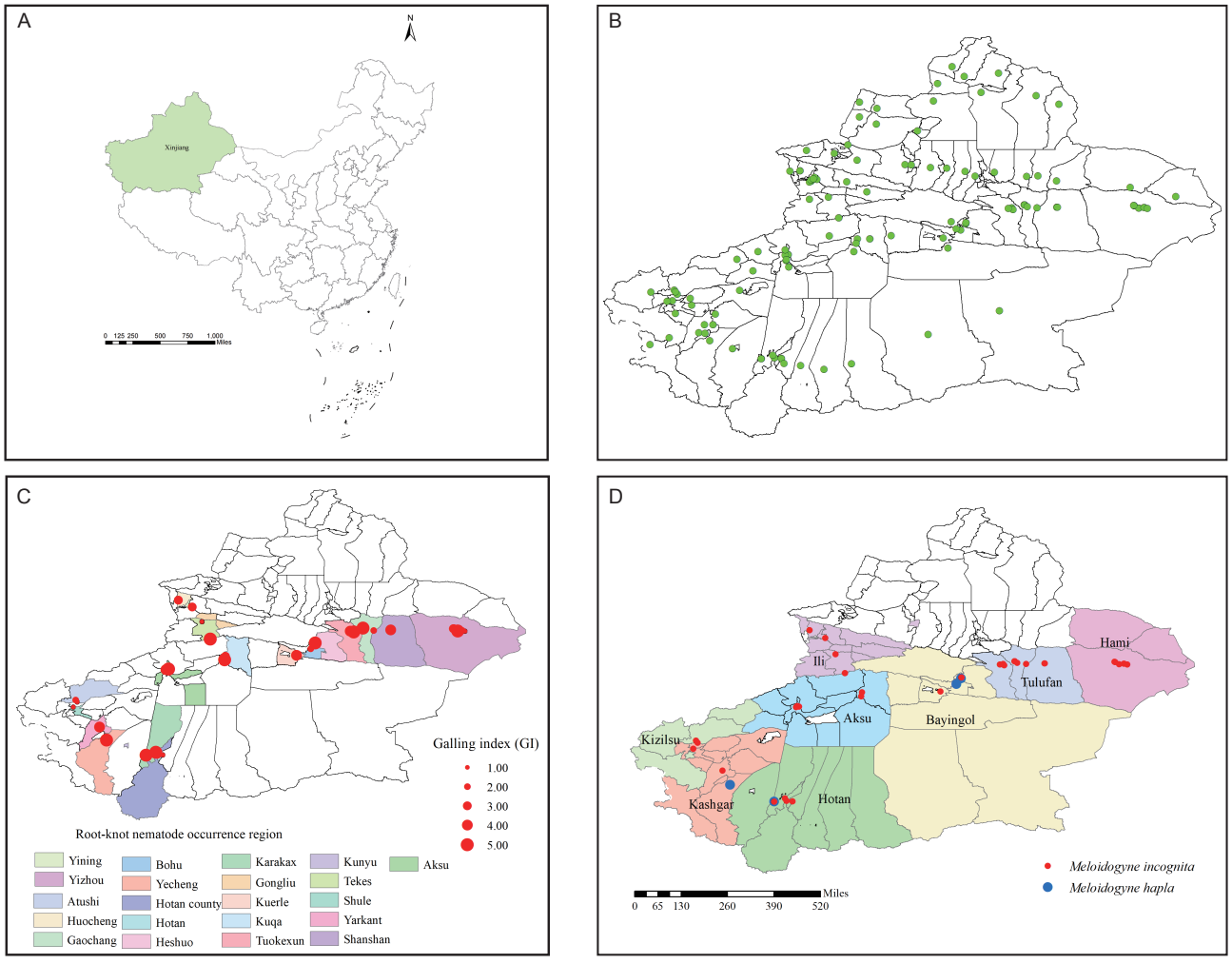
2.7. Gene flow analysis

To evaluate the historical migration patterns of *M*. *incognita* populations, Bayesian search was used based on MIGRATE-N v3.2.1 (Beerli 2006). The complete migration model is achieved through 1×10^5 long chains of record lineages were used, with a sampling increment of 100 times. The running time of each locus is set to 1×10^4 (Shao *et al*. 2022).

3. Results

3.1. Occurrence and damage of vegetable RKNs disease in Xinjiang

The survey results indicated that RKNs disease on vegetable were observed in 21 counties across various cities in Xinjiang, including Hami, Tulufan, Ili, Bayingol, Hotan, Aksu, Kashgar, and Kizilsu (Appendix A; Fig. 1-A–C). In total, 57 out of 130 samples were found to be infested with RKNs, showing an overall disease frequency of 43.85%. In Tulufan, RKN disease poses the most common threat, with a frequency of 100%, followed by Hami and Hotan, which exhibit frequencies of 84.62 and 66.7%, respectively. The severity of RKNs, as evaluated by the gall index, is high in Akesu and Kashgar, with scores ranging from 3.0 to 5.0, followed by Bayingol which ranged from 2.0 to 5.0. The incidence and severity of RKN in 14 geographical areas are presented in Appendix B. The devastating impact of RKNs on various vegetables such as kidney bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), cowpea (*Vigna unguiculata* (L.) Walp), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cabbage (*Brassica rapa* var. glabra Regel.), radish (*Raphanus sativus* L.), squash



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Fig. 1 Occurrence and distribution of root-knot nematodes on vegetables fields in Xinjiang, China. A, the location of Xinjiang where root-knot nematode disease was investigated. B, distribution of all collected samples. C, distribution of root-knot nematode. D, distribution of *Meloidogyne incognita* and *M. hapla*.

(*Cucurbita pepo* L.), balsam pear (*Momordica charantia* L.), purslane (*Portulaca oleracea* L.), towel gourd (*Luffa aegyptiaca*) and celery (*Apium graveolens* L.) (Appendix E). Typically, infected plants exhibit stunted growth and apparent nutrient deficiency, ultimately leading to wilting and death during the later stages of the growing season.

3.2. RKN species identification

We identified two species of RKNs, *M. incognita* and *M. hapla* from 57 samples by analyzing the morphological characteristics of eggs, adult females, males, and juveniles (Fig. 2; Appendix F). The morphology of the perineal pattern of adult females is the most frequently used characteristic for *Meloidogyne* species identification. The typical perineal pattern of *M. incognita* is oval to rounded, with a prominent, squared dorsal arch, wavy striae, and the absence of a lateral field. The perineal pattern of *M. hapla* is rounded, with a low dorsal arch, characteristic punctations near the anus, fine striae, and the presence of a lateral field. The detailed morphometric data is listed in Appendix F.

Molecular analysis was realized by applying the species-specific primer sets in the DNA of individual females, which generated bands of 955 and 462 bp specific for *M. incognita* and *M. hapla*, respectively (Appendix G). Furthermore, based on phylogenetic analysis of the ITS-rDNA, 5 out of 57 sequences clustered with published *M. hapla* sequences, while the remaining 52 sequences clustered with *M. incognita and M. arenaria* (Fig. 3). Those result showed the presence of two species of root-knot nematodes, *M. hapla* and *M. incognita*, in the 57 samples. In conclusion, *M. incognita* was the dominant species in vegetable fields, accounting for 91.23%, followed by *M. hapla* with 8.77%. Furthermore, *M. incognita* is the most widespread species infecting varieties vegetables in facility greenhouses of Xinjiang, whereas the northern root-knot nematode only occurs in Bayingol, Hotan, and Kashgar regions (Fig. 1-D).

3.3. Phylogenetic analysis of *M. incognita*

Phylogenetic trees were constructed separately using the sequences of the ITS-rDNA and *COI* regions, encompassing both Xinjiang and other Chinese *M. incognita* populations. The results revealed clustering of Xinjiang populations

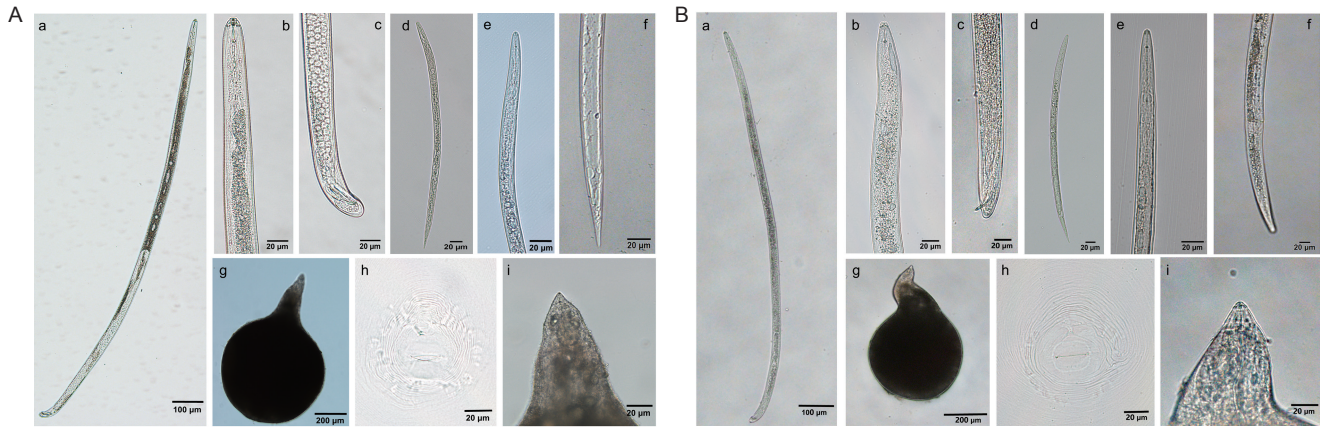


Fig. 2 Morphological characteristics of second instar larvae, males, and females of *Meloidogyne incognita* (A) and *M. hapla* (B) in Xinjiang, China. a, whole male body. b, front part of male body. c, tail part of male body. d, whole second instar larvae. e, front part of second instar larvae. f, rear part of second instar larvae. g, whole female body. h, perineal pattern of female body. i, head of female body.

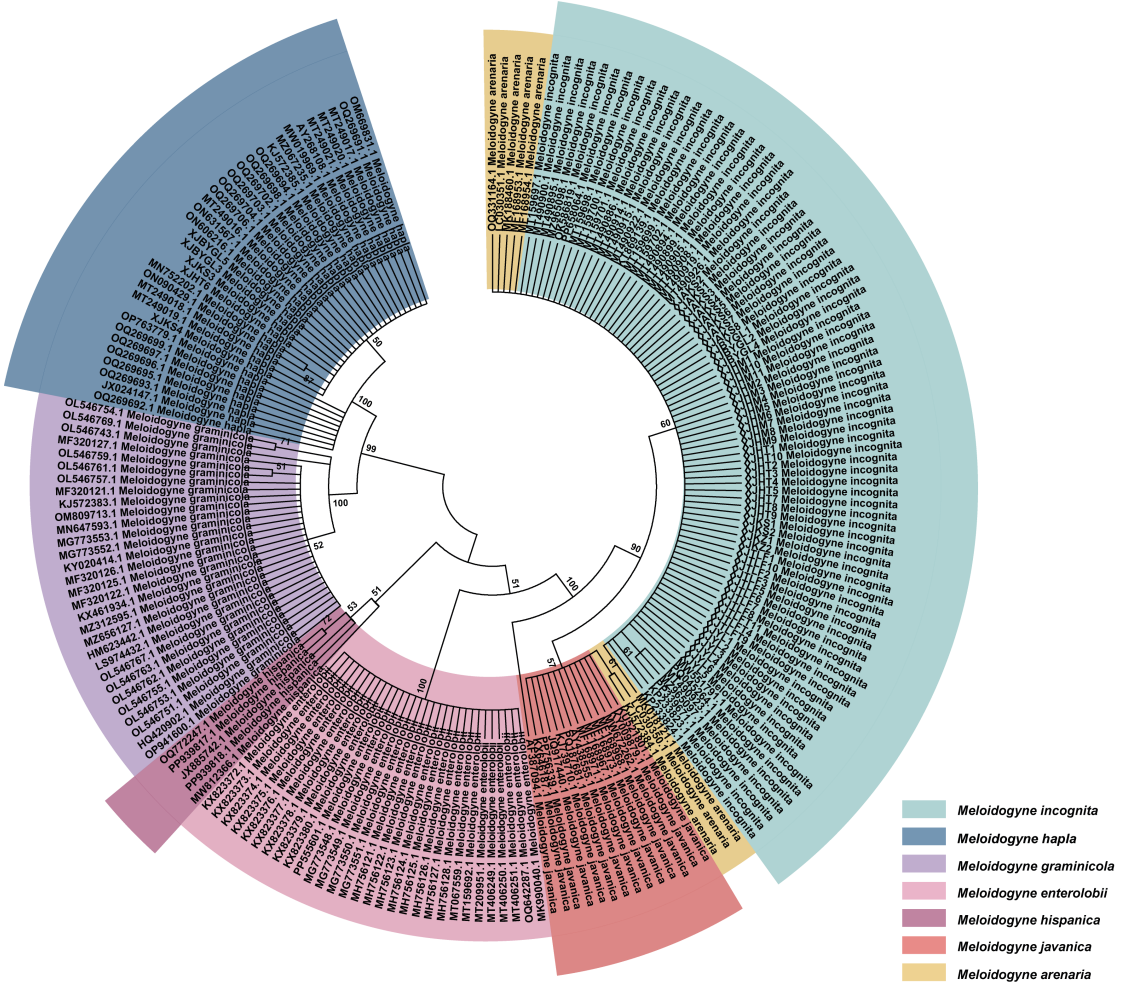


Fig. 3 Phylogenetic relationships of *Meloidogyne* spp. based on the ITS-rDNA sequences by using the maxinum likelihood model.

those from other regions of China, as shown in Fig. 4. This observation hints at the possibility that *M. incognita* individuals from Xinjiang population exhibit stronger phylogenetic affinities with other Chinese populations.

By integrating the phylogenetic tree of *COI* gene (Fig. 5-A) with the haplotype network (Fig. 5-B), we were able to categorize all haplotypes into three distinct groups: Asia, Europe & America, and Africa. These groupings align with the phylogenetic tree representing *M. incognita* populations worldwide. The haplotypes within the Asia group encompass (Hap1, Hap2, Hap4, Hap5, Hap6, Hap7, Hap8, Hap9, Hap10) and represent *M. incognita* populations from China, Philippines,

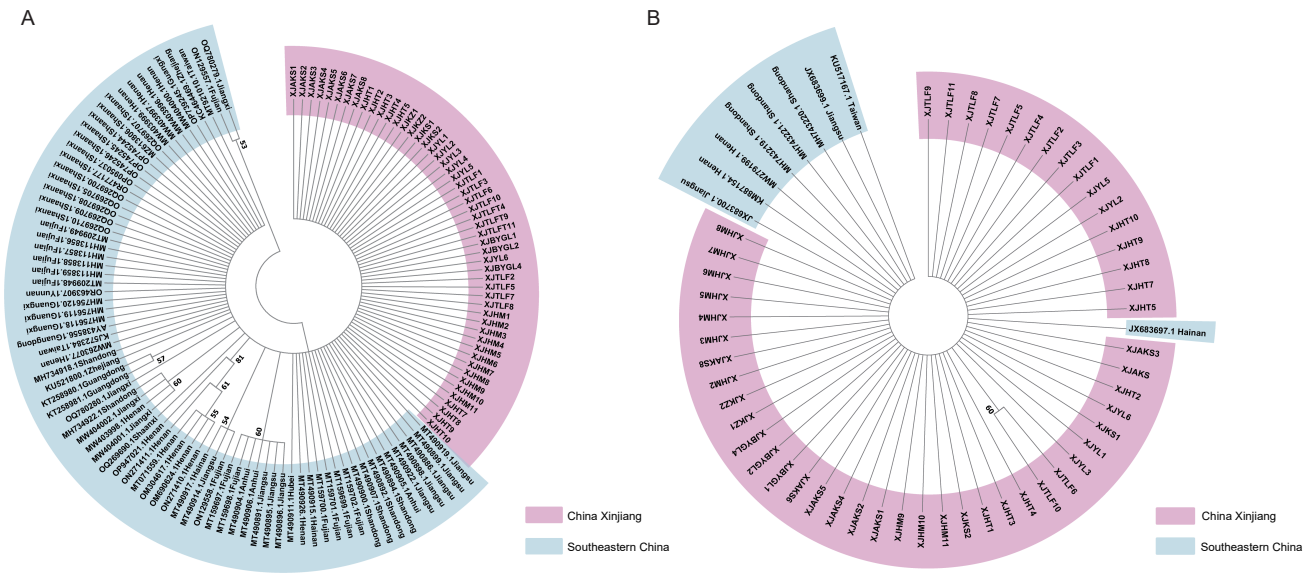


Fig. 4 Phylogenetic analysis of *Meloidogyne incognita* Chinese populations based on ITS-rDNA (A) and COI (B) sequences.

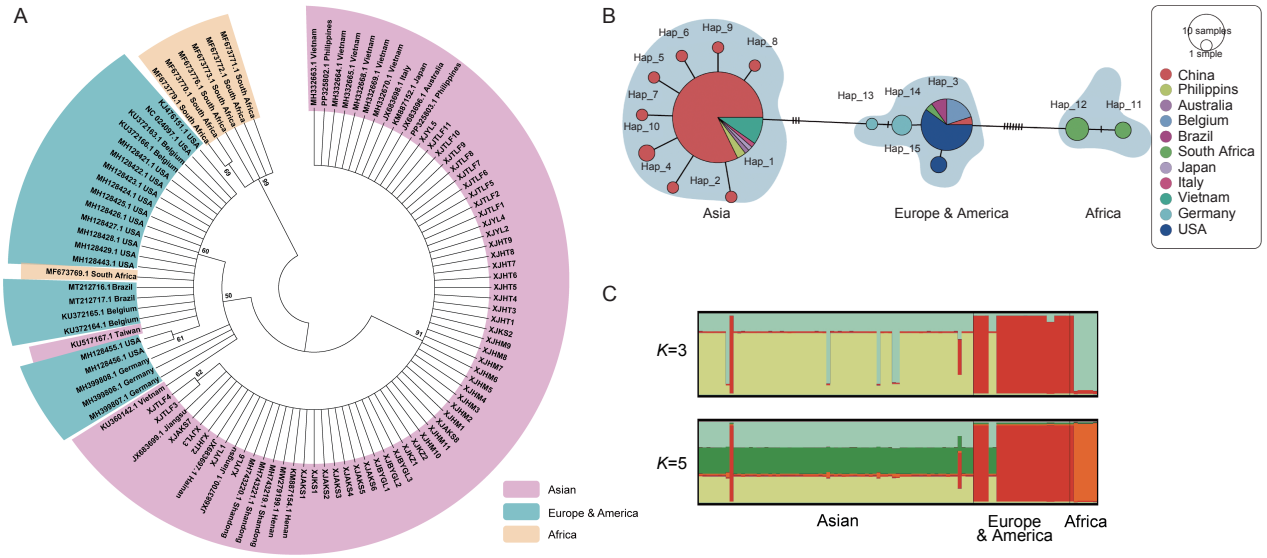


Fig. 5 Phylogenetic analysis of the mitochondrial DNA (mtDNA) haplotypes of *Meloidogyne incognita* worldwide. A, phylogenetic tree of the mtDNA haplotypes of *M. incognita*. The scale indicates the branch length. B, network of mtDNA haplotypes. Each numbered circle (Hap1 to Hap13) represents a unique haplotype. and the size of the circle is proportional to the overall frequency of the haplotype in the entire sample of the species. Each line connecting the haplotype represents a mutational step. Marks on the lines indicate the number of steps. Colors correspond to different sampling locations, and names are given as abbreviations. C, population structure inferred from STRUCTURE runs with the best probability values measured by Δ*K* (*K*=3) and the maximum posterior probability method (*K*=5). Sampling regions are separated by black lines. Distinct clusters are color coded. Clusters with similar colors are more closely related. Within each cluster, an individual sequenced nematode corresponds to a vertical segment that reflects its ancestry. Mixed ancestry is indicated by different colored sectors corresponding to inferred genetic percentages of the corresponding clusters.

Vietnam, Japan, Italy, and Australia (Fig. 5-B). Notably, Hap1 is a haplotype that is shared among 6 countries and is particularly prevalent in Asia. Haplotypes (Hap3, Hap13, Hap14, Hap15) representing *M. incognita* populations from the USA, South Africa, Brazil, Belgium, and Germany were grouped together within the Europe & America category. The haplotypes belonging to the Africa group were exclusively derived from South African *M. incognita* populations, as detailed in Appendix H. The inferred population structure for both *K*=3 and *K*=5 is presented in Fig. 5-C. Regardless of the *K*-value, the results consistently indicate that the haplotypes of *M. incognita* from China's Xinjiang region are genetically proximal to Asia.

3.4. Analysis of nucleotide and haplotype diversity

The diversity of haplotype and nucleotide in 103 *M. incognita* populations were analyzed based on the *COI* genes (Table 1).

The results showed that the haplotype diversity of the total *M. incognita* populations was 0.602, suggesting that the total population has abundant haplotypes. The nucleotide diversity was 0.00643 and the mean nucleotide difference number *K* was 2.864, indicating that the total population was also rich in nucleotides. The Tajima's D (–0.94183), and Fu's Fs values (–2.252) of the total population were less than 0. Among the three clusters of *COI* gene sequences, the highest number of variant loci (10) were detected in the Asia group. The lowest number of variant loci (1) were in the Africa group.

The haplotype diversity (Hd) in the three groups ranged from 0.239–0.533, with the lowest haplotype diversity being 0.239 in the Asia group and the highest haplotype diversity being 0.533 in the Africa group. The range of π values in all groups was between 0.00069–0.01286; the Asia group had the smallest π value, and the Europe & America group had the largest π value. The average number of nucleotide differences *K* varies between 0.309–0.533 among three populations, with the highest being in the Africa group. The neutrality test was performed for three groups of *M. incognita*, in which Tajima's D and Fu's Fs were less than 0 for Asia group, and the test results were not significant.

Table 1 Genetic diversity and neutrality test of *COI* gene among *Meloidogyne incognita* groups

Group	Number of mutations	Haplotype diversity (Hd)	Nucleotide diversity (π)	Tajima's D	Fu's Fs
Asia	10	0.239	0.00069	–2.29100	–10.379
Europe & America	3	0.403	0.01286	–0.90766	–1.350
Africa	1	0.533	0.00120	0.85057	0.625
Overall	22	0.602	0.00643	–0.94183	–2.252

Table 2 *Fst* and Nm among populations of *Meloidogyne incognita* groups based on *COI* gene

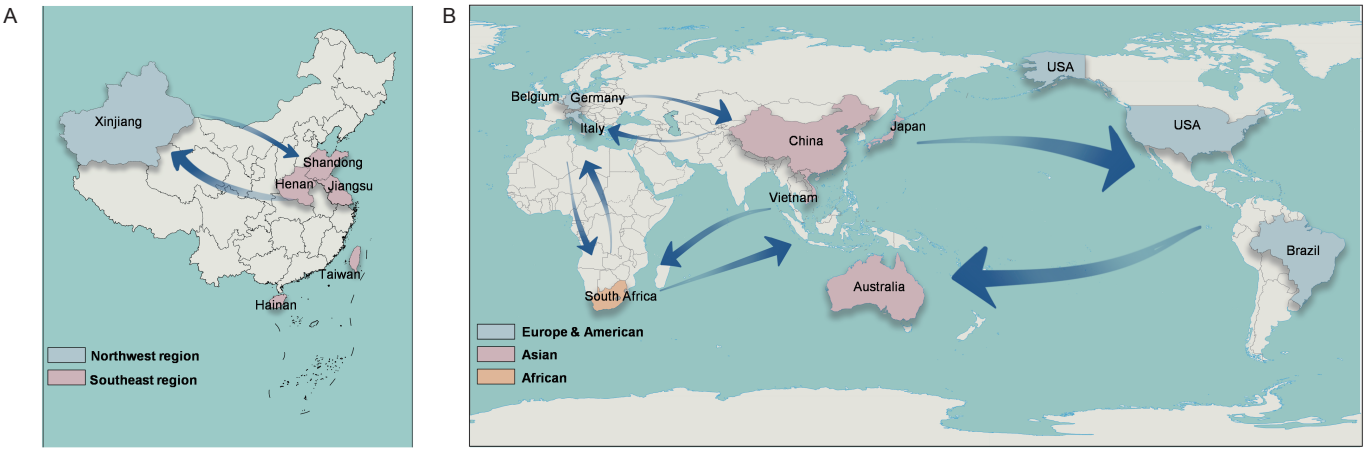
Cluster	Aisa	Europe & America	Africa
Aisa		0.533	0.207
Europe & America	1.273		0.342
Africa	0.159	0.314	

3.5. Gene flow among regions and populations

From a global perspective, the Nm values for the European and American *M. incognita* populations, as well as the Asian population, were 1.273. These Nm values, which ranged from 1 to 4, suggest some genetic exchange among these populations, although it was not significant (Table 2). The Nm values between the Asian population and those from Europe and America, as well as Africa, were 0.533 and 0.207, respectively (both ≤1), indicating that gene exchange between Asia and other regions was limited, potentially due to divergence from genetic drift populations. Notably, the Nm value between the southeastern China population and Xinjiang population in China was 4.06 (>4), which indicates that gene exchange between these two clusters was abundant. Conversely, there was no significant gene exchange between the two Chinese clusters. The gene flow values among Asia, Europe, and Africa populations indicate that the main gene flow patterns of *M. incognita* populations in China involve gene flow from Europe and America to Asia and Africa, as well as within China, particularly between the Southeast regions and Xinjiang (Fig. 6).

4. Discussion

RKNs disease poses a significant challenge to vegetable production worldwide. Presently, root-knot nematode infestations on various crops have been reported in all provinces of China (Jin *et al*. 2022). Xinjiang, with its vast border area, comprises one-sixth of the total landmass of China. Regrettably, the impact of RKNs infestations has escalated in severity in Xinjiang over recent years (Quan and Guo 1997; Du *et al*. 2009; Li *et al*. 2015). Currently, there lacks a comprehensive survey focusing on the distribution and species identification of RKNs on vegetables in Xinjiang. Herein, we present a survey of 130 samples, collected from 86 counties across 14 cities in Xinjiang, aiming to



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Fig. 6 The migration of *Meloidogyne incognita* estimated with MIGRATE-N. A, gene flow within the Chinese populations. B, gene flow among populations across the globe. The arrows represent the direction of migration, and their thickness is proportional to the number of migrants.

comprehensively understand the occurrence, distribution, damage, and species of vegetable root-knot nematodes in the region. The results showed that 57 samples collected from the regions of Hami, Tulufan, Ili, Bayingol, Hotan, Aksu, Kashgar, and Kizilsu in Xinjiang were infected by RKN. Li *et al*. (2015) reported the identification of RKNs in 7 counties of Xinjiang. However, we found that the distribution of RKNs disease in Xinjiang has expanded to 21 counties. This indicates that the RKNs disease is expanding in vegetable-producing regions of Xinjiang. The results of the frequency and severity showed that the severity of root-knot nematode damage is greater in regions like Hami, Hotan, and Tulufan, presumably due to their sandy soil composition and warmer climatic conditions. Studies indicate that RKNs cause greater damage in sandy soil compared to loam soil, with warmer temperatures accelerating their development (Kim *et al*. 2017). In conclusion, our survey findings reveal an abundance of root-knot nematode hosts in Xinjiang. Furthermore, a growing number of greenhouses offer suitable habitats for RKNs during winter. Ultimately, this contributes to escalating damage to vegetable RKNs in Xinjiang.

Until today, over 100 species of root-knot nematodes have been reported, with China reporting the presence of 56 species (Jin *et al*. 2022). Notably, the most common species include *M*. *incognita*, *M*. *hapla M*. *arenaria* and *M*. *arenaria* and *M*. *javanica* (Khan *et al*. 2023). Additionally, over 90% of crop losses attributed to root-knot nematodes can be traced back to the aforementioned four species (Azlay *et al*. 2023). Our identification results demonstrated the presence of *M*. *hapla* at five sites in Bazhou, Hotan, and Kashgar, while the remaining 52 samples belonged to *M*. *incognita*. The findings suggest that the prevalence of *M*. *incognita* and the sparse occurrence of *M*. *hapla* among vegetables in Xinjiang concur with earlier studies (Quan and Guo 1997; Du *et al*. 2009). The *M*. *hapla* was found on chili root in greenhouse in 1991, which is the first recorded of root-knot nematode in Xinjiang, subsequently, *M*. *incognita* was identified on cucumber in Hami City (Du *et al*. 2009). Li *et al*. (2015) survey results showed that 14 samples from different regions of Xinjiang contain both *M*. *incognita* and *M*. *hapla*, *with M*. *incognita* being the dominant population. But unlike our results, 9 of their samples were a mixed population containing two species of *M*. *incognita* and *M*. *hapla*. And our result is that *M*. *incognita* mainly harm greenhouse crops, while *M*. *hapla* mainly harm terrestrial crops, mixed infection by both species was not presented in our survey. Despite collecting 130 samples from 14 cities across Xinjiang, our sampling coverage remains insufficient for the extensive vegetable cultivation area of approximately 4 million mu, potentially accounting for the absence of observed mixing events. These findings suggest a rise in both the frequency and severity of vegetable RNK disease in Xinjiang, the primary species involved remain *M*. *incognita* and *M*. *hapla*. Previous reports indicate that *M*. *hapla* is primarily distributed in cooler regions, where the mean temperature in the coldest month is –15°C (Taylor *et al*. 1982), *M*. *hapla* J2s are capable of infesting crops even at temperatures below 0°C (Vrain *et al*. 1978; Forge and Macguidwin 1990), and *M*. *hapla* eggs are capable of surviving sub-zero temperatures and developing at low temperatures of 6.74°C (Vrain 1978; Tsai *et al*. 2010). With a temperate monsoon climate, Xinjiang experiences an average annual temperature of approximately 8.9°C, it is likely that *M*. *hapla* populations can overwinter in soils on land. The incidence of *M*. *hapla*, although only 8.17%, has the potential to grow at rates comparable to *M*. *incognita* if not controlled.

To investigate the phylogenetic relationships among the *M*. *incognita* Xinjiang population and various populations throughout China, we conducted an analysis of the evolutionary trees constructed from ITS-rDNA and *COI* sequences. Our research has demonstrated genetic distinctiveness of *M*. *incognita* in Xinjiang compared to its populations in other provinces (Fig. 4). A comparable genetic pattern was observed among wheat cyst nematode populations in China (Shao *et al*. 2022). The nematodes exhibit a limited movement range, with geographical isolation being the primary factor driving their genetic differentiation (Picard *et al*. 2004). Moreover, the *M*. *incognita* examined in this study originated from various hosts, however, the genetic differentiation observed was not influenced by the diversity of hosts. The findings from gene flow analysis indicate a significant gene flow from the southeastern provinces (Shandong, Jiangsu, Henan, Taiwan and Hainan) populations towards Xinjiang population. One plausible explanation is that RKNs are spread through long-distance transportation of seedlings and plants with soil, facilitating their dispersal. As an illustration, plant parasitic nematodes were discovered on seedlings transported in Jiangsu province (Zhou and Shen 2003). Additionally, the RKN was identified on strawberry seedlings imported into Beijing (Xi *et al*. 2017). Shandong, Hainan, and other southeastern provinces serve as the primary breeding grounds for vegetable seedlings in China. Annually, significant quantities of seedlings are dispatched from these eastern provinces to Xinjiang *via* logistics channels or online shopping. The restricted gene flow from Xinjiang to other provinces can be attributed to the specific agricultural practices in Xinjiang, where the return of root and stem vegetables to the eastern coastal provinces is hindered by the incurred high logistics costs.

Mitochondrial DNA (mtDNA) serves as a prominent marker for assessing molecular diversity. The *COI* gene has been selected as a standardized molecular marker for taxonomy and identification purposes (Ratnasingham and Hebert 2007; Galtier *et al*. 2009). Nevertheless, multiple studies have demonstrated that the *COI* gene cannot differentiate among tropical root-knot nematode clade, including *M*. *incognita*, *M*. *javanica*, *M*. *arenaria* etc. (Kiewnick *et al*. 2014; Janssen *et al*. 2016; Álvarez-Ortega *et al*. 2019). In our study, a total of 388 *COI* sequences representing 7 species of RKN (including 51 *M*. *incognita* populations, 53 *M*. *javanica* populations, 23 *M*. *arenaria* populations, 6 *M*. *hispanica* populations, 82 *M*. *enterolobii* populations, 114 *M*. graminicola populations and 59 *M*. *hapla* populations) were downloaded from GenBank. Additionally, we performed a phylogenetic tree analysis incorporating 57 sequences obtained from Xinjiang. Our findings were congruent with previous results, indicating that *COI* genes serves as an unreliable discriminator among tropical root-knot nematode species, including *M*. *incognita*, *M*. *javanica*, *M*. *arenaria*,

and *M. hispanica* (Appendix I). Subsequently, a total of 51 published *COI* sequences of *M. incognita* from 10 countries and other provinces in China were analyzed for genetic diversity, along with the sequences obtained from Xinjiang. Twenty-two variable sites and 15 haplotypes were identified from 103 *COI* sequences (Table 2; Appendix H). Additionally, our study reveals distinct clustering patterns within the populations of *M. incognita* across Asia, Europe, the Americas, and Africa. The findings suggest that the *COI* gene, while unsuitable as a molecular marker for distinguishing tropical RKN species, holds potential for elucidating intraspecific genetic diversity within *M. incognita* populations.

In the grouping of haplotype results, Europe (Brazil, Gemany, and Belgium) and the United States were found to be particularly closely related in the same group. It is possible that *M. incognita* populations is exchanged frequently in the trade between the United States and Europe. The Europe-American group is located in the center of the Asian and African group. *Meloidogyne incognita* was first reported on cotton in Alabama in the United States (Sasser 1954). It is presumed that *M. incognita* populations originated in the United States and spread to Asia and Africa, respectively. There are also some genetic differences between *M. incognita* populations in Asian countries, such as China, Philippines and Vietnam, and populations in Europe and the Americas. The main reason for this is that quarantine-imported plants are not able to carry soil and therefore spread more slowly across continents.

5. Conclusion

In this study, we performed the inaugural survey on the incidence, dispersion, and damage caused by root-knot nematodes across 86 counties within 14 cities in Xinjiang. The findings revealed the presence of root-knot nematode damage in greenhouses and land crops across 21 counties in 8 cities, indicating that the occurrence of root-knot nematodes in Xinjiang has been rapidly expanding in the past 10 years. Species identification revealed that *M. incognita* dominate the nematode population in Xinjiang, with *M. hapla* causing localized damage. Genetic diversity analysis indicated that the gene flow of *M. incognita* in Xinjiang originates primarily from nematode populations in southeastern provinces. This study's findings indicate a consistent increase in the detrimental effects caused by root-knot nematodes in Xinjiang. Implementing effective prevention and control measures is imperative to mitigate the spread of root-knot nematodes in Xinjiang.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that we did not use AI in the preparation and writing of this manuscript.

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References

Abad P, Gouzy J, Aury J M, Castagnone-Sereno P, Danchin E G J, Deleury E, Perfus-Barbeoch L, Anthouard V, Artiguenave F, Blok V C, Caillaud M C, Coutinho P M, Dasilva C, De Luca F, Deau F, Esquibet M, Flutre T, Goldstone J V, Hamamouch N, Hewezi T, *et al*. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, **26**, 909–915.

Álvarez-Ortega S, Brito J A, Subbotin S A. 2019. Multigene phylogeny of root-knot nematodes and molecular characterization of *Meloidogyne nataliei* Golden, Rose & Bird, 1981 (Nematoda: Tylenchida). *Scientific Reports*, **9**, 11788.

Azlay L, Boukhari M E M, Mayad E H, Barakate M. 2023. Biological management of root-knot nematodes (*Meloidogyne* spp.): A review. *Organic Agriculture*, **13**, 99–117.

Baidoo R, Joseph S, Mengistu T M, Brito J A, McSorley R, Stamps R H, Crow W T. 2017. Mitochondrial haplotype-based identification of root-knot nematodes (spp.) on cut foliage crops in Florida. *Journal of Nematology*, **48**, 193–202.

Bandelt H J, Forster P A R. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Beerli P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, **22**, 341–345.

Braun-Kiewnick A, Viaene N, Folcher L, Ollivier F, Anthoine G, Niere B, Kiewnick S. 2016. Assessment of a new qPCR tool for the detection and identification of the root-knot nematode *Meloidogyne enterolobii* by an international test performance study. *European Journal of Plant Pathology*, **144**, 97–108.

Brito J A, Subbotin S A, Desaeger J, Achinelly F, Qiu S. 2018. First report of the root-knot nematode (*Meloidogyne javanica*) infecting hops (*Humulus lupulus*) in Florida, USA. *Journal of Nematology*, **50**, 1–2.

Derycke S, Vanaverbeke J, Rigaux A, Backeljau T, Moens T. 2010. Exploring the use of cytochrome oxidase c subunit 1 (COI) for DNA barcoding of free-living marine nematodes. *PLoS ONE*, **5**, e13716.

Desaeger J A, Watson T T. 2019. Evaluation of new chemical and biological nematicides for tomato production and associated double-crops in Florida. *Pest Management Science*, **75**, 3363–3370.

Du L Y, Ayxer A B D L, Gao Y X. 2009. Prevention and control technology of cucumber root-knot nematode disease in Hami, Xinjiang. *Jilin Vegetables*, **2**, 43. (in Chinese)

Eisenback J D, Sasser J, Carter C. 1985. Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). *An Advanced Treatise Meloidogyne*, **1**, 95–112.

Elling A A. 2013. Major emerging problems with minor Meloidogyne species. *Phytopathology*, **103**, 1092–1102.

El-Sagheer A. 2021. Molecular identification of *Meloidogyne javanica* occurring in Upper Egypt based polymerase chain reaction (PCR) assays. *Egyptian Journal of Agronematology*, **20**, 120–127.

Feng G, Dong L, Chen Y, Shang H, Liu Y, Li J, Yang P. 2008. PCR detection of nematode isolated from Panax notoginseng. *Southwest China Journal of Agricultural Sciences*, **21**, 100–102. (in Chinese)

Forge T A, Macguidwin A E. 1990. Cold hardening of *Meloidogyne hapla* second-stage Juveniles. *Journal of Nematology*, **22**, 101–105.

Galtier N, Nabholz B, Glémin S, Hurst G D D. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. *Molecular Ecology*, **18**, 4541–4550.

Ghaderi R, Karssen G. 2020. An updated checklist of *Meloidogyne* Göldi, 1887 species, with a diagnostic compendium for second-stage juveniles and males. *Journal of Crop Protection*, **9**, 183–193.

Golden A M, 1990. Preparation and mounting nematodes for microscopic

observation. In: Amherst M A, ed., *Plant Nematology Laboratory Manual*. University of Massachusetts Agricultural Experiment Station, USA. pp. 197–205.
Hartman K M, Sasser J N. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. *An Advanced Treatise on Meloidogyne*, **2**, 69–77.
Hooper D J. 1986. Extraction of free-living stages from soil, 5–30. In: Southey J F, ed., *Laboratory Methods for Work with Plant and Soil Nematodes*. Her Majesty's Stationery Office, London.
Janssen T, Karssen G, Verhaeven M, Coyne D, Bert W. 2016. Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype based diagnostics and reveals evidence of recent reticulate evolution. *Scientific Reports*, **6**, 22591.
Jin N, Chen Y P, Liu Q, Jian H. 2022. Research progresses in occurrence, diagnoses, pathogenic mechanisms and integrated management of vegetable root-knot nematodes in China. *Journal of Plant Protection*, **49**, 424–438. (in Chinese)
Jones J T, Haegeman A, Danchin E G, Gaur H S, Helder J, Jones M G, Perry R N. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, **14**, 946–961.
Kalyaanamoorthy S, Minh B Q, Wong T K F, Von Haeseler A, Jermiin L S. 2017. Model Finder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**, 587–596.
Katoh K, Standley D M. 2013. Mafft multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.
Khan A, Ali A, Fatima S, Siddiqui M A. 2023. Root-knot nematodes (*Meloidogyne* spp.): Biology, plant-nematode interactions and their environmentally benign management strategies. *Gesunde Pflanzen*, **75**, 2187–2205.
Kiewnick S, Holterman M, van den Elsen S, van Megen H, Frey J E, Helder J. 2014. Comparison of two short DNA barcoding loci (COI and COII) and two longer ribosomal DNA genes (SSU & LSU rRNA) for specimen identification among quarantine root-knot nematodes (*Meloidogyne* spp.) and their close relatives. *European Journal of Plant Pathology*, **140**, 97–110.
Kiewnick S, Wolf S, Willareth M, Frey J E. 2013. Identification of the tropical root-knot nematode species *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using a multiplex PCR assay. *Nematology*, **15**, 891–894.
Kim E, Seo Y, Kim Y S, Park Y, Kim Y H. 2017. Effects of soil textures on infectivity of root-knot nematodes on carrot. *The Plant Pathology Journal*, **33**, 66–74.
Kopelman N, Mayzel J, Jakobsson M, Rosenberg N, Mayrose I. 2015. CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, **2**, 42–49.
Li K M, Dong Y Q, Cao X S, Bayier. 2015. Occurrence and identification of root-knot nematode on green house vegetables in Xinjiang. *Plant Protection*, **41**, 191–194. (in Chinese)
Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.
Liu M Y, Shao H D, Wu Y Y, Peng D L, Yu J W, Jia J P, Huang W K. 2023. *Meloidogyne graminicola* population structure in China suggests a south-to-north expansion. *Plant Disease*, **107**, 2070–2080.
Long H, Sun Y, Chen Y, Pei Y, Feng T, Che H. 2023. Occurrence of root-knot nematodes (*Meloidogyne* spp.) on peppers in Hainan, China, and resistance of field cultivars to *M. enterolobii* and *M. incognita*. *Plant Disease*, **107**, 3148–3154.
Marquez J, Hajihassani A. 2023. Identification, diversity, and distribution of *Meloidogyne* spp. in vegetable fields of South Georgia, USA. *Phytopathology*, **113**, 1093–1102.
Meng Q P, Long H, Xu J H. 2004. PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arena*ria. *Acta Phytopathologica Sinica*, **34**, 204–210. (in Chinese)
Naz I, Khan R A A, Masood T, Baig A, Siddique I, Haq S. 2021. Biological control of root knot nematode, *Meloidogyne incognita*, *in vitro*, greenhouse and field in cucumber. *Biological Control*, **152**, 104429.
Nnamdi C, Grey T L, Hajihassani A. 2022. Root-knot nematode management for pepper and squash rotations using plasticulture systems with fumigants and non-fumigant nematicides. *Crop Protection*, **152**, 105844.
Peng H, Peng D L, Hu X Q, He X F, Wang Q, Huang W K, He W T. 2012. Loop-mediated isothermal amplification for rapid and precise detection of the burrowing nematode, *Radopholus similis*, directly from diseased plant tissues. *Nematology*, **14**, 977–986.
Picard D, Plantard O, Scurrah M, Mugniéry D. 2004. Inbreeding and population structure of the *potato cyst nematode* (*Globodera pallida*) in its native area (Peru). *Molecular Ecology*, **13**, 2899–2908.
Pritchard J K, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
Quan J R, Guo L. 1997. Root-knot nematodes of chilli peppers in Xinjiang. *Xinjiang Agricultural Science*, **1**, 32–34. (in Chinese)
Ratnasingham S, Hebert P D. 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular Ecology Notes*, **7**, 355–364.
Rusinque L, Nóbrega F, Cordeiro L, Lima A, Andrade S, Inácio M L. 2022. Root-knot nematode species associated with horticultural crops in the Island of Azores, Portugal. *Horticulturae*, **8**, 101–109.
Sasser J N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). Technical Bulletin. *Maryland Agricultural Experiment Station*, **77**, 1–30.
Shao H D, Xue Q, Yao K, Cui J K, Huang W K, Kong L A, Li C R, Li H M, Peng D L, Smiley R W, Peng H. 2022. Origin and phylogeography of Chinese cereal cyst nematode *Heterodera avenae* revealed by mitochondrial COI sequences. *Phytopathology*, **112**, 1988–1997.
Shao H D, Zhang P, You C P, Li C R, Feng Y, Xie Z W. 2020. Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* in mulberry based on the mitochondrial COI gene. *Ecology and Evolution*, **10**, 5391–5401.
Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.
Takahata N, Palumbi S R. 1985. Extranuclear differentiation and gene flow in the finite island model. *Genetics*, **109**, 441–457.
Taylor A L, Sasser J N. 1978. *Biology, Identification and Control of Root-Knot Nematodes* (*Meloidogyne* species). North Carolina State University Graphics, Raleigh, NC.
Taylor A L, Sasser J N, Nelson L A. 1982. *Relationship of Climate and Soil Characteristics to Geographical Distribution of Meloidogyne Species in Agricultural Soils*. International Meloidogyne Project. p.65.
Tigano M, De Siqueira K, Castagnone-Sereno P, Mulet K, Queiroz P, Dos Santos M, Carneiro R M D G. 2010. Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species. *Plant Pathology*, **59**, 1054–1061.
Tsai C F, Tsay T T, Chen P. 2010. The effect of soil temperature on the penetration and development of three root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. hapla*. *Plant Pathology Bulletin*, **19**, 235–242.
Vrain T C. 1978. Influence of chilling and freezing temperatures on infectivity of *Meloidogyne incognit*a and *M. hapla*. *Journal of Nematology*, **10**, 177–180.
Vrain T C, Barker K R, Holtzman G I. 1978. Influence of low temperature on rate of development of *Meloidogyne incognita* and *M. hapla* larvae. *Journal of Nematology*, **10**, 311–313.
Vrain T C, Wakarchuk D A, Levesque A C, Hamilton R I. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. *Fundamental and Applied Nematology*, **15**, 563–573.
Xi X, Xing D, Fu Q, Chen X, Hang C, Lu Z. 2017. Pathogen identification of root-knot nematode disease in eight batches of strawberry seedlings transferred to Beijing. *China Plant Protection Guide*, **37**, 45–48. (in Chinese)
Ye W, Robbins R T, Kirkpatrick T. 2019. Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) from Arkansas, USA. *Scientific Reports*, **9**, 15680.
Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li W X. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, **20**, 348–355.
Zhou H, Shen P G. 2003. The treatment of 'root velamen nematicide' to parasitic nematodes within plant roots for entry-exit garden plants. *Plant Quarantine*, **4,** 208–212. (in Chinese)

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