Regulatory Mechanisms
in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Molecular-genetic analysis of *Meloidogyne* (Heteroderidae) species from Surxondaryo Region, Uzbekistan based on ITS markers

S. Choriyev*, A. Khurramov*, O. Amirov**, D. Mardonayeva*,
S. Bobokeldiyeva*, B. Asliddin***, G. Narzullaeva*****, N. Khusanov****

**Termez State University, Termez, Uzbekistan*
***Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan*
****Denov Institute of Entrepreneurship and Pedagogy, Denov, Uzbekistan*
*****National University of Uzbekistan named after Mirzo Ulugbek, Tashkent, Uzbekistan*
******Termez Branch of Tashkent Medical Academy, Termez, Uzbekistan*

Article info
Received 08.11.2025
Received in revised form 02.12.2025
Accepted 23.12.2025

Termez State University, Barkamol avlod st., 43, Termez, 190111, Uzbekistan. Tel.: +998-91-581-05-01. E-mail: choriyev.siroj@mail.ru

Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, Bogishamol st., 232b, Tashkent, 100053, Uzbekistan.

Denov Institute of Entrepreneurship and Pedagogy, Bog' st., 112, Denov District, Surkhandarya Region, Uzbekistan.

National University of Uzbekistan named after Mirzo Ulugbek, University st., 4, Tashkent, Uzbekistan.

Department of Medical Biology and Histology, Termez Branch of Tashkent Medical Academy, Islam Karimov st., 64, Termez, 132000, Uzbekistan.
Tel.: +99-871-289-04-65.
E-mail: bioinformzology@gmail.com

Choriyev, S., Khurramov, A., Amirov, O., Mardonayeva, D., Bobokeldiyeva, S., Asliddin, B., Narzullaeva, G., & Khusanov, N. (2025). Molecular-genetic analysis of Meloidogyne (Heteroderidae) species from Surxondaryo Region, Uzbekistan based on ITS markers. Regulatory Mechanisms in Biosystems, 16(4), e25217. doi:10.15421/0225217

This study investigated the molecular-genetic characteristics of *Meloidogyne* species from Surxondaryo region, Uzbekistan, using ITS (Internal Transcribed Spacer) nucleotide sequences. A total of 765 root samples from peanut, carrot, sugar beet, cucumber, and tomato were collected and analyzed. Nematodes were morphologically identified, and genomic DNA was extracted from mature females. The 5.8S–ITS2 region was amplified using TW81 and AB28 primers, sequenced, and analyzed phylogenetically using Maximum Likelihood methods with IQ-TREE 2 and 1000 bootstrap replicates. Multiple sequence alignments were performed with MAFFT, and genetic diversity indices, including haplotype diversity (Hd), nucleotide diversity (π), Tajima’s D, and Kimura 2-parameter (K2P) distances, were calculated in R using ape and pegas packages. Phylogenetic analyses revealed four main clusters corresponding to *M. arenaria*, *M. incognita*, *M. javanica*, and *M. konaensis*, with high bootstrap support (≥70%), confirming their monophyletic nature and clear inter-species differentiation. K2P distance analyses showed very low intra-species divergence (0.0015–0.0095) and high inter-species differentiation (0.040–0.055), indicating strong molecular conservation within species. Haplotype analysis revealed high diversity (Hd = 0.937), while nucleotide diversity was low (π = 0.052), suggesting recent population expansion, close evolutionary relationships, or selective pressures shaping the genetic structure. Overall, the results demonstrate that the ITS marker is a reliable molecular tool for species identification and population-genetic studies in *Meloidogyne*. The inclusion of Uzbek isolates provides molecular confirmation of their taxonomic status and establishes a foundation for future ecological and genetic research.

Keywords: *Meloidogyne*; ITS; phylogeny; genetic diversity; K2P; haplotype; Uzbekistan.

Introduction

In recent years, the rapid increase in the global human population has intensified challenges related to food security and the need to enhance agricultural productivity. From this perspective, plant-parasitic nematodes pose a significant threat to global food security due to their polyphagous nature and ability to damage a wide range of crops. Nematodes adversely affect plant growth and development by feeding on host tissues, leading to substantial reductions in crop yield (Somasekhar et al., 2011; Mendy et al., 2017). Climate change, particularly global temperature rise, directly influences the ecological and physiological dynamics of nematode populations. Elevated soil temperatures and alterations in plant physiology accelerate nematode life cycles, increase reproduction rates, and exacerbate plant infestation levels.

Root-knot nematodes (*Meloidogyne* Göldi, 1892) are among the most destructive plant pathogens globally. They infect over 2,000 mono- and dicotyledonous plant species and can cause an average loss of approximately 12% in local crop yields worldwide, resulting in annual economic losses estimated at around 100 billion USD (Bardgett, 2014; Cabrera et al., 2016; Forghani et al., 2020). Therefore, accurate identification of species composition and the development of effective management strategies are of critical importance for sustainable agriculture (Whitehead, 1968; Hussey, 1979; Sasser, 1980; Zeng et al., 2012).

Advances in molecular-genetic techniques in recent years have provided new approaches for studying nematodes at the species and population levels. Mitochondrial and nuclear DNA markers enable the detection of genetic variation that may not be evident morphologically, providing higher resolution for species and population differentiation (Hyman, 1990; Blok et al., 2009; Ye et al., 2015; Kavuluko et al., 2016; Khanal et al., 2016). Even in cases where morphological differences are subtle within *Meloidogyne* species, molecular-genetic analyses reveal distinct genetic lineages, which are essential for accurate taxonomic classification and ecological monitoring (Carneiro, 2005; Hunt et al., 2009; Ye et al., 2019). Thus, molecular-genetic investigations of *Meloidogyne* species are crucial not only for clarifying their systematic classification but also for developing scientifically based management strategies to protect agricultural crops from nematode infestations. The objective of this study was to conduct a molecular-genetic analysis of *Meloidogyne* Göldi, 1892 species – specifically *M. javanica* (Treub, 1885) Chitwood, 1949, *M. incognita* Lordello, 1956, and *M. arenaria* Chitwood, 1952 – from the Surxondaryo Region of Uzbekistan using nucleotide sequences of the ribosomal DNA 5.8S–ITS2 region. This approach aims to elucidate species- and population-level genetic diversity, investigate evolutionary relationships, and provide a solid scientific foundation for future ecological and phytopathological studies.

Materials and methods

The study was conducted during the spring, summer, and autumn seasons of 2024–2025 in Surxondaryo region of the Republic of Uzbekistan, specifically in Jarqo‘rg‘on (67.415957º E 37.356305 º N), Qumqo‘rg‘on (67.704670º E 37.938029º N), Sherobod, Angor (67.197502º E 37.403216º N), Oltinsoy, Denov, Sho‘rchi districts, and the city of Termez, focusing on both farmer-managed and private household fields. The research targeted peanut (*Arachis hypogaea*), carrot (*Daucus carota*), sugar beet (*Beta vulgaris*), as well as greenhouse-grown cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*). A total of 765 samples of plant roots infected with root-knot nematodes were collected using both transect and stationary observation methods. For molecular-genetic analyses, infected root samples were preserved in 70% ethanol solution. Nematodes were extracted from roots and rhizosphere soils following the modified "funnel" method described by Baermann. Permanent slides were prepared using the Seinhorst method. Identification of nematode species and sexes was performed using an N-300M trinocular microscope and reference identification keys and atlases. Morphological identification relied on the examination of permanent and temporary preparations, focusing on head and tail morphometrics, structure of the red esophagus, and cuticle lines (Choriyev et al., 2024; Khuramov et al., 2024).

For molecular-genetic procedures, root samples were washed with distilled water, and three mature female nematodes were isolated per sample. The nematodes were placed in 1.5 mL Eppendorf tubes. Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA). The ribosomal DNA 5.8S–ITS2 region was amplified using the TW81 forward (5´-GTTTCCGTAGGTGAACCTGC-3´) and AB28 reverse (5´-ATATGCTTAAGTTCAGCGGGT-3´) primers, commonly applied in molecular nematode taxonomy (Curran et al., 1994). Polymerase chain reaction (PCR) was performed under the following conditions: initial denaturation at 94 °C for 5 minutes; denaturation at 95 °C for 45 seconds; primer annealing at 55 °C for 45 seconds; elongation at 72 °C for 1 minute 40 seconds; final extension at 72 °C for 5 minutes. The denaturation, annealing, and elongation steps were repeated for 35 cycles (Curran et al., 1994). PCR products were confirmed on 1% agarose gels at 120 V and purified according to the manufacturer's instructions using the "Sileks M" reagent kit (Moscow, Russia).

Sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 chemistry, and products were analyzed on an ABI PRISM 3100-Avant automated sequencer (Moscow, Russia). For phylogenetic analysis, sequenced DNA of *Meloidogyne incognita*, *M. arenaria*, *M. signifera*, and *M. javanica*, along with publicly available sequences from the National Center for Biotechnology Information (NCBI) database, were used (NCBI, www.ncbi.nlm.nih.gov).

Multiple sequence alignments were generated using MAFFT software (Katoh & Standley, 2013). Consensus sequences were constructed using IQ-TREE 2 (Minh et al., 2020). Phylogenetic trees were inferred using the Maximum Likelihood (ML) method, and statistical support was assessed through 1000 bootstrap replicates (Felsenstein, 1985). *Abbreviata caucasica* (accession number MN956809) was used as an outgroup for rooting the phylogenetic tree.

Genetic diversity was evaluated based on the number and frequency of haplotypes, overall diversity indices, haplotype sequences, Tajima's D test (Tajima, 1989), and Kimura 2-parameter (K2P) genetic distance (Kimura, 1980). All analyses were performed in the R statistical environment using the ape (Paradis & Schliep, 2019) and pegas (Paradis, 2010) packages.

Results

The phylogenetic tree constructed based on ITS (Internal Transcribed Spacer) nucleotide sequences clearly reflected the evolutionary relationships among *Meloidogyne* species and their geographically diverse isolates. The tree is presented in a cladogram format, with node values representing support levels (%) calculated through bootstrap analysis. Overall, the predominance of high bootstrap values (≥70%) confirms the robustness and reliability of the phylogenetic structure (Figure 1).

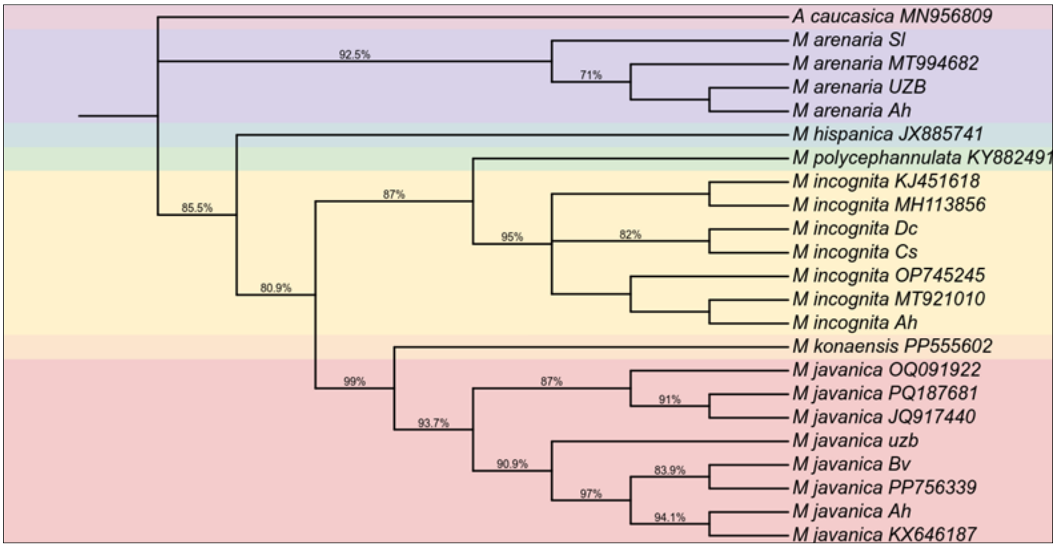

Fig. 1. Phylogenetic analysis of ITS region in *Meloidogyne* species and their geographically diverse isolates

The phylogenetic analysis revealed four main clusters in the tree, corresponding to distinct species or species complexes within the genus *Meloidogyne*.

The first cluster, located at the upper part of the tree, included isolates belonging to the *M. arenaria* group. This cluster comprised *M. caucasica* (MN956809) and several *M. arenaria* isolates (SL, MT994682, UZB, and Ah). The cluster was strongly supported with a 92.5% bootstrap value, indicating its monophyletic nature. Some sub-branches within the cluster exhibited lower bootstrap support (71%), suggesting relatively minor genetic differentiation among certain isolates.

The second major cluster, situated in the middle of the tree, included *M. incognita* and closely related species. Isolates within this cluster included *M. hispanica* (JX885741), *M. polycephannulata* (KY882491), and several *M. incognita* isolates (KJ451618, MH113856, Dc, Cs, OP745245, MT921010, and Ah). Nodes within this cluster were supported by bootstrap values ranging from 80.9% to 95.0%, indicating close genetic relationships while also reflecting internal diversification. The complex internal structure of this cluster confirms the high genetic variability within the *M. incognita* complex.

A distinct, separate cluster was formed by *M. konaensis* (PP555602), which was supported by a 99% bootstrap value, highlighting its clear genetic differentiation from other clusters. Nonetheless, it remains evolutionarily related to the major clusters, representing a distinct but closely related lineage within the genus *Meloidogyne*.

The fourth and largest cluster, located at the lower part of the tree, included isolates of *M. javanica*. This group comprised isolates OQ091922, PQ187861, JQ917440, uzb, Bv, PP756339, Ah, and KX646187. Nodes within the cluster were strongly supported by bootstrap values ranging from 83.9% to 99.0%, with the highest values (99% and 97%) indicating a well-supported monophyletic structure. The placement of the Uzbek isolate (uzb) within this cluster confirms its molecular identity as *M. javanica*.

Molecular differentiation among Meloidogyne species and their isolates was assessed based on ITS nucleotide sequences using the Kimura 2-parameter (K2P) model (Fig. 2). A heatmap and clustered distance matrix were generated to visualize inter- and intra-species evolutionary relationships both numerically and graphically. K2P analysis indicated that *Abbreviata caucasica* (MN956809) is highly divergent from all *Meloidogyne* species, with genetic distances ranging from 0.687 to 0.707, reflecting its distant phylogenetic relationship. In the heatmap, these high values were represented by dark colors, confirming the separate evolutionary lineage of *A. caucasica*. The genetic distance between the *M. arenaria* group and the *M. incognita* and *M. javanica* groups ranged from 0.040 to 0.055, indicating clear molecular differentiation among species. Specifically, the distance between *M. arenaria* isolates (SL, Ah, UZB, MT994682) and *M. incognita* isolates was approximately 0.045–0.048 K2P.

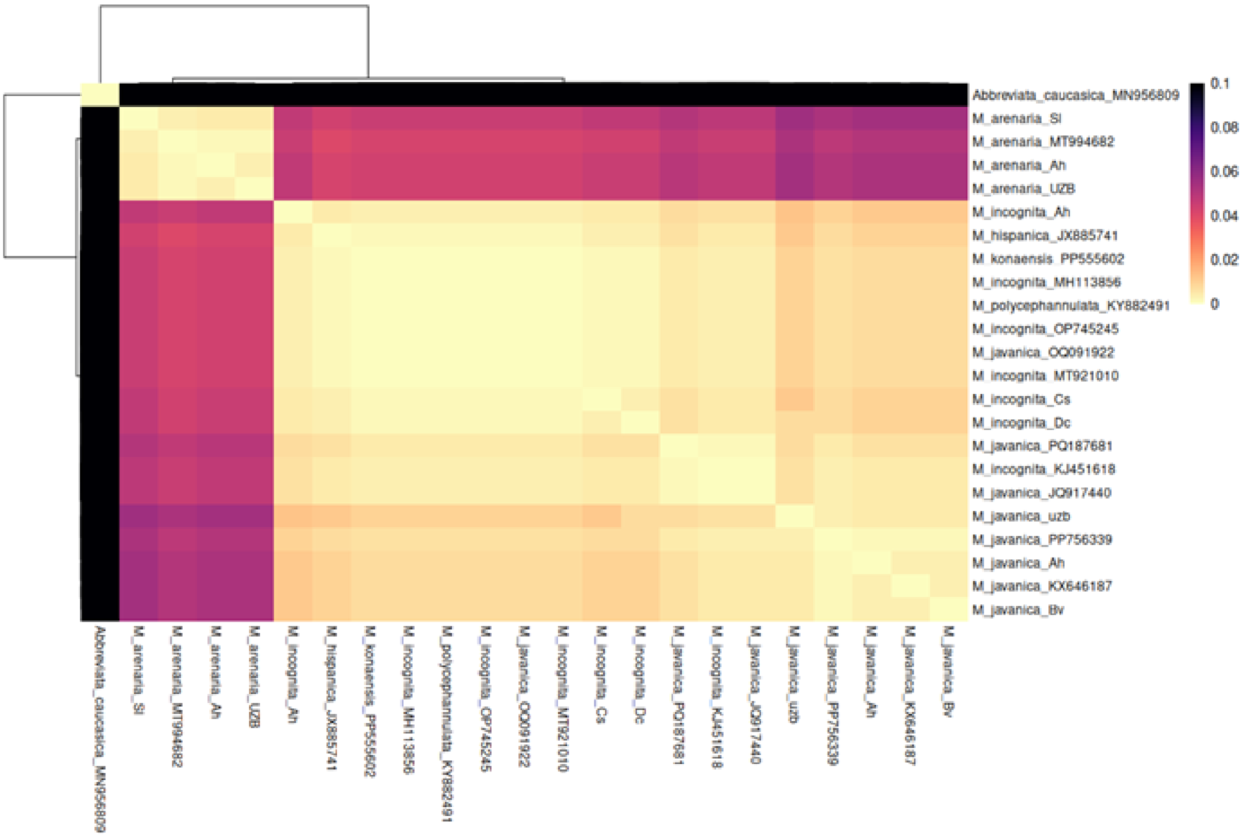

Fig. 2. Genetic divergence among *Meloidogyne* isolates and the outgroup based on ITS sequences using the kimura 2-parameter (K2P) model

Intra-species genetic distances were generally very low, indicating a high degree of molecular similarity among isolates. Within the *Meloidogyne arenaria* group, K2P distances ranged from 0.0015 to 0.0047, reflecting nearly identical ITS sequences among isolates. Notably, the Uzbek isolate (*M. arenaria* UZB) exhibited minimal genetic differentiation compared to other *M. arenaria* isolates. For the *M. incognita* complex (Ah, Cs, Dc, MT921010, OP745245, MH113856, KJ451618), genetic distances ranged from 0 to 0.0063, with some isolates showing zero divergence. This indicates a highly conserved ITS region within the species and supports the monophyletic nature of *M. incognita*. The close association of *M. hispanica* and *M. polycephannulata* isolates with this cluster, as indicated by very small distances, confirms their close evolutionary relationship with the *M. incognita* complex. The *M. javanica* group formed the largest cluster, with K2P distances among isolates ranging from 0 to 0.0095. The Uzbek isolate (*M. javanica* uzb) showed very low genetic divergence (≈0.003–0.007) relative to other *M. javanica* isolates, confirming its molecular identity within this species. Although *M. konaensis* (PP555602) displayed very low K2P distances (≈0–0.003) to *M. incognita* and *M. javanica* groups, it remained a distinct branch in the phylogenetic tree. This suggests that, despite its close evolutionary relationship with these species, it represents an independent genetic lineage. Genetic diversity within *Meloidogyne* isolates was evaluated using haplotype and nucleotide diversity indices.

Overall, K2P genetic distance analysis corresponded closely with the phylogenetic tree results, confirming clear molecular boundaries among major *Meloidogyne* species. Very low intra-species genetic distances indicate high molecular similarity among isolates, whereas high inter-species distances reflect independent evolutionary trajectories. These findings support the ITS marker as an effective molecular tool for species identification and population-genetic studies in *Meloidogyne*. Haplotype and nucleotide diversity analyses based on ITS sequences revealed a high haplotype diversity (Hd = 0.937), indicating the presence of numerous distinct haplotypes and their widespread occurrence within the population. In contrast, nucleotide diversity (π) was low (0.052), suggesting limited nucleotide-level variation and high sequence similarity among isolates. The combination of high haplotype diversity and low nucleotide diversity reflects close evolutionary relationships among isolates, recent population expansion, or the influence of selective pressures shaping the genetic structure.

Discussion

The ITS-based phylogenetic tree and genetic distance analyses provided a detailed view of evolutionary relationships among *Meloidogyne* species and their geographically distinct isolates. The four main clusters identified in the cladogram confirmed inter-species differentiation and monophyletic molecular groupings. Predominantly high bootstrap values (≥70%) support the robustness and statistical reliability of the phylogenetic structure. The first cluster included *M. arenaria* isolates, supported by a 92.5% bootstrap value, confirming a monophyletic group. Some sub-branches had lower support (71%), indicating minor molecular differences among isolates. This finding aligns with previous reports confirming relatively uniform genetic makeup within *M. arenaria* (Castagnone-Sereno et al., 2013). The second cluster comprised *M. incognita* and closely related species, with nodes supported by 80.9–95% bootstrap values, reflecting internal diversification. The close association of *M. hispanica* and *M. polycephannulata* isolates with *M. incognita* confirms their evolutionary proximity. Minor intra-cluster genetic differences may reflect population-level variation or local adaptation. *Meloidogyne konaensis* (PP555602) formed a distinct cluster with 99% bootstrap support, indicating clear molecular differentiation from other clusters while remaining evolutionarily linked to major lineages. This suggests the presence of a new but closely related phylogenetic line within the genus. The fourth cluster included *M. javanica* isolates, supported by bootstrap values of 83.9–99%. The Uzbek isolate (uzb) clustered within this group, confirming its molecular identity as *M. javanica*.

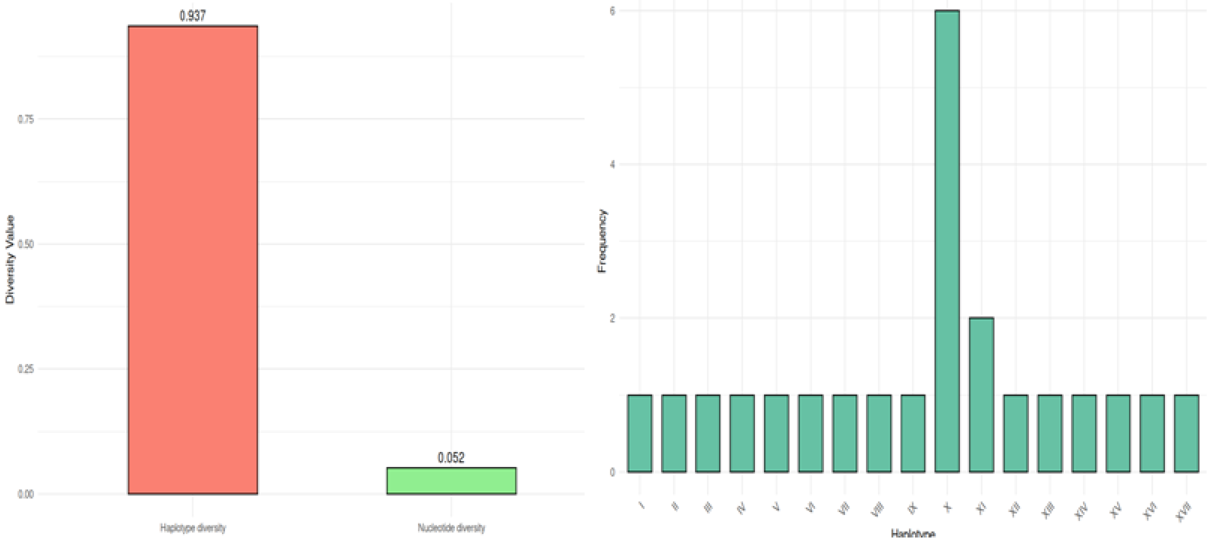

Fig. 3. Assessment of genetic diversity in *Meloidogyne* isolates based on haplotype and nucleotide variation

This observation is consistent with previous molecular studies showing that *M. javanica* isolates form a genetically close and monophyletic group (Blok et al., 2008). K2P genetic distance analyses corroborated the phylogenetic results. *Abbreviata caucasica* was clearly differentiated from all *Meloidogyne* species, confirming its distant evolutionary relationship. Average inter-species distances among *M. arenaria*, *M. incognita*, and *M. javanica* ranged from 0.040 to 0.055, demonstrating clear molecular differentiation. In contrast, intra-species K2P distances were very low, highlighting high molecular similarity among isolates: 0.0015–0.0047 for *M. arenaria*, 0–0.0063 for the *M. incognita* complex, and 0–0.0095 for *M. javanica*. These low distances confirm the conserved nature of the ITS marker and the monophyletic structure within species. Haplotype and nucleotide diversity analyses were consistent with phylogenetic and K2P results. The combination of high haplotype diversity (Hd = 0.937) and low nucleotide diversity (π = 0.052) indicates recent population expansion, close evolutionary origin, or selective pressure shaping the genetic structure. Overall, these findings confirm the ITS marker as an effective molecular tool for species identification and population-genetic studies in *Meloidogyne*.

Conclusion

The results of this study based on ITS (Internal Transcribed Spacer) nucleotide sequences clearly elucidated the phylogenetic and genetic relationships among *Meloidogyne* species and their geographically distinct isolates. Phylogenetic analyses identified four main clusters, revealing both inter- and intra-species molecular differentiation. Isolates of *M. arenaria*, *M. incognita*, *M. javanica*, and *M. konaensis* formed distinct monophyletic groups, allowing clear determination of their genetic relatedness and evolutionary connections. K2P genetic distance analyses confirmed significant inter-species differentiation, while low intra-species distances indicated high molecular similarity among isolates. Haplotype and nucleotide diversity assessments further highlighted genetic stability within species and evidence of recent population expansion. Overall, these findings confirm the utility of the ITS marker as an effective molecular tool for species identification and the study of population-genetic structures in *Meloidogyne*. Additionally, the inclusion of Uzbek isolates provides molecular confirmation of their taxonomic status, laying a solid foundation for future ecological and population-genetic investigations.

This work was supported by the "Academic Mobility" program under project number AK-25-19. We sincerely thank the staff of the Zoology Institute Laboratory, Uzbekistan Academy of Sciences, for providing all necessary technical equipment and facilitating optimal working conditions during the preparation of this study.

References

Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. Nature, 515, 505–511.

Blok, V. C., & Powers, T. O. (2009). Biochemical and molecular identification. In: Perry, R. N., Moens, M., & Starr, J. L. (Eds.). Root-knot nematodes. CABI Publishing, Wallingford. Pp. 98–118.

Blok, V. C., Phillips, M. S., & Fargette, M. (2008). Genetic diversity and phylogeny of *Meloidogyne javanica* populations. Nematology, 10(3), 345–354.

Cabrera, J., Barcala, M., Fenoll, C., & Escobar, C. (2016). The power of omics to identify plant susceptibility factors and to study resistance to root-knot nematodes. Current Issues in Molecular Biology, 19, 53–72.

Carneiro, R., Dickson, D., Jeyaprakash, A., Adams, B., & Tigano, M. (2005). Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. Nematology, 7(6), 851–862.

Castagnone-Sereno, P., Bongiovanni, M., Wajnberg, E., & Dalmasso, A. (2013). Genetic structure and evolutionary relationships among populations of *Meloidogyne arenaria*. Journal of Nematology, 45(2), 123–133.

Choriyev, S., Khurramov, A., Khurramov, S., & Mardonayeva, D. (2024). Ecological analysis of peanut nematodes in Surkhondaryo Region. In: BIO Web of Conferences, 100, 04006.

Curran, J. A., Argyle, D. J., Cox, P., Onions, D. E., & Nicolson, L. (1994). Nucleotide sequence of the equine interferon gamma cDNA. DNA Sequence, 4(6), 405–407.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39(4), 783–791.

Forghani, F., & Hajihassani, A. (2020). Recent advances in the development of environmentally benign treatments to control root-knot nematodes. Frontiers in Plant Science, 11, 1125.

Hunt, D. J., & Handoo, Z. A. (2009). Taxonomy, identification and principal species. In: Perry, R. N., Moens, M., & Starr, J. L. (Eds.). Root-knot nematodes. CABI Publishing, Wallingford. Pp. 55–97.

Hussey, R. S. (1979). Biochemical systematics of nematodes – a review. Helminthological Abstracts, Series B, Plant Nematology, 48, 141–148.

Hyman, B. C. (1990). Molecular diagnosis of *Meloidogyne* species. Journal of Nematology, 22(1), 24–30.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution, 30(4), 772–780.

Kavuluko, J., Gichuki, C., Wanjohi, W., & Runo, S. (2016). Molecular characterization and genetic variation of root-knot nematode (*Meloidogyne* spp.) in selected legume production areas of Eastern Kenya. American Scientific Research Journal for Engineering, Technology, and Sciences, 18(1), 67–83.

Khanal, C., Robbins, R. T., Faske, T. R., Szalanski, A. L., McGawley, E. C., & Overstreet, C. (2016). Identification and haplotype designation of *Meloido-*

gyne spp. of Arkansas using molecular diagnostics. Nematropica, 46(2), 261–270.

Khuramov, A., Bobokeldieva, L., Choriyev, S., Rakhmatullaev, B., Khimmatov, N., Raimov, S., Narzullaeva, G., Bobokeldiyeva, S., Mukhiddinova, M., & Karshieva, M. (2024). A comprehensive study of phytonematodes of grape plants in the conditions of the Surkhandarya Valley, Uzbekistan. Biodiversitas Journal of Biological Diversity, 25(11), 3.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16(2), 111–120.

Mendy, B., Wang'ombe, M. W., Radakovic, Z. S., Holbein, J., Ilyas, M., Chopra, D., Holton, N., Zipfel, C., Grundler, F. M. W., & Siddique, S. (2017). *Arabidopsis* leucine-rich repeat receptor–like kinase NILR1 is required for induction of innate immunity to parasitic nematodes. PLoS Pathogens, 13(4), e1006284.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37(5), 1530–1534.

Paradis, E. (2010). pegas: An R package for population genetics with an integrated-modular approach. Bioinformatics, 26(3), 419–420.

Paradis, E., & Schliep, K. (2018). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, 35(3), 526–528.

Sasser, J. N. (1980). Root-knot nematodes: A global menace to crop production. Plant Disease, 64(1), 36–47.

Somasekhar, N., & Prasad, J. S. (2011). Plant – nematode interactions: Consequences of climate change. In: Venkateswarlu, B., Shanker, A. K., Shanker, C., & Maheswari, M. (Eds.). Crop stress and its management: Perspectives and strategies. Springer, Dordrecht. Pp. 547–564.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123(3), 585–595.

Whitehead, A. G. (1968). Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. The Transactions of the Zoological Society of London, 31(3), 263–401.

Ye, W., Robbins, R. T., & Kirkpatrick, T. (2019). Molecular characterization of root-knot nematodes (*Meloidogyne* spp.) from Arkansas, USA. Scientific Reports, 9, 15680.

Ye, W., Zeng, Y., & Kerns, J. (2015). Molecular characterisation and diagnosis of root-knot nematodes (*Meloidogyne* spp.) from turfgrasses in North Carolina, USA. PLoS One, 10(11), e0143556.

Zeng, Y., Ye, W., Tredway, L., Martin, S., & Martin, M. (2012). Taxonomy and morphology of plant-parasitic nematodes associated with turfgrasses in North and South Carolina, USA. Zootaxa, 3452(1), 1–46.