Short Communication

Genetic Characterization of Species of the Genus Mesorhabditis (Rhabditida: Rhabditidae) Osche, 1952

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Abstract | This study presents a genetic characterization and phylogenetic analysis of nematode species belonging to the genus *Mesorhabditis* Osche, 1952, collected from wild rosehip (*Rosa canina* L.) in the Surkhandarya region of southern Uzbekistan. Specimens of *M. spiculigera*, *M. franseni*, and *M. irregularis* were identified using integrated morphological and molecular approaches. The internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified, sequenced, and used as a molecular marker to assess species-level differentiation and evolutionary relationships. Phylogenetic reconstruction based on Maximum Likelihood analysis revealed that *Mesorhabditis* species form several distinct monophyletic clades with varying levels of bootstrap support, indicating well-defined evolutionary lineages within the genus. Pairwise genetic distance analysis further confirmed molecular divergence among species and supported their taxonomic validity. *Parasitorhabditis obtusa* was used as an outgroup and showed the highest genetic divergence, validating its phylogenetic position outside the genus *Mesorhabditis*. The close genetic relationships observed among certain species, particularly between M. spiculigera and *M. irregularis*, suggest recent divergence events. Overall, the results demonstrate the effectiveness of ITS rDNA markers for species identification, phylogenetic inference, and taxonomic clarification within *Mesorhabditis*, contributing valuable molecular data for nematode systematics and biodiversity studies in Uzbekistan.

Received | March 27, 2026; **Accepted** | May 07, 2026; **Published** | June 12, 2026
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Citation | Mukhlisa, T., B. Abdujabbor, A. Oybek, I. Azamat, E. Mehmonjon and J. Guzal. 2026. Genetic characterization of species of the genus mesorhabditis (Rhabditida: Rhabditidae) Osche, 1952. *Pakistan Journal of Nematology*, 44(1): 77-86.
DOI | https://dx.doi.org/10.17582/journal.pjn/2026/44.1.77.86
Keywords | Mesorhabditis, Phylogenetic analysis, rDNA, Monophyletic clades, Genetic distance, Evolutionary relationships, Nematodes, Taxonomy

Introduction

Nematodes are organisms that live in water and soil, and although most of them cause significant economic damage, some species participate in nutrient cycling in the soil and help regulate natural ecological balance (Jabbar *et al.*, 2024; Koppenhofer and Fuzy, 2007). A small portion of nematodes

feed on plants and aquatic algae (first trophic level), others are grazers that consume fungi and bacteria (second trophic level), and still others feed on other nematodes (higher trophic levels). Various nematodes inhabit several trophic levels of the soil food chain. Nematodes are primarily found in the surface layer of the soil (Yadav *et al*., 2018). Some nematode species feed on microorganisms, contributing to nutrient cycling in the soil and the mineralization of compounds accumulated in microbial cells (Neidig *et al*., 2010). Bacterivorous nematodes contribute (directly or indirectly) to approximately 8% to 19% of nitrogen mineralization in conventional and integrated agricultural systems (Beare and Feller, 1997). The abundance of bacterivorous nematodes indicates a high quantity of easily decomposable organic matter (Sohlenius and Bostrom, 2001). Species belonging to the genus *Mesorhabditis*, discovered by Osche (1952) and Dougherty (1953), are widely distributed worldwide and are found in soils around decaying plant residues, beneath plant bark, in tree cavities, manure, and sediment layers along basin shores. Among the 34 species of this genus, six have been recorded in association with insects. Of these, the species *M. labiata* is the best studied, being associated with nine insect species from different groups. Following this, *M. irregularis* has been found in association with three insect species, and *M. inarimensis* with two insect species. *M. franseni*, *M. longistomis*, and *M. spiculigera* have each been recorded with only one insect species. Correctly understanding the systematic and evolutionary relationships among nematodes is important for their practical applications and for legal protection issues. The insufficient study of genetic diversity within species and populations reduces the efficiency of work in this area. In general, the taxonomic position of nematodes is determined based on morphological characteristics; however, solely relying on morphological traits is not always sufficient to accurately identify which species a newly discovered specimen belongs to. For this reason, analyzing genetic diversity among existing populations and developing precise diagnostic methods is of particular importance. This is especially relevant when assessing the potential use of nematodes as saprophytes for decomposing organic matter. The genus *Mesorhabditis* was proposed by Osche (1952). Sudhaus (1976) considered *Mesorhabditis* as a subgenus of the genus *Rhabditis* and recorded 16 valid species within this group. Andrassy (1976), based on certain minor morphological differences observed in male individuals, separated the genus Bursilla from *Mesorhabditis* (Osche, 1952) Dougherty, 1953 as a new genus. These differences are characterized by well-developed bursae and a greater number of sexual papillae in *Mesorhabditis*, whereas in Bursilla, the bursa is rudimentary and the number of sexual papillae is lower. Sudhaus (2011) synonymized the genus Bursilla (Andrássy, 1976) with *Mesorhabditis* (Osche, 1952; Dougherty, 1953) and divided this genus into two groups, namely the Monhystera group and the Spiculigera group. He listed 34 valid species within this genus. The *Monhystera* group is characterized by the following features: the pharynx has a zipper-like structure; the distance between the vulva and anus is shorter than the distance between the anus and tail; the tail is conical and gradually tapers; male individuals are rare; the bursa is rudimentarily developed. The genus *Mesorhabditis* includes species in nature with both typical sex ratios of males and species in which males are very rare (Letunic and Bork, 2021). Like many nematode species, they have traditionally been described based on morphological traits, but neither hybridization tests nor molecular markers (genetic markers) have been applied, and these animals have not even been kept in laboratory conditions (https://pmc.ncbi.nlm.nih.gov/articles/PMC7433073/). At present, numerous scientific studies are being conducted on the molecular genetics of vertebrate and invertebrate animals within the fauna of our republic. In particular, research on parasitic nematodes of vertebrate animals has been carried out by Ikromov *et al*. (2025); Aliyev *et al*. (2025); Seytveliyeva *et al*. (2026). In addition, scientific investigations on the molecular genetic characterization of vertebrate animals are being conducted by Amirov *et al*. (2025); Adhamjon *et al*. (2025); Kuchboev *et al*. (2025). The aim of this study is to carry out a molecular genetic characterization of species of the genus *Mesorhabditis* Osche (1952) in the southern Surkhandarya region of the Republic.

Materials and Methods

Morphological research methods

In this study, during 2025, nematodes belonging to the species *Mesorhabditis spiculigera, M. franseni*, and *M. irregularis* were collected from rosehip (*Rosa canina* L.) in the districts of Sariosiyo, Termiz, and Boysun in Surkhandarya region (Table 1).

Table 1: *Areas where nematodes of the genus Mesorhabditis were collected.*

No.	Plant species	Plant organ	Region (District)	Coordinate point(s)	Nematode species	Number of collected nematodes (specimens)
1	Rosehip (*Rosa canina* L.)	Root	Sariosiyo	37.8865009, 66.9351784; 38.4217921, 67.9724267; 38.4025774, 67.9609157	*Mesorhabditis spiculigera*; *Mesorhabditis franseni*; *Mesorhabditis irregularis*	150
2	Rosehip (*Rosa canina* L.)	Root	Termiz	37.3488982; 67.3447448; 37.3125639, 66.6567993; 37.3399159, 67.2341377	*Mesorhabditis spiculigera*; *Mesorhabditis franseni*; *Mesorhabditis irregularis*	90
3	Rosehip (*Rosa canina* L.)	Root	Boysun	38.3317694; 67.0802875; 38.3318916; 67.0081856; 38.2819281, 67.285988	*Mesorhabditis spiculigera*; *Mesorhabditis franseni*; *Mesorhabditis irregularis*	60
Total						300

The Bereman funnel method was used to isolate phytoparasitic nematodes from plant organs and rhizosphere soil. The species composition of the phytoparasitic nematodes was studied using the MBR-3 microscope. To identify the species, the universally accepted De Man (De Man, 1880) formula and morphometric parameters obtained according to its modification by Micoletzky were used. Permanent slides were prepared based on the Seinhorst method (Seinhorst, 1959), with more than 100 permanent and temporary slides made. For this work, various light microscopes available at the Molecular Zoology Laboratory of the Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, were used, along with the ML 2000 (Meiji) digital camera, Olympus CX31 and CK2V microscopes, Levenhuk T-Series microscopes, and cameras.

DNA extraction

Genomic DNA was isolated from biological material preserved in 70% ethanol using the DNeasy Blood and Tissue Kit (Qiagen, November 2023). DNA concentration was quantified with a Thermo Fisher Scientific spectrophotometer. The purified DNA samples were stored at –20 °C for subsequent PCR analysis.

PCR amplification

PCR amplification was performed to amplify a fragment of the ITS of ribosomal DNA (rDNA) gene, a standard genetic marker for the molecular identification of amphibian species. The PCR reaction mix consisted of: 26.4 µl double-distilled water, 4 µl 10× Taq buffer, 0.8 µl dNTPs, 2 µl of each primer (AB28 (ATA TGC TTA AGT TCA GCG GGT) ва TW81 (GTT TCC GTA GGT GAA CCT GC),(Curran at el., 1994), 4 µl DNA template, and 0.8 µl Taq polymerase, in a total volume of 40 µl. The thermal cycling conditions were: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 51°C for 40 seconds, and extension at 72 °C for 80 seconds; with a final elongation at 72°C for 5 minutes (Kocher *et al*., 1989). Presence of PCR products was verified by electrophoresis on a 1.0% agarose gel at 100 V. DNA fragments were excised and purified using reagents supplied by Sileks M (Moscow, Russia), following the manufacturer's instructions. Sequencing of DNA fragments was performed with the ABI PRISM® BigDye™ Terminator v3.1 kit, and sequencing services were provided by GATC Biotech AG. Analysis of the obtained nucleotide sequences was conducted using software packages such as BioEdit, ClustalX2, DNAstar™, and PAUP4 (Hall, 1999; Larkin *et al*., 2007).

Phylogenetic tree construction

In this study, a total of 23 nucleotide sequences representing species of the genus *Mesorhabditis* were analyzed (Table 2). Among them, nine sequences were newly generated from specimens collected in Uzbekistan and deposited in GenBank under accession numbers PX677369–PX677376. The remaining sequences were retrieved from the GenBank database to represent the genetic diversity of the genus.

Multiple sequence alignment was generated with the MAFFT algorithm (Katoh *et al*., 2002), with further manual editing of specific segments carried out in BioEdit (version 7.0.5.2; Hall, 1999) to enhance the accuracy of the alignment. Phylogenetic trees were reconstructed using the Maximum Likelihood (ML) approach implemented in IQ-TREE 2 (Minh *et al*., 2020). The optimal substitution model was automatically selected with the Model Finder module. Node support was assessed through 1,000 bootstrap

replicates (Felsenstein, 1985). The final phylogenetic tree was visualized using the iTOL (Interactive Tree of Life) online tool (Letunic and Bork, 2021), which allowed a clear and customizable representation of phylogenetic relationships.

Table 2: *GenBank accession numbers of Mesorhabditis species used in this study.*

No.	Species Name	GenBank accession No.	Source
1	*Mesorhabditis irregularis*	PX677371	Uzbekistan
2	*Mesorhabditis spiculigera*	PX677362	Uzbekistan
3	*Mesorhabditis irregularis*	PX677376	Uzbekistan
4	*Mesorhabditis irregularis*	PX677375	Uzbekistan
5	*Mesorhabditis franseni*	PX677372	Uzbekistan
6	*Mesorhabditis franseni*	PX677373	Uzbekistan
7	*Mesorhabditis franseni*	PX677374	Uzbekistan
8	*Mesorhabditis spiculigera*	PX677370	Uzbekistan
9	*Mesorhabditis spiculigera*	PX677369	Uzbekistan
10	*Mesorhabditis simplex*	MT710248	Genbank
11	*Mesorhabditis monhystera*	MT710274	Genbank
12	*Mesorhabditis monhystera*	MT710271	Genbank
13	*Mesorhabditis microbursaris*	MT710259	Genbank
14	*Mesorhabditis cranganorensis*	MT710263	Genbank
15	*Mesorhabditis cranganorensis*	MT710262	Genbank
16	*Mesorhabditis longespiculosa*	MT710275	Genbank
17	*Mesorhabditis longespiculosa*	EU195980	Genbank
18	*Mesorhabditis vernalis*	MT710255	Genbank
19	*Mesorhabditis belari*	MT710234	Genbank
20	*Mesorhabditis belari*	EF417149	Genbank
21	*Mesorhabditis franseni*	MT710246	Genbank
22	*Mesorhabditis franseni*	MT710245	Genbank
23	*Parasitorhabditis obtusa*	MF288651	Genbank

Results

Based on sequencing analyses, the nucleotide sequences of the mitochondrial ITS region of *Mesorhabditis spiculigera* (samples A, B, C) with a length of 474 base pairs were successfully obtained, and the phylogenetic relationships of the genus *Mesorhabditis* (Osche, 1952), were inferred (Figure 1). The phylogenetic tree constructed using the Maximum Likelihood (ML) method revealed a clear branching pattern, reflecting both interspecific and intraspecific genetic relationships. To root the tree, *Parasitorhabditis obtusa*, belonging to the genus *Parasitorhabditis*, was used as an outgroup.

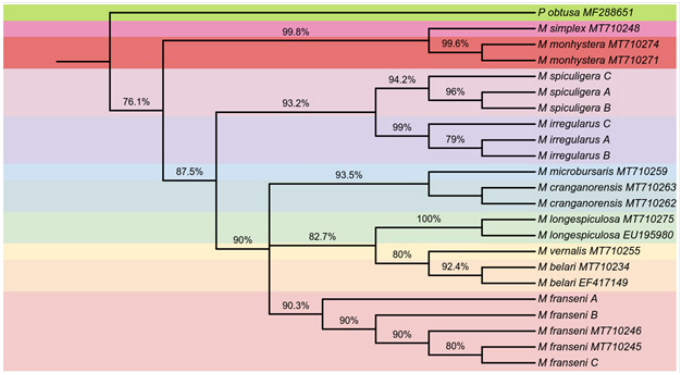

Figure 1: *Phylogenetic relationships of species belonging to the genus Mesorhabditis* Osche, 1952 *based on the ITS gene of rRNA.*

In the phylogenetic analysis, species of the genus *Mesorhabditis* formed several well-supported monophyletic clusters. The *P. obtusa* outgroup (MT288651) occupied a basal position, exhibiting no direct genetic affinity with the ingroup species, with a bootstrap support of 76.1%, indicating moderate reliability. Within the genus, *M. monhystera* (MT710274, MT710271) formed a highly supported cluster (bootstrap = 99.6%), which further clustered with *M. simplex* (MT710248) with an overall bootstrap of 99.8%, demonstrating very high interspecific genetic cohesion. Intraspecific variation was observed in *M. spiculigera* (A, B, C), where samples A and B clustered together (bootstrap = 96%) and subsequently merged with C, yielding an overall bootstrap support of 93.2%. Similarly, *M. irregularus* (A, B, C) exhibited clustering of A and B (bootstrap= 79%) and subsequent inclusion of C (overall bootstrap= 93.2%), reflecting high intraspecific genetic divergence. Other species clusters were also well resolved: *M. microbursaris* (MT710259) clustered with *M. cranganorensis* (MT710263, MT710262) with a bootstrap of 93.5%, confirming close genetic relatedness. *M. longespiculosa* (MT710275, EU195980) formed a fully supported cluster (bootstrap = 100%) before joining other lineages (bootstrap = 82.7%). *M. vernalis* (MT710255) and *M. belari* (MT710234, EF417149) clustered with bootstrap values of 92.4% and 80%, respectively, indicating moderate to high interspecific support. Notably, *M. franseni* displayed multiple internal clusters: A and B (bootstrap = 90%), subsequently merged with MT710246 (bootstrap = 90%), while MT710245 and C formed a separate lineage (bootstrap = 80%), yielding an overall cluster support of 90.3%, highlighting intraspecific genetic diversification. Bootstrap values above 70% were considered statistically reliable, and the majority of

clusters exhibited support between 80% and 100%, indicating robust phylogenetic resolution. Observed intraspecific divergence in *M. franseni* underscores the presence of genetic variation within the species. The use of color-coded branches and clusters in the phylogenetic tree facilitated the visual distinction of species and their sample groups. Overall, these results demonstrate that the mitochondrial ITS region serves as a highly reliable molecular marker for distinguishing *Mesorhabditis* species and provides critical insights into both intraspecific genetic variation and interspecific phylogenetic relationships.

Genetic distance analysis and K2P results

In this study, evolutionary divergence among 23 *Mesorhabditis* samples was assessed based on ITS/28S rRNA gene fragments using the Kimura two-parameter (K2P) model. Pairwise genetic distances were calculated to generate a 23 × 23 distance matrix, which was subsequently visualized as a heatmap. The K2P analysis revealed clear and structured patterns of both intra- and interspecific molecular divergence within the genus *Mesorhabditis*.

Identical and closely related samples. Several pairs of samples exhibited K2P genetic distance values of 0.000, indicating complete sequence identity across the analyzed ITS/28S rRNA regions. These included: *M. longespiculosa* (EU195980 and MT710275), *M. monhystera* (MT710271 and MT710274), *M. cranganorensis* and *M. microbursaris* (MT710262, MT710263, MT710259) and *M. belari* (EF417149 and MT710234). These results suggest that the respective samples either belong to the same species or represent lineages that have diverged very recently. Furthermore, the absence of detectable genetic divergence among some taxa may indicate potential synonymy or highlight the need for taxonomic re-evaluation within the genus.

Intraspecific diversification

Intraspecific genetic divergence was generally low, as reflected by small K2P distance values. Specifically: *M. irregularus* samples A, B, and C showed distances ranging from 0.008 to 0.014, *M. spiculigera* samples A, B, and C ranged from 0.004 to 0.008 and all *M. franseni* samples and *M. vernalis* (MT710255) exhibited distances between 0.002 and 0.008. These low levels of intraspecific divergence indicate limited genetic differentiation within species and are consistent with the relatively slow evolutionary rate of ITS/28S rRNA loci. Notably, the extremely small genetic distances between *M. franseni* and *M. vernalis* suggest that these taxa may represent recently diverged evolutionary lineages or intraspecific variants.

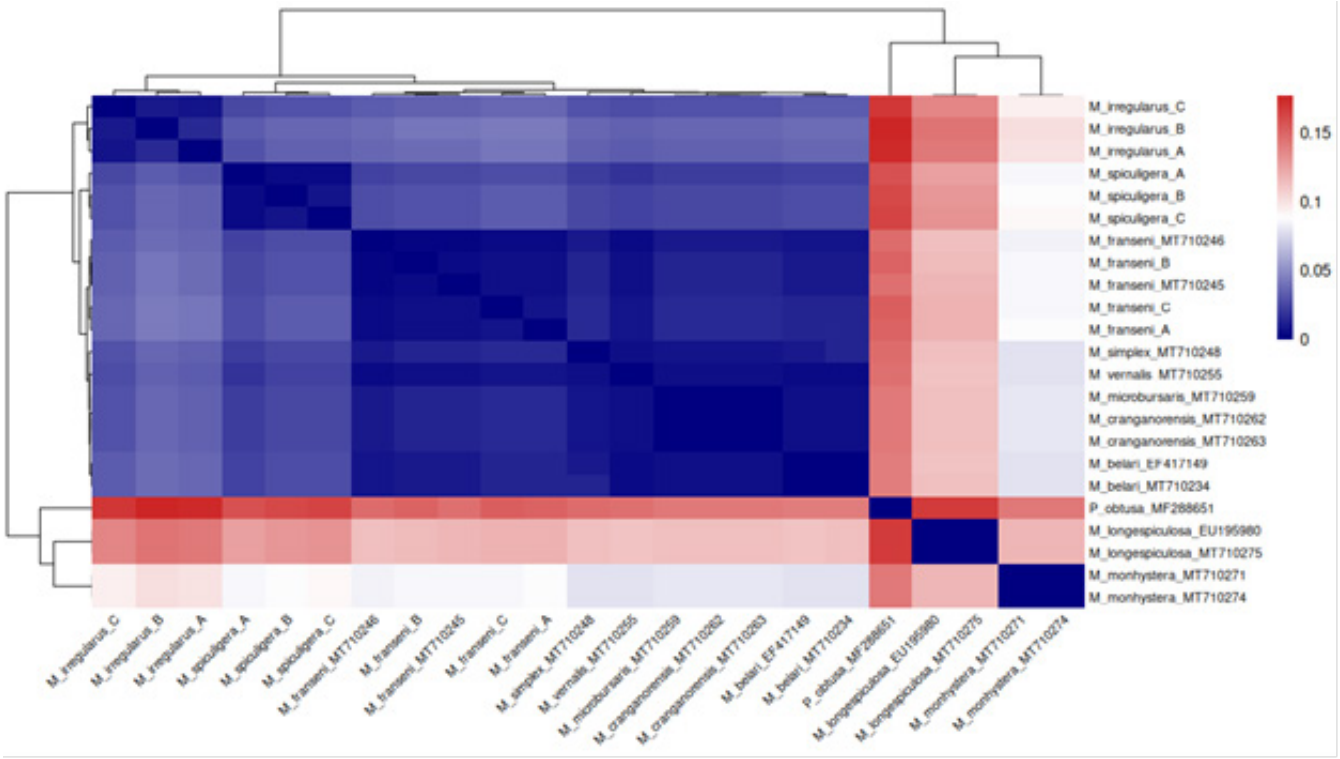

Figure 2: *Genetic divergence among Mesorhabditis species, including samples from Uzbekistan. Specimens labeled with suffixes (A, B, C) represent individuals collected from Uzbekistan.*

Interspecific divergence

Pairwise K2P distances between species clearly reflected interspecific divergence and supported the delineation of major clades within *Mesorhabditis*. In particular: The distance between *M. longespiculosa* and *M. monhystera* was approximately 0.118, distances between *M. irregularus* and *M. spiculigera* ranged from 0.030 to 0.036 and distances among *M. simplex, M. belari, M. cranganorensis*, and *M. microbursaris* ranged from 0.006 to 0.014. These values demonstrate that ITS/28S rRNA markers possess strong diagnostic power for resolving species boundaries within *Mesorhabditis* and provide robust molecular support for the observed phylogenetic structure.

Comparison with the outgroup

The outgroup taxon *Parasitorhabditis obtusa* (MF288651) exhibited substantially higher K2P distances relative to all *Mesorhabditis* samples, ranging from 0.140 to 0.177. This pronounced divergence confirms its phylogenetic distance from *Mesorhabditis* and supports its suitability as an outgroup for rooting phylogenetic reconstructions.

Evolutionary implications

Based on the K2P genetic distance analysis, several key evolutionary conclusions can be drawn: Intraspecific divergence within *Mesorhabditis* is generally low (≈0.002–0.036), which is consistent with expectations for ITS/28S rRNA loci. Interspecific genetic distances (≈0.08–0.12) provide clear molecular thresholds for species delimitation and taxonomic resolution. The genetic distance patterns support the recognition of the following major phylogenetic clades within *Mesorhabditis*: Clade I: *M. longespiculosa,* Clade II: *M. Monhystera,* Clade III: *M. irregularus* + *M. spiculigera*, Clade IV: *M. simplex* + *M. belari* + *M. cranganorensis* + *M. Microbursaris,* Clade V: *M. franseni* + *M. vernalis*. Overall, the K2P genetic distance analysis provides strong molecular evidence for the internal phylogenetic structure and evolutionary diversification of *Mesorhabditis*, forming a solid foundation for subsequent phylogenetic, taxonomic, and ecological interpretations.

Discussion

Phylogenetic resolution of Mesorhabditis based on ITS rRNA sequences

The present study provides a comprehensive molecular and morphological assessment of *Mesorhabditis* species collected from Uzbekistan, integrating ITS/28S rRNA sequence data, K2P genetic distance analyses, and classical morphometric identification. The Maximum Likelihood phylogenetic reconstruction based on the ITS rRNA region revealed a well-resolved and robust topology, supporting the monophyly of the genus *Mesorhabditis* and clearly distinguishing both interspecific and intraspecific relationships. The use of *Parasitorhabditis obtusa* as an outgroup resulted in a stable tree rooting, with consistently high genetic distances (K2P= 0.140–0.177) relative to *Mesorhabditis* species. This level of divergence is consistent with previous nematode phylogenetic studies, where *Parasitorhabditis* has been shown to be evolutionarily distant from *Mesorhabditis* within *Rhabditidae* (Sudhaus, 2011; Kiontke *et al.*, 2011). The moderate bootstrap support at the basal node further reflects the deep divergence between the ingroup and outgroup taxa.

Intraspecific genetic variation and population structure

Low intraspecific divergence was observed across most *Mesorhabditis* species, with K2P distances generally ranging from 0.002 to 0.036. Such low levels of variation are typical for ribosomal DNA regions, which evolve relatively slowly and are subject to concerted evolution (Hillis and Dixon, 1991; Baldwin *et al.*, 1995). The clustering patterns observed in *M. spiculigera, M. irregularus,* and *M. franseni* indicate limited genetic differentiation among populations collected from geographically distinct regions within Surkhandarya. Notably, *M. franseni* exhibited higher internal structuring compared to other species, forming multiple subclusters with strong bootstrap support. This pattern suggests the presence of genetically differentiated populations, potentially driven by local adaptation, ecological heterogeneity, or restricted gene flow. Similar intraspecific diversification has been reported in other free-living nematodes occupying heterogeneous soil environments (Kiontke and Sudhaus, 2006; Félix *et al.*, 2014). These findings highlight the importance of integrating population-level sampling when assessing nematode diversity.

Species boundaries and potential taxonomic implications

Several species pairs exhibited zero genetic distances, including *M. cranganorensis and M. microbursaris*, as well as multiple accessions of *M. longespiculosa, M. monhystera, and M. belari*. Complete sequence identity across the analyzed ITS/28S rRNA regions

strongly suggests conspecificity or extremely recent divergence. This pattern is consistent with previous studies reporting synonymy or cryptic species complexes within *Mesorhabditis* (Sudhaus and Kiontke, 2007). The extremely low genetic divergence observed between *M. franseni* and *M. vernalis* further supports the hypothesis that these taxa may represent closely related sister species or intraspecific variants. However, given the conservative nature of rRNA loci, additional fast-evolving markers (e.g., mitochondrial COI) and broader morphological comparisons would be required to fully resolve their taxonomic status.

Phylogenetic structure and evolutionary relationships

Both the ML phylogeny and the K2P distance matrix consistently supported the recognition of five major clades within *Mesorhabditis*. The concordance between tree topology and genetic distance patterns reinforces the reliability of the ITS/28S rRNA region as a molecular marker for species delimitation within the genus. Similar phylogenetic structuring has been reported in related rhabditid nematodes, where ITS regions provide sufficient resolution at the species level while maintaining alignment stability (Floyd *et al*., 2002; Blaxter *et al*., 2005). The observed interspecific K2P distances (≈0.08–0.12) fall within the range commonly reported for species-level divergence in nematodes, supporting the validity of current species delimitations (Subbotin *et al*., 2010). The relatively lower divergence between *M. irregularus* and *M. spiculigera* suggests a more recent common ancestry, potentially reflecting adaptive radiation within similar ecological niches.

Integration of molecular and morphological data

The molecular findings of this study are strongly supported by classical morphological identification based on De Man indices and morphometric analyses. The congruence between molecular phylogeny and morphological characters reinforces the taxonomic reliability of the identified species and demonstrates the effectiveness of integrative taxonomy in nematode systematics (Dayrat, 2005; De Ley and Blaxter, 2004). The collection of specimens from rosehip (Rosa canina) roots across multiple districts further suggests that these *Mesorhabditis* species are well adapted to rhizosphere environments. Soil-associated nematodes are known to exhibit both ecological specialization and cryptic diversity, underscoring the importance of combining molecular tools with traditional morphology (Bongers and Ferris, 1999).

Implications for nematode biodiversity and future research

This study provides the first comprehensive molecular characterization of *Mesorhabditis* species from Uzbekistan, contributing valuable data to the global understanding of rhabditid nematode diversity. The results demonstrate that the ITS/28S rRNA region is a reliable marker for resolving phylogenetic relationships at both interspecific and intraspecific levels within *Mesorhabditis*. Future studies should incorporate additional molecular markers, expanded geographic sampling, and ecological data to further elucidate evolutionary processes shaping diversity within the genus. Such integrative approaches will be essential for refining species boundaries, uncovering cryptic diversity, and improving our understanding of nematode evolution in soil ecosystems.

Conclusion

This study provides an integrative molecular and morphological characterization of *Mesorhabditis* species associated with Rosa canina in southern Uzbekistan. Phylogenetic reconstruction based on ITS/28S rRNA sequences, supported by K2P genetic distance analyses, confirmed the monophyly of the genus and resolved five well-defined evolutionary clades with strong statistical support. Low intraspecific and clear interspecific genetic distances demonstrate that the ITS/28S rRNA region is an effective marker for species delimitation within *Mesorhabditis*. While several taxa showed identical or near-identical sequences, suggesting recent divergence or possible taxonomic overlap, *M. franseni* exhibited notable internal genetic structuring, indicating population-level differentiation. The congruence between molecular results and morphological identification validates the integrative taxonomic approach applied in this study. Overall, these findings contribute new molecular reference data for *Mesorhabditis* and improve our understanding of nematode diversity in Central Asia, providing a solid basis for future phylogenetic, taxonomic, and ecological investigations.

Acknowledgement

This article is funded by the project (AK–25–55) implemented within the framework of the "Academic Mobility" program. We express our sincere gratitude to the staff of the laboratories of the Institute of Zoology, Academy of Sciences of Uzbekistan, for providing all necessary technical equipment and

favorable working conditions during the research and the preparation of this article.

Novelty Statement

The analysis results showed that *P. obtusa* (MF288651) exhibited the greatest genetic distance relative to all *Mesorhabditis* species, confirming its selection as an outgroup in the phylogenetic analysis. At the same time, the low genetic distances among species within the genus *Mesorhabditis* indicate their close phylogenetic relationships and the presence of independent evolutionary lineages for some species. The low genetic distances between samples collected from Uzbekistan and corresponding species from other geographic regions allow for a more precise understanding of their taxonomic identity and phylogenetic structure.

Author's Contribution

Turopova Mukhlisa contributed to the conceptualization and design of the study, data collection, and drafting the manuscript. Bekmurodov Abdujabbor contributed to data analysis and interpretation. Amirov Oybek supervised the study, provided critical revisions, and approved the final manuscript. Ismatov Azamat contributed to methodology development and technical support. Egamberdiyev Mehmonjon assisted in data curation and manuscript editing. Jumaqulova Guzal contributed to literature review and referencing.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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