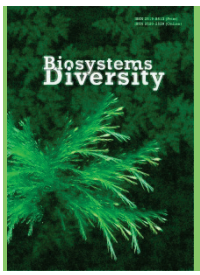

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Morphological and molecular characterization of root-knot nematodes from Uzbekistan

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Plant-parasitic nematodes are of great economic importance as widespread harmful plant pests of the world. Among them, root-knot nematodes, especially species of the genus *Meloidogyne*, cause significant damage to agriculture. In this paper the species composition, morphological and molecular genetic characteristics of root-knot nematodes in vegetable crops of the Zarafshan Valley of Uzbekistan were studied. As a result of morphological studies, three species belonging to the genus *Meloidogyne* were found in Samarkand region of the Zarafshan Valley; these species were *Meloidogyne javanica*, *M. incognita* and *M. hapla* identified by concentric lines in the anal-vulvar part of sexually mature female nematodes. The results show that the anal arch of *M. javanica* and *M. hapla* is low, and that of *M. incognita* is high. In order to additionally characterize the species of the discovered root-knot nematodes, we carried out their molecular identification. However, nucleotide sequence analysis in the 5S–ITS2 region of rDNA showed no differences between *M. javanica* and *M. incognita*, the overall difference being to 0.6%. These data indicate that both these taxa belong to the one species based on 5S–ITS2 region, but this datum should be supplemented by additional research with other genes. The sequence differences between the species *M. javanica* or *M. incognita* with species *M. hapla* amounted to 23%. Species analysis based on the analysis of morphological and molecular genetic indicators of root-knot nematodes is important because it was conducted for the first time in Central Asia. The studies conducted indicate that *M. hapla*, *M. javanica* and *M. incognita* are common on tomato farms in Payaryk, Akdarya and Jambay districts of Samarkand region of Uzbekistan.

Keywords: *Meloidogyne*; root-knot nematodes; tomato; morphology; morphometric; molecular; rDNA, 5S–ITS2, Central Asia.

Introduction

In all countries with developed agriculture, plant nematology has been significantly developed. In recent years, damage to cultivated plants by plant nematodes and crop loss rates in different countries vary from 25% to 70%, and the damage caused is estimated at about 125 billion dollars per year (Chitwood, 2003; Mesa-Valle et al., 2020). Parasitic nematodes cause significant damage to irrigated crops, especially in subtropical and tropical regions with hot and dry climates. In some countries, these nematodes kill from 10% to 20% of the crop, and in severely damaged fields, the entire crop dies (Subedi et al., 2020).

Root-knot nematodes (RKN) are the most common plant parasitic nematodes and the damage caused by them to crops is estimated to be 80–110 billion dollars per year (Nikol et al., 2011; Elling, 2013; Jones et al., 2013). Tomato (*Solanum lycopersicum*) products in Ghana are devastated by RKN and yield losses about 70% have been experienced in farmers' fields (Nyaku et al., 2018; Oluwatoyin, 2022). RKN occur in Turkey and parasitise potatoes, causing significant economic damage to potato production (Demirbaş et al., 2020).

RKN are very diverse; according to some data, there are more than 90 species of plant-damaging nematodes in the world (Hunt & Handoo, 2009; Janssen et al., 2017). Four nematode species (*Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*) are the most important nematode pests in tomato fields (Jacquet, 2005). The first three species are widely distributed in the warmer tropical regions of the world; however, *M. hapla* is more prevalent in the temperate regions (Naz et al., 2012).

In Uzbekistan, an infection of plants by RKN was first established in 1935. In the studies conducted in the following years, their distribution in the fields of Tashkent, Namangan, Fergana and Surkhandarya regions and the Republic of Karakalpakstan was established. Five species of RKN (*Meloidogyne hapla*, *M. incognita*, *M. javanica*, *M. arenaria*, *M. acrita*) have been found in Uzbekistan according to morphometric study (Narzullayev, 2022). It is important to choose the correct and accurate method to identify different species of nematodes, including RKN (Den Nijs & Van den Berg, 2012). RKN and other nematodes have been identified based on morphological characters, host-plant response, isozyme analyses and molecular techniques (Kiewnick et al., 2014; Álvarez-Ortega et al., 2019; Sato et al., 2019; Kuchboev et al., 2020; Ibrokhimov et al., 2023). Currently, a number of molecular methods have been developed to identify species of RKN. Among them, polymerase chain reaction (PCR) based diagnostics have provided rapid, accurate, and sensitive tools for the detection of RKN (Kiewnick et al., 2014). Recently, about 50 species of *Meloidogyne* have been molecularly identified with ribosomal DNA (two expansion segments (D2 and D3) of the 28S rDNA, ITS1 and 18S) and mitochondrial DNA genes (COI, COII, 16S) as a tool for species identification (Adam et al., 2007; Janssen et al., 2017; Archidona-Yuste et al., 2018). Adam et al. (2007) developed a useful molecular diagnostic key for identification of seven economically important RKN species (*M. javanica*, *M. incognita*, *M. arenaria*, *M. mayaguensis*, *M. hapla*, *M. chitwoodi*, *M. fallax*) commonly found in many nematological research and diagnostic laboratories. This key uses several molecular diagnostic techniques to identify these seven common species.

In the recent past, the identification of species of RKN was based on the study of their morphological characteristics, in particular the size of the body, the head capsule, the stylet and the structure of the lines on the anal-vulvar plate. However, the microscopic size of nematodes and the similarity of morphological features between species cause significant difficulties in classifying root knot nematodes (Brito et al., 2004). In particular, in 2017, based on phylogenetic analysis based on mtDNA fragments, a species belonging to this genus, *M. aberrans*, was recorded as a new species for science (Tao et al., 2017), while in 2019, rDNA and mtDNA analyses of *M. natalie* proved it to be an independent species (Álvarez–Ortega et al., 2019). Morphological, morphometric, and DNA sequence analyses were performed to identify species of RKN in the central and

western zones of Punjab, India. A result of the study, *M. javanica*, *M. incognita* species were identified in the Central agroclimatic zone (Kaur et al., 2016).

The objective of our study is morphometric and molecular characterization of RKN in vegetables crops of Samarkand region of the Zarafshan Valley, Uzbekistan.

Materials and methods

Study area and data collection. Materials were collected in 2018–2021 in vegetable crop fields of farms and household plots in Akdarya, Jambay and Payaryk districts of Samarkand region of the Zarafshan Valley (Fig. 1, Table 1). Samples for morphological and molecular study used nematodes from tomato roots infected with RKN.

Morphometric analysis. Concentric lines in the anal-vulvar portion of sexually mature female nematodes are the main criterion for morphological identification of species. Differences between species are determined based on morphometric parameters (Bogali et al., 2020). The location on the cuticle around the anus and vulva, i.e., the anal-vulvar plate in female nematodes of concentric lines, is often used to determine species of RKN (Manzoor et al., 2022).

For morphological research, samples of damaged roots were fixed in 6–7% formalin solution. Identification of nematode species was performed based on morphological characters. For this purpose, permanent preparations in glycerol-gelatine and temporary preparations in glycerol were prepared from the anal-vulvar plate of 10 sexually mature female nematodes. For this purpose, one part of the affected root was placed in a Petri dish with water and female nematodes that had begun to lay eggs were extracted from the root using an entomological needle under a stereoscopic microscope. The part of the body where the nematode's anus and vulva were located was then cut with an ophthalmic scalpel and transferred to a watch glass with a lactophenol solution (1:2:1 mixture of a lactic acid, glycerol, distilled water, and some crystals phenol). Under a microscope, the specimen in solution was cleaned of internal organs and left overnight in lactophenol solution and pure glycerol. Permanent preparations in glycerol-gelatine and temporary preparations in sterile glycerol were made to study cuticle lines. At the same time, morphometric measurements of adult females and their larvae were performed (Manzoor et al., 2022).

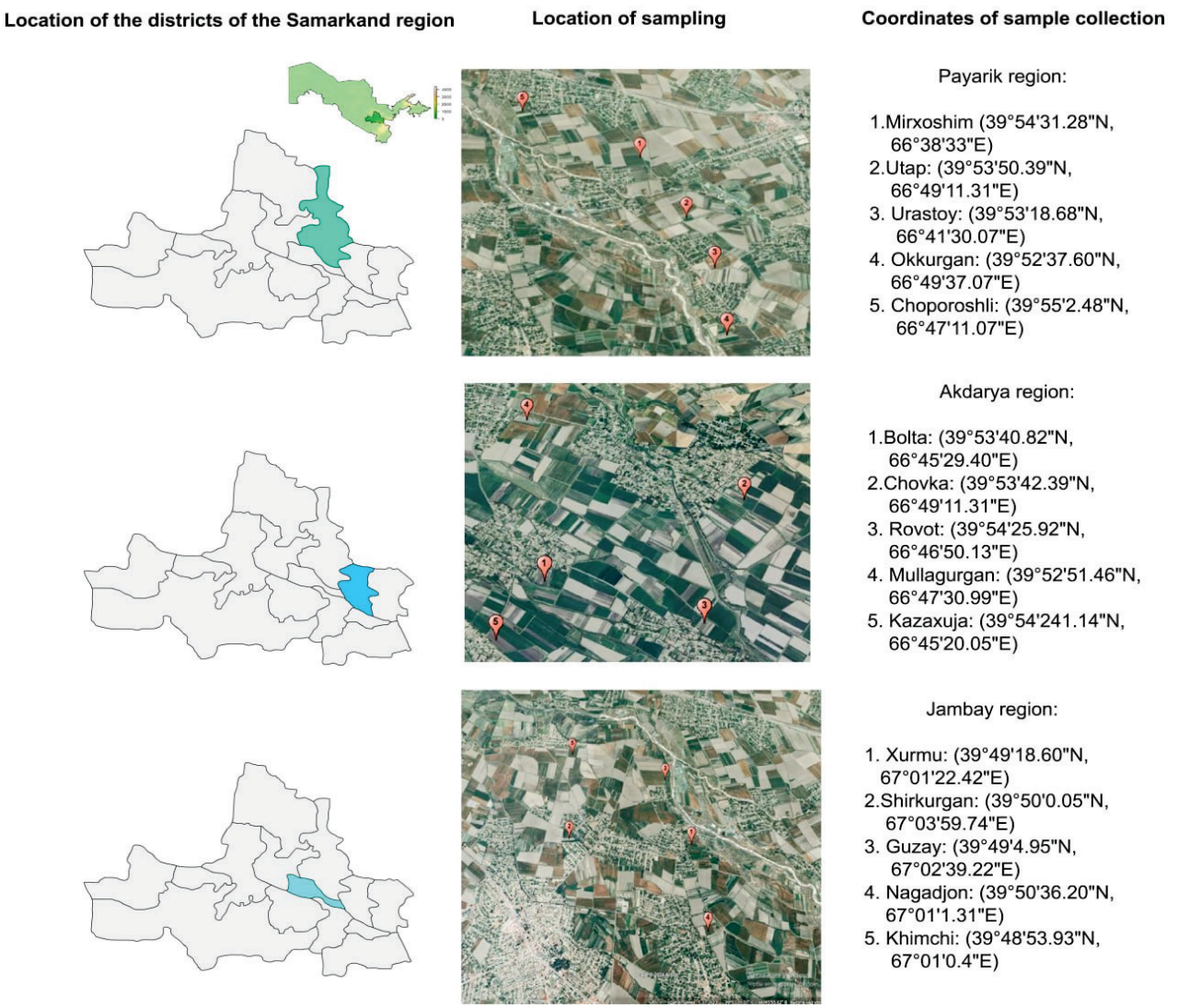

Fig. 1. Map with locations and coordinates of sample collection of the districts of the Samarkand region in Uzbekistan

Concentric lines on the cuticle around the anus and vulva of an adult female nematode, that is, on the anal-vulvar plate, were used to identify the species of nematodes based on their morphological characteristics (Mehlhorn, 2008; Eisenback & Triantaphyllou, 2020). Usually, in the cuticle of the anal-vulvar plate of female cyst-forming nematodes, concentric ridges (stripes) similar to fingerprints surround the anus and vulva. Side lines cross the sides of the forks on both sides. The pedicles located above the lateral lines form the anal arch, and the pedicles located below them form the ventral arch.

Most of the measurements were recorded using a 40^x and 100^x objective lens. Only for curved structures that could not be measured accurately with ocular micrometer was the camera lucida method used. The following measurements (with codes) were recorded in different stages of *Meloidogyne* sp.: L = total body length (μm), MBW = maximum body

width (μm), STYL L = stylet length (μm), DPGO = distance from stylet knobs to opening of dorsal pharyngeal gland (μm), EXC PORE = distance from anterior end to excretory pore (μm), PHX–C = distance from anterior end up to cardia (μm), PHX–G = distance from anterior end up to gland overlap (μm), TL = tail length (μm), HYL TL = length of hyaline portion of tail (μm), ABW = anal body width (μm), SPL = length of spicules (μm), GUB = length of gubernaculum (μm), VUL L = vulval length (μm); IPD = interphasmidial distance (μm); AVD = distance between anus and vulva (μm) de Man's ratios i.e., a, b, b′, c, and c′ were calculated (Bogale et al., 2020).

Table 1

Root-knot nematode populations and their host plants, location, coordinates and altitudes from samples collected in research area

Species	Code	Locality (region/district)	Host plant	Latitude (N)	Longitude (E)	Altitude, m
M. hapla	hap1	Samarkand/ Akdarya	tomato	39º58'06"	66º43'03"	546
	hap2	Samarkand/ Jambay	tomato	39º43'41"	67º04'47"	671
M. javanica	jav1	Samarqand/ Akdarya	tomato	39º53'48"	66º41'10"	553
	jav2	Samarkand/ Jambay	cucumber	39º42'43"	67º03'52"	663
	jav3	Samarqand/ Jambay	bell pepper	39º42'43"	67º03'52"	663
M. incognita	inc1	Samarkand/ Payaryk	tomato	39º59'18"	66º51'14"	609
	inc2	Samarkand/ Payaryk	bell pepper	39º59'18"	66º51'14"	609

DNA extraction, PCR amplification, sequencing, and statistical analysis. For molecular study of RKN samples stored in a 70% alcohol solution were used. Samples were washed with distilled water and from adult female nematodes genomic DNA was isolated. DNA extraction was done using the DNeasy & Tissue Kits (Qiagen) following the manufacturer's instructions. Genomic DNA aliquots stored at –20 °C in 1.5 mL tubes.

PCR was performed in a 25 μL volume using 2 μL of extracted DNA solution and 25 pmol of each of the primers TW81 (5′- GTTTCC GTAGGTGAACCTGC - 3′) and AB28 reverse (5′ - ATATGCTTA AGTTCAGCGGGT- 3′) for amplification of domain of 5,8S-ITS2 rDNA (Ikromov et al., 2020). The PCR mix contained 3 mM $MgCl_2$, 1xPCR buffer, 0.2 mM each dNTP, and 0.25 μL (1.25 units) of Taq polymerase (Qiagen, Germany). The PCR was performed according to the following scheme: stage 1, DNA denaturation at 94 °C for 5 minutes; stage 2, DNA denaturation at 95 °C for 45 seconds; stage 3, DNA primer softening at 55 °C for 45 seconds, stage 4, elongation for 1 minute 40 seconds at 72 °C; and stage 5, chain elongation for 5 minutes at 72 °C. From the second to the fourth step, the process is repeated up to 35 times per cycle.

The presence of DNA in PCR products was determined by electrophoresis at 120 V in a 1.0% agarose gel. We used kits of reagents produced by Silex Company (Russia) by the manufacturer's instructions.

Sequencing was carried out at the Institute of Molecular Biology of the Russian Academy of Sciences (Moscow). The chromatograms (in ab1 format) obtained from the center was analyzed using the Chromas 2.6.6 program (Technelisium Ltd. South Brisbane, Australia, 2018) and translated into fast format. The resulting nucleotide sequences were analyzed using the BLAST algorithm, which made it possible to determine the range of species of parasitic organisms closest to the studied forms. The nucleotide sequence was analysed using special programs Bioedit (Hall, 1999) and Clustal W (Thompson et al., 1994).

Phytohelminthological, comparative morphological, morphometric, and ecological methods of investigation, as well as program Biostat, 2007 3.8, Statistica v.6.0 (Stat Soft Inc. USA, 2007) were used during the studies.

Results

As a results of morphological study of RKN on tomato farms in districts of Samarkand region three species were recorded belonging to the genus *Meloidogyne*; the Javanese RKN – *M. javanica*, the northern RKN – *Meloidogyne hapla* and the southern RKN – *M. incognita*. The *M. incognita* were detected in samples taken from Payaryk district, and the other two species, *M. hapla* and *M. javanica* were isolated from samples collected from vegetable crops of Akdarya and Jambay districts (Table 1, Fig. 1). The obtained morphometric data on the structure and size of females, males and their larvae of the three compared species did not reveal any features characteristic of a particular species (Fig. 2). Observations in spring and summer showed no signs of damage to tomato roots by any certain species.

Only in the last days of the summer and fall observations was it found that the galls formed by the nematode *M. hapla* were slightly smaller than those of the other two species (Fig. 2c). However, this difference may be due to the intensity of plant root damage. For this reason, morphometric parameters of RKN and visual signs of tomato root damage may not always be reliable evidence for determining the species of RKN.

An adult female nematode that begins to lay eggs has concentric grooves (lines) in the anal-vulvar plate's cuticle surrounding the anus and vulva, resembling fingerprints. The furrows are crossed on both sides by lateral lines. The furrows above the lateral lines form the anal arch, and the furrows below them form the abdominal arch (Fig. 3). If the distance between the vulva and the uppermost sulcus is 2.7 times the vulval opening length, then the anal arch is considered high, and if this distance is less than 2.3 times, the anal arch is low.

Among the species found on tomatoes, the anal arch of *M. javanica* and *M. hapla* is considered low, and that of *M. incognita* is high. The following identifies gall nematode species damaging tomatoes in Samarkand region based on the structure of concentric grooves in the cuticle of the anal-vulvar plate.

1 (5). The anal-vulvar arch is low. Lines on the cuticle of the anal-vulvar plate are smoothly curved to the sides.

2 (3). Two lateral lines crossing concentric lines of the anal-vulvar plate are very well visible. The lines often extend beyond the concentric lines of the anal-vulvar plate in Javan RKN (*M. javanica*, Fig. 3b, 4c, 4d).

3 (2). The lateral lines are poorly visible but they can be identified by irregular fractures of concentric lines in the cuticle of the anal-vulvar plate.

4 (5). Near the appendages of the tail, a dotted structure is visible. Concentric lines at lateral lines of the anal-vulvar plate are smooth or slightly wavy and somewhat haphazard. Sometimes in the area of lateral lines, the upper caudal and lower anal arches join and form an angle, in the northern RKN (*M. hapla*, Fig. 3a, 4a, 4b).

5 (1). Arches of the anal-vulvar plate high. Arcs of the anal-vulvar plate have angular or irregularly angular shapes and consist of many wavy or irregularly wavy lines – southern RKN (*M. incognita*, Fig. 3c, 4f, 4g).

Meloidogyne hapla Chitwood, 1949. Females: The length of the body with the neck is 678–1200 μm, the width is 305–560 μm (Fig. 2a). Neck length 102–215 μm, width 68–82 μm; stylet 14–16 μm. The body is pear–shaped. The cuticle is weakly ringed. The inside of the head is sclerotized. The head cuticle consists of two rings. The length of the red tibia is 78–112 μm, its posterior part is expanded; compressed in front of the metacorporal bulb. Metacarpal bulb is spherical, strongly sclerotized. The outlet of the dorsal laryngeal gland opens into the esophagus 4.6–5.0 μm from the stylet. Stylet 14–17 μm; its rounded head is 2 μm high and 4 μm wide.

The arc of the anal-vulvar plate is low, flat and circular from the dorsal side (Fig. 3a). Lateral lines are not distinct, but may have branched lines instead; the arcs of the anal – vulvar plate are almost uniformly circular (Fig. 4a, b).

Larvae of the second age: L = 450–470 μm; a = 8–13; b = 7.4–7.7; c = 8–9. Cuticle with small rings. The height of the head capsule is 1.7–2.1 μm, the width at its base is 5–6 μm. The dorsal gland hole opens into the esophageal cavity at a distance of 3–4 μm from the stylet head. The tail is 47–50 μm, the tip is conically sharpened (Fig. 2b).

Fig. 2. A root-knot nematodes and tomato roots damaged by them: *a*, *b*, *c* – *Meloidogyne hapla*; *d*, *e*, *f* – *M. javanica*; *g*, *h*, *i* – *M. incognita*; *a*, *d*, *c* – female nematodes; *b*, *e*, *h* – their larvae; *d*, *f*, *i* – damaged tomato roots

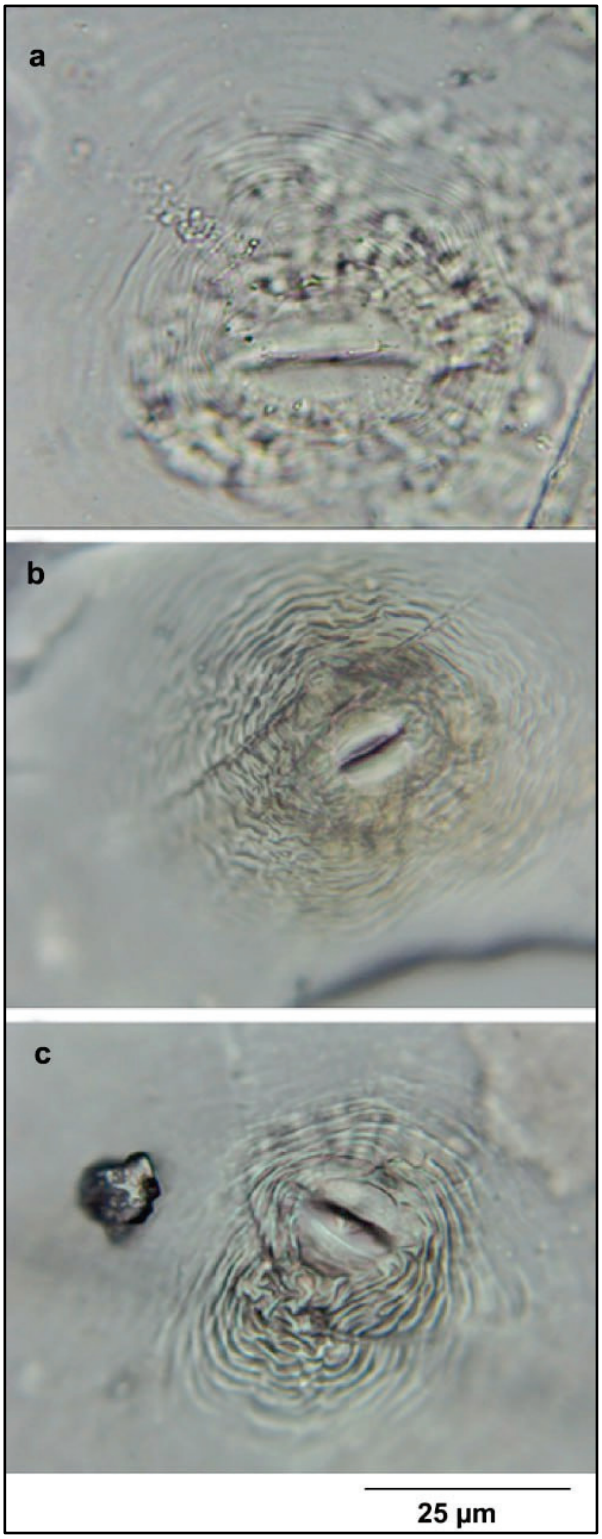

Fig. 3. Microphotography at the same magnification of a perineal pattern of a female of the root-knot nematodes: *a – Meloidogyne hapla*; *b – M. javanica*; *c – M. incognita*

Meloidogyne javanica Chitwood, 1949. Females: L = 700–1300 µm, width 311–581 µm (Fig. 2d). The body is pear-shaped, rarely spherical with a protruding neck. The rear end of the body is smoothly rounded and slightly elongated. Head capsule height 14.4–16.8 µm, one ring. Stylet head ovate is 1.4–2.6 µm high, 3.8–5.2 µm wide. The separation opening of the dorsal gland is located at a distance of 30.0–43.0 µm from the tip of the head. The distance between phasmids is 11.8–22.0 µm. The anal-vulvar plate is usually round in shape (Fig. 3b). Side lines are very clearly visible; four lines. Lateral lines divide the arches of the anal-vulvar plate into dorsal and ventral sectors. There are several lines around the vulva and anus, several smooth and flat, slightly wavy lines under the vulva, several smooth wavy lines, and interrupted wavy lines (Fig. 4 c, d). The tail rudiment is located at a distance of 10.9 (8.6–12.0) µm from the anal opening.

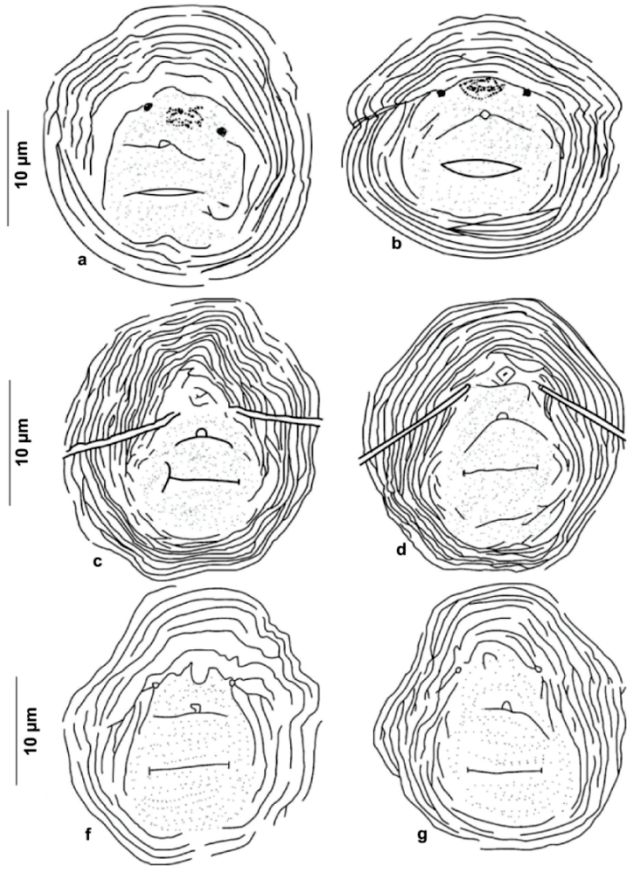

Fig. 4. Structure of lines in the cuticle of the anal-vulvar plate of root-knot nematodes: *a, b – Meloidogyne hapla*; *c, d – M. javanica*; *f, g – M. incognita*

Larvae of the second age: L = 450–560 µm; a = 28–35; b = 5.9–7.2; c = 6.5–11.3 (Fig. 2e). The head is not clearly separated from the body. Stylet length 10.5–13.6 µm, width 1.8–3.0 µm, head height 1.2–2.0 µm. Phasmids are located in the middle part of the tail, at a distance of 24.0–37.0 µm from the tip of the tail. The separation hole is located at a distance of 84.5 µm from the tip of the head. The length of the tail is 42.8–68.5 µm; the tip is slightly pointed. The width of the body in the area of the anal opening is 5.7–11.1 µm.

Meloidogyne incognita Chitwood, 1949. Females: L = 670–900 µm, width 300–480 µm (Fig. 2g). Head capsule 2 µm high, 4–5 µm wide; front end not protruding, smooth. The dorsal laryngeal duct opens into the larynx at a distance of 2–4 µm from the head of the stylet. The cuticle of the tail rudiment area does not have a punctate structure. The anal-vulvar plate is oval irregularly oval in shape (Fig. 3c). Plate arches are high, often asymmetrical in structure; folds are located close to each other in the form of additional lines. The tail rudiment forms a curly fold. The lateral lines are indistinct, with the appearance of short double lines (Fig. 4f, 4g).

Larvae of the second age: L = 360–400 µm; a = 30–33; b = 5.8–6.6; c = 8.2–9.2 (Fig. 2h). There are 4 cuticular rings on the head. Heads are round, 1.5 µm high, 2 µm wide. The dorsal laryngeal duct opens into the esophageal cavity at a distance of 2.0–2.5 µm from the stylet head. The length of the tail is about 50 µm, the tip is flat. The hyaline part of the tail is 8–9 µm.

Morphological studies indicate that *M. hapla*, *M. javanica* and *M. incognita* are common among tomatoes in the Zarafshan valley of Uzbekistan.

Family Meloidogynidae Skarbilovich, 1959

Genus *Meloidogyne* Coeldi, 1887

Species: *Meloidogyne hapla* Chitwood, 1949

Meloidogyne incognita Chitwood, 1949

Meloidogyne javanica Chitwood, 1949

Molecular analysis of species.

To reliably estimate the species of the detected RKN in the Zarafshan Valley, Samarkand region, it is advisable to carry out their molecular identification.

The gall nematodes were identified as *M. incognita*, *M. javanica*, and *M. hapla* based on the structure of concentric lines in the aforementioned anal-vulvar plate and the nucleotide sequences 5,8S–ITS2 rDNA fragments were compared and differences between these species were established for molecular studies (Fig. 5). The results of the molecular genetic studies performed were compared with information from the Genbank of the National Center for Biotechnology Information (NCBI) to clarify the species. As a result, the differences in nucleotide arrangement between *M. javanica* and *M. incognita* consisted of three nucleotides, i.e., the difference was 0.6% (Fig. 4). For this reason, the affirmation of *M. incognita* and *M. javanica* belongs to the same species based only on 5,8S–ITS2 region of the rDNA and this requires further study. The differences between the following species *M. javanica* and *M. hapla*, consisted of 117 nucleotides, which was 22.9%; the differences between *M. incognita* and *M. hapla* consisted of 116 nucleotides, which was 22.5%. This explains that *M. hapla* is a valid species.

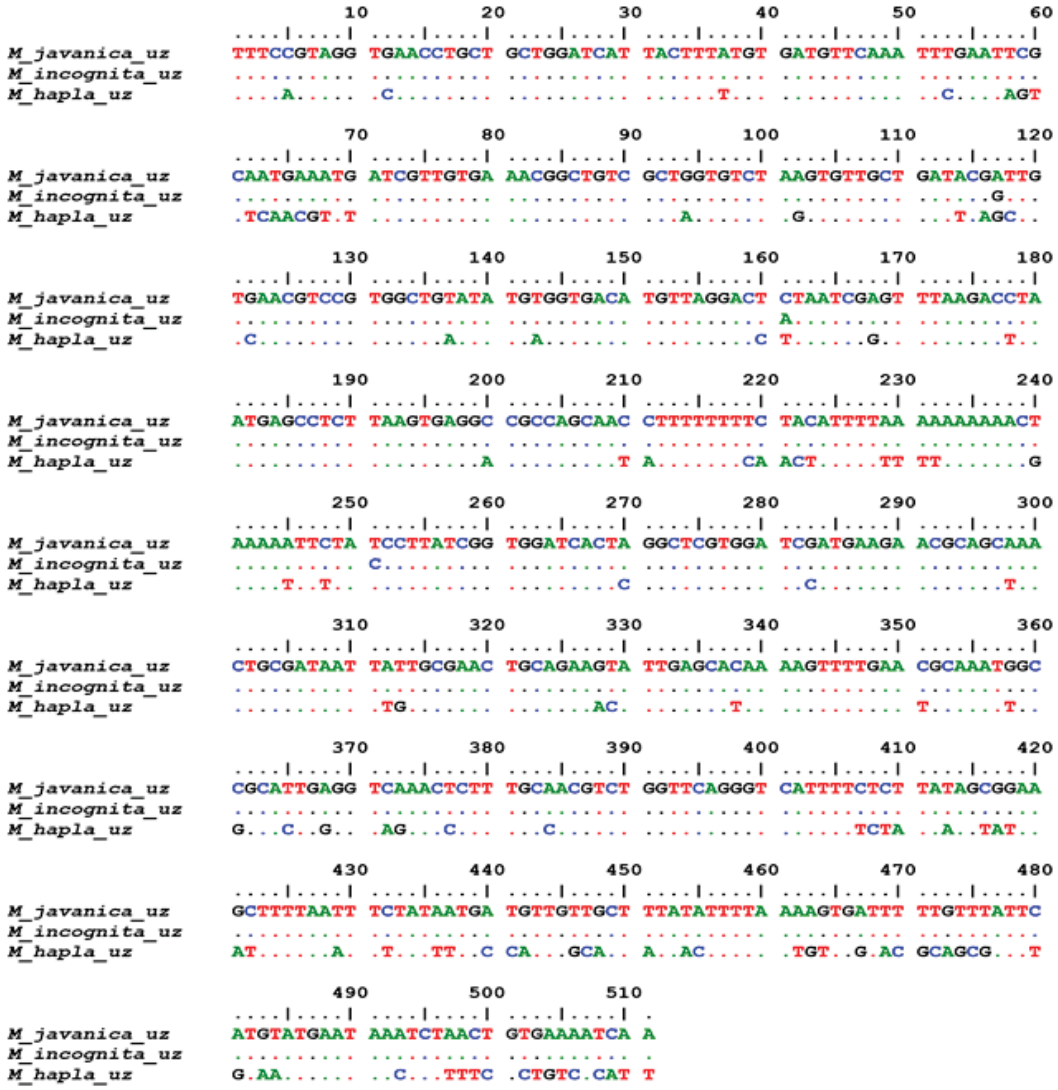

Fig. 5. Comparison of nucleotide sequence of fragments of the 5,8S–ITS2 region of ribosomal DNA samples of *M. javanica*, *M. incognita* and *M. hapla* (direction from 5' to 3'– end)

Discussion

Studies in the Zarafshan Valley so far have reported only one species of RKN (*M. hapla*) (Narzullayev, 2022). In these studies, the identification of nematode species was based solely on morphometric parameters. Despite the similarity of climate and soil composition, species not found in Uzbekistan have been recorded in other Central Asian countries (for example, *M. artiellia* Sagitov, 1987). However, these results were not corroborated by the PCR method.

Modern methods of controlling plant-parasitic nematodes require that they be based on data on their systematics and biology. The RKN species are morphologically very similar to each other and identification to the species level is difficult. Therefore, DNA analysis is required in conjunction with the morphological method to identify these nematode species. Brito et al. (2004) recorded that the RKN *M. mayaguensis* was first discovered in Florida, USA; the concentric lines on the anal-vulvar plate of this species are morphologically similar to *M. incognita*. To distinguish the species from each other, the 16S mtDNA gene, the 18S gene in rDNA, and the ITS1 gene of the nuclear genome in *M. mayaguensis* isolates were studied, obtained in Puerto Rico, and their sequence compared. Intraspecific variability was not observed. The molecular identification of *M. mayaguensis* is necessary because *M. mayaguensis* occurs together with *M. arenaria*, *M. incognita* and *M. javanica* in populations. Because of some difficulties in morphological identification of these species, molecular identification is considered economically crucial.

Powers (2005) conducted nematode studies, including morphological and molecular studies, in potato fields in the Central USA. As a result, no significant differences were found when comparing the nucleotide sequence of the 18S rDNA gene of *M. arenaria*, *M. incognita* and *M. javanica*; however, differences in the mtDNA gene region provided greater spe-

cies specificity, and intraspecific variation was observed among many isolates. None of the mtDNA variants identified during the study resulted in a change in the PSR/RFLP protocol used to identify *M. chitwood*. At the same time, the author recommends using new markers in species identification and evaluation.

The sequence and results of the primers used to identify RKN and other nematodes are consistent with the results of previous studies (Correa et al., 2013; Janssen et al., 2017; Kuchboev & Krücken, 2022). The species analysis we conducted based on the analysis of morphological and molecular genetic indicators of root-knot nematodes is important because it was conducted for the first time in Central Asia.

Conclusion

In this study, three species *Meloidogyne javanica*, *M. incognita* and *M. hapla* belonging to the genus *Meloidogyne*, collected in the Samarkand region of the Zerafshan Valley of Uzbekistan, were identified based on morphological characteristics. Molecular genetic studies showed that the differences between *M. javanica* and *M. incognita* consisted of three nucleotides, with a total difference of 0.6%. These data indicate that both taxa belong to the one species based on 5S–ITS2 region, but this datum requires to be supplemented by additional research on other genes. The difference between *M. javanica* and *M. hapla* consisted of 117 nucleotide differences, which is 22.9%; the difference between *M. incognita* and *M. hapla* consisted of 116 nucleotide differences, which was 22.5%. To evaluate the validity of these species, we recommend additional studies using additional genetic markers. The nucleotide sequence data obtained from the studies were placed in the GeneBank (NCBI), and access numbers were obtained (*M. javanica* – ON150871; *M. hapla* – ON150876, ON150878; *M. incognita* – ON197188).

The cost of the research was borne by the researchers themselves. We thank the junior scientist Humoyun Sobirov at the laboratory of Molecular Zoology of the Institute of Zoology of the Academy of Sciences of Uzbekistan for help with the technical work and collection of biological materials.

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