Research Article

Morphological and Molecular Studies Nematode of the Species *Parascaris equorum* (Ascaridida)

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Abstract | This article presents the results of research on the morphometric and molecular characteristics of the nematode *Parascaris equorum* collected from horses of the Fergana Valley, Uzbekistan. The total length of the male *Parascaris equorum* nematode was 175.5±3.86 mm, and that of the female was 293.7±4.83 mm. For molecular genetic studies from the collected samples, genomic DNA was extracted using the head of male of *P. equorum* species. QIAamp DNA Mini Kit reagents (QIAGEN, Germany) were used for genomic DNA isolation. According to the results of molecular genetic study, the nucleotide sequence belonging to the ITS1-5.8S-ITS2 region of the ribosomal DNA of *P. equorum* species was analyzed, and it was found that 100% nucleotide similarity with *P. equorum* species (MH030605) and 95.85% similarity with the species *P. univalens* (MK209648) in the GenBank database (NCBI).

Keywords | Nematode, Horse, Helminth, Ribosomal DNA, ITS2, *Parascaris equorum*

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INTRODUCTION

Horses are susceptible to various diseases, and many invasive diseases are recorded in them. Helminths are parasites in various organs and tissues, causing serious damage to animal health. It also causes rapid fatigue, reduced work efficiency and endurance, and reduced growth (Kanijazov and Shakarboev, 2023), and development of young animals (Arkhipov *et al*., 2017; Skrjabin and Petrov, 1964).

Parascaris equorum is a nematode of the *Parascaris* (Yorke and Maplestone, 1926) genus that infects horses and has been found in Sudan, Egypt, Iran, Great Britain, Australia, China and other countries (Beasley *et al*., 2015; Chang *et al*., 2015; Easton *et al*., 2016; Ismail *et al*., 2016; Morsy *et al*., 2016; Tavassoli *et al*., 2016). Severely infected horses, especially foals, with *P. equorum* nematode cause enormous economic losses to livestock farms (Khasanova, 2013). Generally, parascariasis is caused by the nematode *P. equorum*, but *Parascaris univalens* has been reported as the dominant species affecting horses in the United States, Switzerland, and Sweden (Jabbar *et al*., 2014; Martin *et al*., 2018). Ingestion of infective eggs in the environment is the major route of transmission (Boyle and Houston, 2006).

Moreover, this species is one of the rare nematodes which induce absolute acquired immunity (Craig *et al*., 2007) and is a common and ubiquitous parasite that persists for many years in stables and on pasture in spite of good hygiene and anthelmintic control programs. These worms cause various degrees of damage, and they decrease the performance,

production, and productivity in the animals mainly in the reduction of body weight or failure to gain weight or even increase the mortality in acute case (Boyle and Houston, 2006).

Parascaris equorum species is widespread in horses of the Ferghana Valley and was detected in 22 of 83 fully helminthologically examined horses. The intensity of invasion was 26.5%.

These identified species *P. equorum* and *P. univalens* are morphologically identical, and these species can be identified by molecular genetic analysis (Nielsen *et al*., 2014; Goday and Pimpinille, 1986). Morphological and molecular genetic analysis of *P. equorum* and *P. univalens* species in some literatures revealed that they belong to the same species (Gao *et al*., 2018).

Currently, molecular taxonomy methods are used to identify nematode species. By analyzing the nucleotides of the ITS region of the ribosomal DNA of nematodes, it is determined that the species are independent species (Ibrokhimov *et al*., 2023; Ikromov *et al*., 2023; Kuchboev *et al*., 2020; Dallas *et al*., 2000; Zarlenga *et al*., 1998).

The aim of this research work is the morphometric and molecular genetic analysis of the nematode *Parascaris equorum*, belonging to the genus *Parascaris* parasites of horses of the Fergana Valley of Uzbekistan.

MATERIALS AND METHODS

Collection of genetic material

In order to carry out the research work, the nematode species *P. equorum* belonging to the genus *Parascaris* was collected from 22 horses from the regions of the Fergana Valley of the Republic of Uzbekistan (Figure 1).

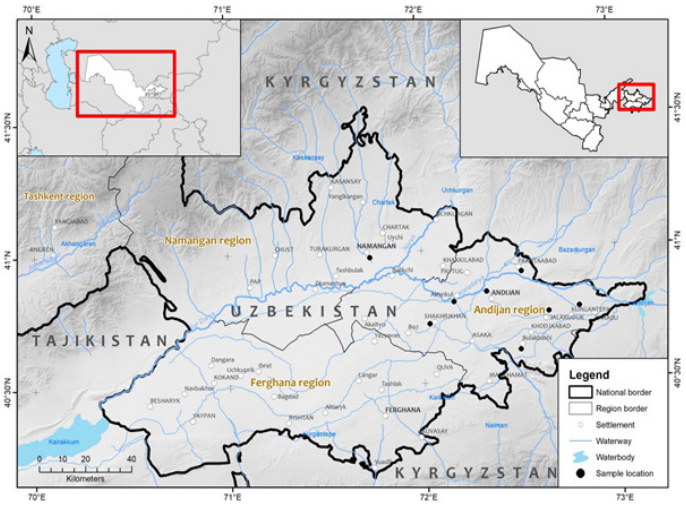

Figure 1: *Parascaris equorum* nematode collection areas (Ferghana Valley, Uzbekistan).

Complete and incomplete helminthological dissection method of Skrjabin (1928) was used for collecting helminthological samples. From 2 to 52 samples were collected from the small intestines of horses. Before dissecting a dead horse, its body is examined, then the skin is removed and the subcutaneous tissues are examined, the abdomen and chest are opened in turn, and each organ is placed in separate containers (buckets). Small and large intestines are placed in some containers and water is poured. Then the intestines are thoroughly washed and the dung inside is put into a bowl.

The works of Ivashkin and Dvoinos (1984) were used for the morphological identification of the collected helminthological samples. Helminthology, comparative morphology, morphometry research methods (Demidov, 1987; Ivashkin *et al*., 1971) and Biostat 2007 programs were used during the work. NSZ-405 (HDCE-X5N) microscope was used for species identification.

Molecular genetic method

For molecular genetic studies from the collected samples, genomic DNA was extracted using the head of male of *P. equorum* species. QIAamp DNA Mini Kit reagents (QIAGEN, Germany) were used for genomic DNA isolation.

Primers that read nucleotides belonging to the ITS1-5.8S-ITS2 region of ribosomal DNA, which are widely used in the molecular genetic identification of nematodes, were used for polymerase chain reaction (PCR) (Subbotin *et al*., 2001). In the PCR, 16,1 µl of water, 2 µl of 10x PCR buffer, 0,4 µl of dNTP, 2 µl of each primer (TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) and reverse primer AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′), 0.4 µl of Taq polymerase were added to make a total mixture of 20 µl. PCR was performed in the following steps; at 98 °C for 30 s, 40 cycles of 98 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s were followed by a final incubation at 72 °C for 10 min (Kuchboev et Krücken, 2022).

The presence of DNA in PCR products was determined by electrophoresis on a 1,0 % agarose gel with a voltage of 100 V. DNA amplification and DNA extraction from the gel were performed using a reagent kit manufactured by Silex M (Moscow, Russia) following the manufacturer's instructions.

DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit, and reaction products were sequenced at GATC Biotech AG.

For the phylogenetic analysis of the species *P. equorum* and *P. univalens* belonging to the genus *Parascaris*, which are the object of research, in the course of our scientific research, the sample collected from the Fergana Valley

region and the ITS gene sequence of 36 species belonging to this genus in the National Center for Biotechnology Information (NCBI, GenBank) were used. The resulting sequences were compared using the T-Coffee multiple sequence alignment tools software tool (https://mafft.cbrc.jp/alignment/software/source.html). The data obtained as a result of the comparison were edited using the Geneious Prime 2024.0.3 (free version) program, and based on them, a maximum likelihood (ML) phylogenetic tree was built using the IQ-TREE -1.6.12 program (Trifinopoulos *et al*., 2016). The phylogenetic tree was visualized using iTOL web software (https://itol.embl.de/login.cgi) (Letunic and Bork 2021). The species *Ascaris suum* (MH030604) was taken as an outgroup (Table 1).

Table 1: Species of the genus *Parascaris* from the Genbank database.

S. No.	Nematode species	ITS	Country
1.	*Parascaris equorum*	PP373800	Uzbekistan
2.	*Parascaris equorum*	MH030605	USA
3.	*Parascaris equorum*	JN617987	China
4.	*Parascaris equorum*	OM876362	China
5.	*Parascaris equorum*	OM876361	China
6.	*Parascaris equorum*	OM876360	China
7.	*Parascaris equorum*	MT579850	China
8.	*Parascaris equorum*	MK209647	China
9.	*Parascaris equorum*	MK209646	China
10.	*Parascaris equorum*	MG882035	China
11.	*Parascaris equorum*	MG882021	China
12.	*Parascaris equorum*	MG882015	China
13.	*Parascaris equorum*	MG882023	China
14.	*Parascaris univalens*	MZ577207	Germany
15.	*Parascaris univalens*	MZ577206	Germany
16.	*Parascaris univalens*	MZ577195	Germany
17.	*Parascaris univalens*	MZ577205	Germany
18.	*Parascaris univalens*	MZ577204	Germany
19.	*Parascaris univalens*	MZ577202	Germany
20.	*Parascaris univalens*	MZ577186	Germany
21.	*Parascaris univalens*	MZ577185	Germany
22.	*Parascaris univalens*	MZ577190	Germany
23.	*Parascaris univalens*	MZ577188	Germany
24.	*Parascaris univalens*	MZ577199	Germany
25.	*Parascaris univalens*	MZ577200	Germany
26.	*Parascaris univalens*	MZ577198	Germany
27.	*Parascaris univalens*	MZ577201	Germany
28.	*Parascaris univalens*	MZ577192	Germany
29.	*Parascaris univalens*	MZ577187	Germany
30.	*Parascaris univalens*	MZ577196	Germany
31.	*Parascaris univalens*	MZ577193	Germany
32.	*Parascaris univalens*	MZ577191	Germany
33.	*Parascaris univalens*	MZ577194	Germany
34.	*Parascaris univalens*	MZ577197	Germany
35.	*Parascaris univalens*	MZ577184	Germany
36.	*Parascaris univalens*	MZ577189	Germany
37.	*Parascaris univalens*	MZ577203	Germany

RESULTS AND DISCUSSION

Morphological and morphometric analysis

The total body length of *Parascaris equorum* for male nematodes is 152-197 (average 175.5±3.86) mm and the width of the body is 4.7-5.2 (average 4.93±0.045) mm. Spicules are uniform, 2.2-2.5 (average 2.4±0.035) mm long. The stoma is surrounded by three large lips, divided into one dorsal and two lateroventral lips. There is a difference in the structure of the lips of male and female nematodes, and the lips of females are larger.

The length of females was 274-320 (average 293.7±4.83) mm and width 7.6-8 (average 7.85±0.038) mm. The vulva is located in the second quarter of the body, 65-70 (average 67.3±0.44) mm from the tip of the head. Egg length 0.08-0.099 (average 0.089±0.002) mm, width 0.075-0.089 (average 0.079±0.001) mm (Table 2, Figure 2).

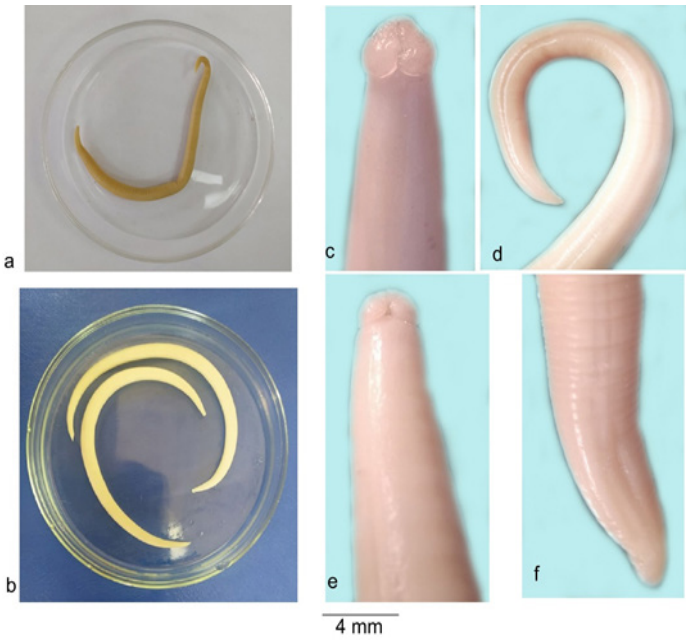

Figure 2: Appearance of the nematode *P. equorum*. Explanation: a, general appearance of the male; b, general appearance of the female; c and d, head and tail of a male; e and f, head and tail of a female.

These morphometric data are close to the research conducted by Morsy *et al*. (2016), in which the length of male *P. equorum* nematode is 12-15 (average 14±2) cm, and the length of females is 13-18 (average 16±2) cm. However, the morphometric differences in the obtained results can be explained by the dependence of the nematodes on the ecological habitat of the host.

In order to identify these species at the species level, it is important to study the nucleotides of the ITS region of their ribosomal DNA.

Table 2: Morphometric dimensions of the nematode species *Parascaris equorum*, (mm), n=10.

Signs	Lim	(M±m)
Male nematodes		
Body length	152-197	175.5±3.86
Maximum body width	4.7-5.2	4.93±0.045
Length from tip of tail to cloaca	1.2-1.7	1.41±0.054
Esophagus length	7.7-8.3	7.96±0.078
Esophagus width	1.1-1.3	1.22±0.022
Spicule length	2.2-2.5	2.4±0.035
Female nematodes		
Body length	274-320	293.7±4.83
Maximum body width	7, 6-8	7.85±0.038
The distance of the vulva from the tip of the head	65-70	67.3±0.44
Esophagus length	9.6-10.8	10.15±0.099
Esophagus width	1.6-1.9	1.71±0.024
Egg length	0.08-0.099	0.089±0.002
Egg width	0.075-0.089	0.079±0.001

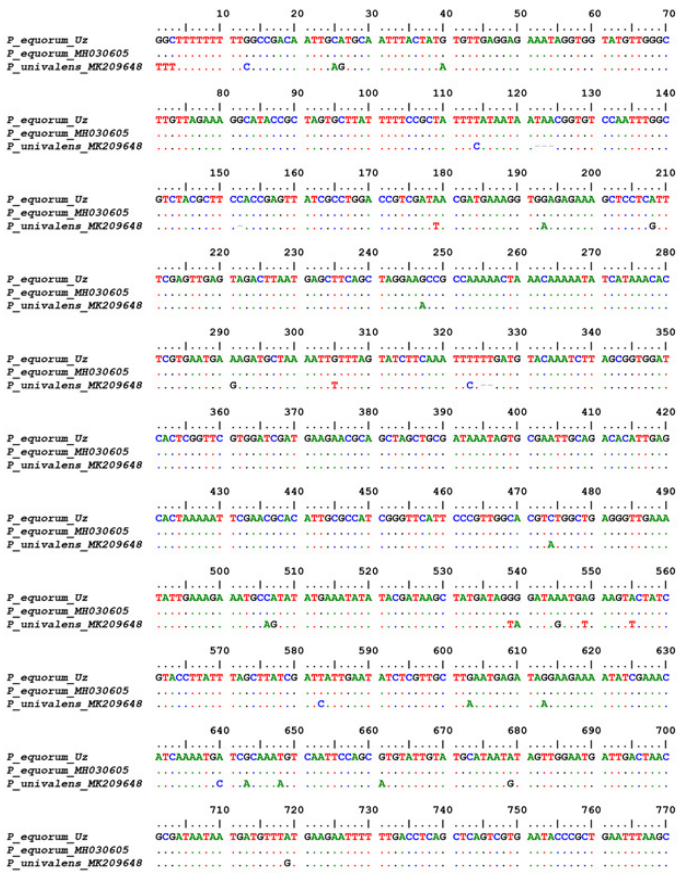

Figure 3: Nucleotide sequence of the ITS region of rDNA of nematode of the species *P. equorum*.

Molecular-genetic identification

According to the results of the conducted molecular genetic research, the nucleotide sequence of 770 pairs of bases belonging to the ITS region of the rDNA of the *P. equorum* species belonging to the genus *Parascaris* was extracted and compared with the nucleotide sequence of the *P. equorum* (MH030605) and *P. univalens* (MK209648) species of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) (Figure 3).

No differences were noted between the nucleotides of the *P. equorum* species and the *P. equorum* species (MH030605) in the GenBank database NCBI.

P. equorum species were compared with the nucleotides of *P. univalens* (MK209648) in the GenBank database. There are 32 nucleotide differences between the nucleotides of these *P. equorum* and *P. univalens* (MK209648) species, including the exchange of G-guanine in the *P. equorum* species and T-thymine in the *P. univalens* species in the 1, 2, 305 and 539 nucleotides, C-cytosine in the *P. equorum* species and T-thymine in the *P. univalens* species in the 3 nucleotide, G-guanine in the *P. equorum* species and C-cytosine in the *P. univalens* species in the 13 nucleotide, C-cytosine in the *P. equorum* species and A-adenine in the *P. univalens* species in the 25, 474 and 506 nucleotides, A-adenine in the *P. equorum* species and G-guanine in the *P. univalens* species in the 26, 208, 291, 507, 545, 679 and 719 nucleotides, G-guanine in the *P. equorum* species and A-adenine in the *P. univalens* species in the 40, 193, 247, 540, 603, 613, 643 and 661 nucleotides, T-thymine in the *P. equorum* species and S-cytosine in the *P. univalens* species in the 114, 323 and 583 nucleotides, A-adenine in the *P. equorum* species and T-thymine in the *P. univalens* species in the 179, 549 and 555 nucleotides, A-adenine in the *P. equorum* species and C-cytosine in the *P. univalens* species in the 640 nucleotide, T-thymine in the *P. equorum* species and A-adenine in the *P. univalens* species in the 648 nucleotide.

Importantly, all the *Parascaris* species clustered based on the concatenated amino acid sequences of 12 protein-coding genes. Interestingly, *P. univalens* (Switzerland and USA isolates) and *P. equorum* (Japan and China isolates) were not classified into the same branches. *A similar* result was reported previously for *A. suum* China isolate and *A. suum* USA isolate (Liu *et al*., 2012). The clustering of the four *Parascaris* species in a clade with high statistical support in the present study indicates that *P. equorum* and *P. univalens* are very closely related and may even be the same species.

Phylogenetic tree

A phylogenetic family tree was constructed based on the molecular-genetic analysis of the *P. equorum* species belonging to the *Parascaris* genus and the nucleotides belonging to the rDNA ITS region of the species obtained from the genebank database of this genus (Figure 4).

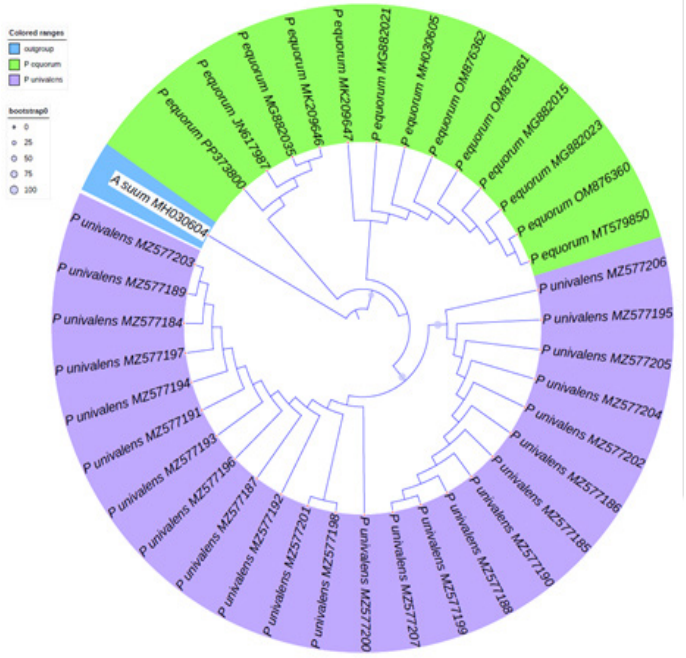

Figure 4: Phylogenetic tree of species belonging to the genus *Parascaris* based on the ML method.

According to the family tree constructed on the basis of nucleotide sequences belonging to the ITS region, the species *P. equorum* and *P. univalens* belonging to the genus *Parascaris* are united into 2 separate monophyletic groups.

In the first group, the *P.equorum* species formed 75-100% bootstrap loading compared to the main joint, and formed 2 small groups within it. The genetic distance between representatives of the species is in the range of 0.000-0.004.

In the second group, the samples of *P. univalens* species also formed 2 subgroups, and these subgroups created a bootstrap loading of 75%. The genetic distance between representatives of the species is in the range of 0.000-0.008.

The nucleotide sequence obtained as a result of molecular genetic studies of *Parascaris equorum* was deposited in the GenBank database (NCBI) and the accession number (PP373800) was obtained.

CONCLUSIONS AND RECOMMENDATIONS

Male and female individuals of the nematode *P. equorum* were studied morphometrically, and based on the obtained results, the nucleotide sequence of the rDNA ITS region of this species was analyzed, and nucleotides were compared with the species of *P. univalens* (MK209648) in the NCBI database. The difference between total nucleotides was found to be 4.15%. In this study, it was found that the *Parascaris equorum* species belonging to the *Parascaris* genus is distributed in the Fergana Valley.

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NOVELTY STATEMENT

Morphological and molecular genetic identification of *Parascaris equorum* nematode was carried out for the first time in Uzbekistan. It was found that the structure of the lips of female nematodes differs dramatically in size from that of males. According to the results of the molecular genetic research, the nucleotide sequence of 770 base pairs belonging to the ITS region of the rDNA of *Parascaris equorum* species was extracted and placed in the National Center for Biotechnology Information (NCBI) and received the accession number (PP373800).

AUTHOR'S CONTRIBUTION

Materials were collected, morphologically studied and statistically analyzed by TS. Molecular analysis was done by OA and AK. The analysis of the collected materials and the preparation of the manuscript of the article were carried out by ES. The authors read and approved the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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