SJIF Impact Factor (2025): 8.688| ISI I.F. Value: 1.241| Journal DOI: 10.36713/epra2016 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 10 | Issue: 4 | April 2025 - Peer Reviewed Journal

MOLECULAR GENETIC IDENTIFICATION OF THE SPECIES RAPHIDASCARIS ACUS (BLOCH, 1779) BELONGING TO THE GENUS RAPHIDASCARIS (ANISAKIDAE: ASCARIDATA)

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Article DOI: https://doi.org/10.36713/epra20874
DOI No: 10.36713/epra20874

ABSTRACT

Anisakid nematodes of the genus Raphidascaris (Railliet & Henry, 1915) are known to be parasites that inhabit the digestive tracts of various types of fish, which can be found in marine, brackish, and freshwater environments across the globe. One notable species within this genus is Raphidascaris acus, which is recognized as a cosmopolitan species. This particular nematode has been reported in association with a diverse range of host species, highlighting its widespread distribution and adaptability. It has recently been demonstrated that numerous nematode species are, in reality, species complexes, as evidenced by precise morphological and genetic investigations. This species acts as a parasite within the digestive systems of fish found in the water bodies of the Khorezm region. This variation can be attributed to the interaction of environmental factors affecting the host and the environment in which the helminth resides.

KEYWORDS: Raphidascaris Acus, Male, Female, Nucleotide, Nematode, Genetic Investigations, Morphological.

INTRODUCTION

The nematode species Raphidascaris acus (Bloch, 1779) belonging to the genus *Raphidascaris* Railliet & Henry, 1915 is a widespread parasite of predatory fish living in freshwater bodies. This species is a cosmopolitan species with diverse morphological characters [Smith 1984, Moravec 1994, Nagasawa et al. 2007]. More than 29 species of this genus have been identified worldwide (https://www.gbif.org/ru). The species *R. acus* is a biohelminth, the development cycle of which occurs with the participation of primary (oligochaetes, copepods and many other invertebrates), secondary (dragonfly larvae, springtails, beetles, benthic carpsimon fish) intermediate hosts and the main host - predatory fish [Vasiliev G.V., 1989].

Currently, genetic markers are used to identify species and address issues of polymorphism within them [Hebert et al. 2003]. These markers are mainly studied based on markers developed for the ITS1, 5.8S, and ITS2 regions of the ribosomal rDNA of species [Chilton et al. 1995, Zhu et al. 1998, 2000, 2001; D'Amelio et al. 2000, Zhang et al. 2007, Fang et al. 2010]. Liu Y and other scientists conducted research based on morphological analysis and genetic markers of nematodes of the Anisakidae family [Li et al. 2012a, b; Shamsi et al., 2008].

The aim of this research work is to characterize the ribosomal rDNA of the nematode species *Raphidascaris acus* (Bloch, 1779) of the genus *Raphidascaris* Railliet & Henry, 1915 of our Republic based on nucleotides belonging to the ITS domain.

MATERIAL AND METHODOLOGY

This study will be conducted in the Republic of Uzbekistan in the years 2023-2025, covering the following areas: *crucian carp ,silver* and *carp white* Amur from the villages of Yukaridormon, Urgench district and Akhunboboev, Yangiarik district. Fish was collected from the digestive systems. (Figure 1)

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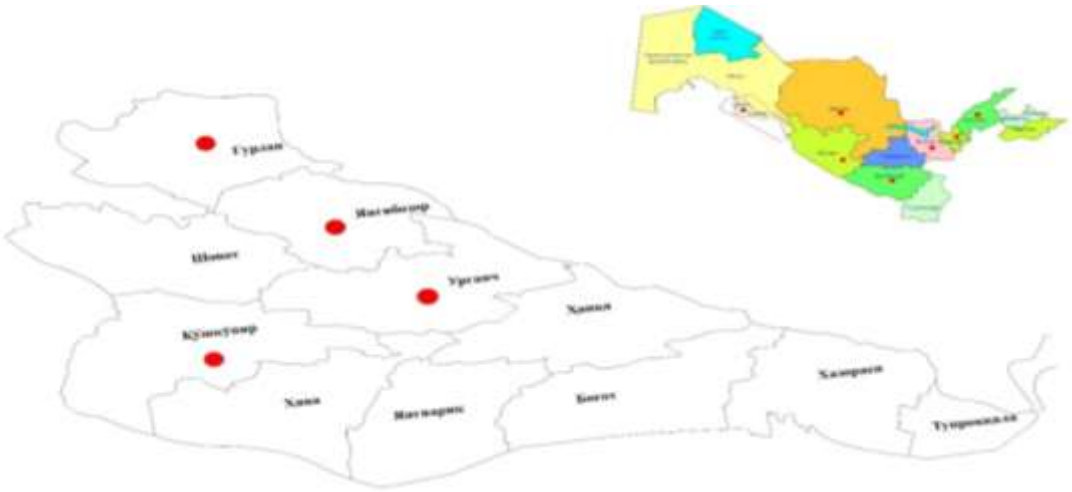


Figure 1. Areas where the study was conducted

14 head of Chipor rudd fish were examined, 3 of which had 1-12 specimens, and 4 of which had 1-15 specimens, a total of 42 specimens of the nematode *R. acus* were collected.

Scriabin and Bykhovskoy-Pavlovskoy methods were used to fully dissect the fish, as well as the generally accepted methods of collecting and processing helminthological samples [Bykhovskaya-Pavlovskaya, 1985, Dogel V.A. 1962, Markevich A.P. 1951].

MOLECULAR ANALYSES

To isolate genomic DNA from nematode tissue, each sample was incubated with 20 µl of NaOH (0.25M) for 12 hours at room temperature, then heated to 95°C for 3 minutes, and 10 µl of Tris-HCl was added to the samples, vortexed, and then centrifuged for 2 minutes. After centrifugation, 4 µl of HCl (1:15) was added, vortexed and centrifuged again, then 5 µl of Triton (2%) was added. Then, heated to 95°C for 3 minutes and stored at -20°C.

Nucleotides of ITS fragments of nematode ribosomal DNA (rDNA) were isolated using AV28 forward (ata tgc tta agt tca gcg ggt) and TW81 reverse (gtt tcc gta ggt gaa cct gc) primers used in molecular taxonomy. Polymerase chain reaction (PTsR) was carried out according to the following scheme: 1 - step - DNA denaturation at 94°C for 5 minutes, 2 - step - DNA denaturation at 95°C for 45 seconds, 3 - step - softening of primers in DNA at 55°C for 45 seconds, Step 4 – elongation at 72°C for 1 minute 40 seconds, Step 5 – chain elongation at 72°C for 5 minutes. From the second to the fourth step, the process was repeated up to 35 times in a loop form.

The presence of DNA in the PCR products was determined by electrophoresis on a 1.0% agarose gel at 120 V. A set of reagents manufactured by Silex M (Moscow, Russia) was used for DNA amplification and DNA extraction from the gel, following the manufacturer's instructions.

Sequencing of purified DNA was performed using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit, and reaction products were recorded on an ABI PRISM 3100-Avant automated sequencer (Moscow, Russia).

Analysis of the obtained nucleotide sequences was carried out using the special computer programs Bioedit, Clustal W and DNAstarTM, PAUP4.

THE OBTAINED RESULTS AND THEIR ANALYSIS

According to the results of the morphological study, the L3 stage nematode larvae of the genus *Raphidascari*s are medium-sized, with a white, cylindrical body, transverse stripes, and three prominent anterior lips. The mouth is triangular in shape.

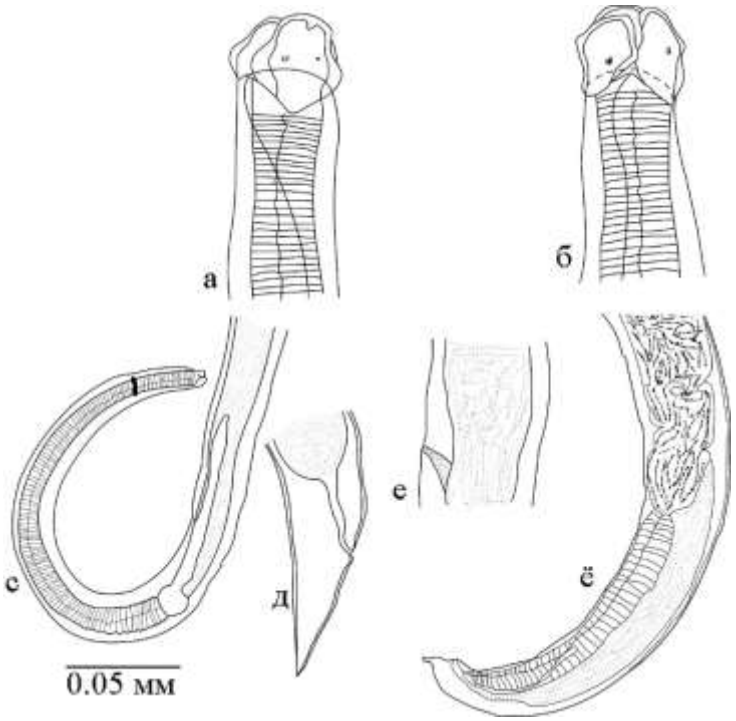
The total body length of males is 22.5–31.3, (26.5±1.6), width 0.49–0.78, (0.53±0.07), and the length of the anus is 2.1–3.2 mm, (2.5±0.12). Spicules are equal in length, 0.61-0.89 mm (0.72±0.6), the tail is hook-shaped, 1.3-2.1 mm (1.4±0.8) in length (1-table).

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c	**Nematode characteristics**	Male lim (M±m)	Female lim (M±m)
1	Body length	22,5–31,3, (26,5±1,6)	19,7-38,4, (27,3±1,2)
2	Body width	0,49–0,78, (0,53±0,07)	0,39-1,2, (0,9±0,04)
3	The distance of the nerve ring to the anterior border	0,43-0,75, (0,59±0,4)	0,42–0,54, (0,48±0,2)
4	Esophagus length	2,1-3,2, (2,5±0,12)	2,2–4,7, (3,1±1,7)
5	The length of the tail	1,3-2,1, (1,4±0,8)	2,2–3,8, (2,6±0,9)
6	The length of the spicule	0,61-0,89, (0,72±0,6)	-
7	Egg size	-	0,06-0,11×0,045-0,055

Morphometric measurements of L3 stage nematode larvae of the genus Raphidascaris (n=10, mm)

The length of the female is 19.7-38.4 mm (27.3±1.2), the width is 0.39-1.2 mm (0.9±0.04), the length of the ovary is 2.2-4.7 mm, (3.1±1.7). Eggs are 0.06-0.11×0.045-0.055 mm in size (Figure 1.)



Morphological appearance of a third-stage larva of the genus Raphidascaris.

Note: a, b– head part, c- front part of female, d- tail part of female, e- vulva, yo- tail part of male

*M*orphometric measurements of L3-stage nematode larvae belonging to the genus *Raphidascari*s (Mikhak Jahantab & Mohammad Haseli & Zivar Salehi, 2014, Avdeev V.V., 1987) were taken in close agreement with the data presented in the literature, and it was determined that this larval species may be the species *Raphidascaris acus* (Bloch, 1779) belonging to the genus *Raphidascari*s Railliet & Henry, 1915.

According to the results of the molecular genetic study, a nucleotide sequence of 691 base pairs belonging to the ITS region was isolated from the rDNA of L3-stage nematode larvae of the genus *Raphidascari*s, found in roundhead fish, silver bream, carp, and eel.

The sequence data was analyzed using bioinformatics software and compared with the species sample (*Raphidascari*s *acus* – MK271790) (Keskin M., 2018) in the National Center for Bioinformatics Information (NCBI) gene bank (Figure 2).

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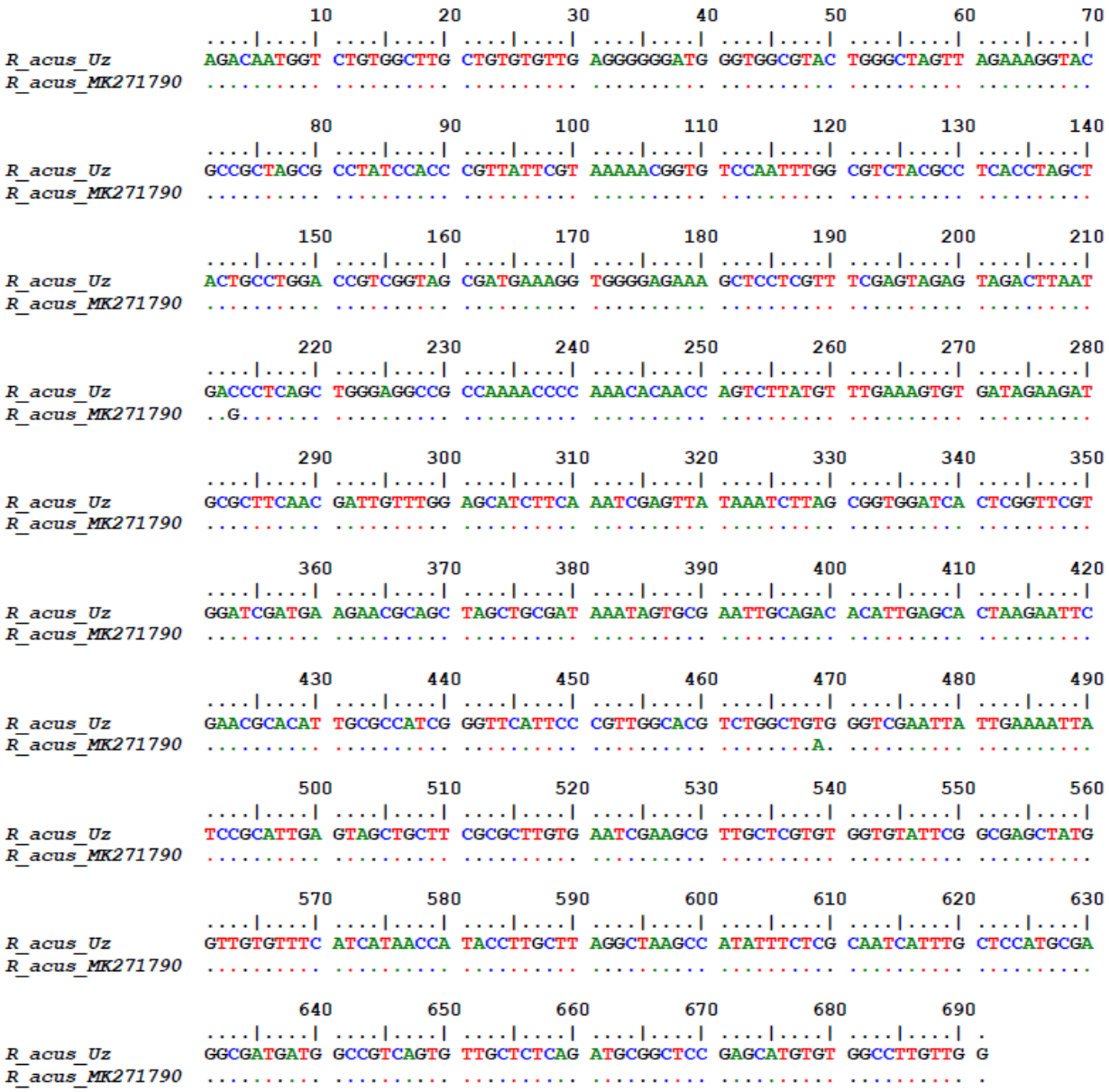


Figure 2. Comparative comparison of nucleotide sequences belonging to the ITS region of the rDNA of the nematode *Raphidascaris acus* larvae.

According to the analysis of bioinformatics programs, there were two nucleotide differences between the larvae of the nematode *R. acus* belonging to the genus *Raphidascari*s, found in roundhead fish, silver bream, carp and eel, and the *R. acus* – MK271790 sample obtained from the NCBI database. These differences were found to be the substitution of C-cytosine in the *R. acus* sample at nucleotide 213, and G-guanine in the *R. acus* sample at nucleotide 469, and A-adenine in the *R. acus* sample at nucleotide 469. The differences between nucleotides in this *R. acus* sample can be explained by the environmental conditions and host species of the places where this nematode is found.

The data obtained as a result of molecular genetic research were deposited in the National Bioinformatics Information Center and accession numbers were obtained (*Raphidascaris acus* - PV194978).

The type of L3 stage nematode larvae belonging to the genus *Raphidascari*s, found in the body cavity, liver, intestinal wall and ducts of Khorezm roundhead fish, silver bream, carp and eel of our republic, was determined and studied using morphological and molecular genetic methods. As a result, it was determined that these larval specimens correspond to the species *Raphidascaris acus* (Bloch, 1779).

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According to the data obtained as a result of molecular genetic research, there were two nucleotide differences between the nucleotides of the nematode *R. acus* and the *R. acus* sample - MK271790 obtained from the National Center for Biotechnology Information, and the total nucleotide difference was 0.2%.

CONCLUSION

These species were analyzed in the present paper clearly belongs to the genus *Raphidascaris*. The morphology of our specimens agrees well with the description of *R. acus*. Accurate identification of a parasite at any stage of its development has important implications for studying parasite epidemiology and resolving taxonomic problems. Different studies have demonstrated that the ITS regions and 5.8S provide useful genetic markers for the accurate identification of sibling species and morphospecies within ascaridoid species.

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