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# MOLECULAR-GENETIC AND PHYLOGENETIC ANALYSIS OF THE NEMATODE *RHABDIAS ENGELBRECHTI* FOUND IN SOUTH UZBEKISTAN

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**Abstract**

For this molecular genetic research, helminthological samples were collected from the lungs of 17 samples of *Pelophylax terentievi* (Mezhzherin, 1992) distributed in the Kashkadarya and Surkhondarya regions in the spring and summer of 2024. The difference between the nucleotides of the *R. engelbrechti_uz* (PQ219671) sample belonging to the *Rhabdias* genus collected from the southern regions of our republic and the *R. engelbrechti* (MG428406) sample obtained from the NCBI database was 0.4%. The nucleotide sequence obtained from the molecular-genetic study of *Rhabdias engelbrechti* belonging to the *Rhabdias* genus was placed in the *National Center for Biotechnology Information*. To the best of our knowledge, this species was recorded for the first time in the territory of the Republic of Uzbekistan.

***Keywords****: Rhabdias engelbrechti; helminth; molecular phylogeny; ribosomal DNA; ITS region*

## 1. Introduction

The species *Rhabdias engelbrechti* Kuzmin, Halajian, Tavakol, Luus- Powell & Tkach, 2017 belong to the genus Rhabdias, representatives of this genus are distributed in all zoogeographic regions except Antarctica, and they are parasites in the lungs of amphibians and reptiles and it has been determined that it consists of about 80 species by now [2, 3, 5].

Two species belonging to the genus *Rhabdias* (*R. bufonis, R. rubrovenosus*) are distributed in our republic, and these species were recorded in the lungs of representatives of the *Rana, Bufotes* and *Pelophylax* families [1, 4, 9].

According to Kuzmin Y. et al., 2017 mitochondrial DNA sequence *COI* and ribosomal DNA sequence 12S, ITS, and 28S region of the species *R. engelbrechti*, belonging to the genus *Rhabdias*, which was identified in the frog *Phrynomantis bifasciatus* (Microhylidae) distributed in Africa in 2017, were characterized based on the nucleotide sequence.

**The purpose of this research work** is a molecular-genetic description of the *Rhabdias engelbrechti* species found in the lungs of *Pelophylax terentievi* (Mezhzherin 1992) distributed in the southern region of

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our republic.

## 2. Materials and methods

*Molecular genetics method*: ITS fragments of ribosomal DNA were isolated from the nematode species mentioned above for molecular genetics research. For this, samples of 3 individuals of *Rhabdias engelbrechti* nematodes were taken and genomic DNA was isolated.

The GeneJet Genomic DNA Reagent Kit was used to extract genomic DNA from nematode tissue samples [6, 8].

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.

PCR recipe: primary DNA denaturation at 94˚C for 3 min, followed by 9 cycles consisting of denaturation at 94˚C – 1 min, annealing at 54˚C – 1 min 30 s and elongation at 72˚C – 1 min 30 s; then 24 cycles, consisting of denaturation at 94˚C – 45 s, annealing at 54˚C – 45 s and chain elongation at 72˚C – 2 min; followed by final elongation at 72˚C for 5 minutes. The results of the PCR reaction were checked by electrophoresis of 1 ml of the product in a 1% agarose gel (100 V, 80–100 mA, approximately 30–40 min).

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

Analysis of the received nucleotide sequence was carried out using Bioedit, Clustal W, and DNAstarTM, a PAUP4 special computer program.

*Constructing a phylogenetic tree:* Nucleotide sequences of helminths of the genus *Rhabdias* were sequenced and DNA sequences obtained from the *National Center for Biotechnology Information* database [10] were used and these sequences were manually aligned using Genius Prime Software. The edited, consensus sequences were calculated using Mega X computing software. Primer data from this program and additional sequences from the GenBank database were aligned using MAFFT v.7 online software using default settings and Clustal Omega 1.2.2 software and edited with Genius Prime software.

Nucleotide sequences belonging to the ITS region of the obtained ribosomal DNA (rDNA) maximum (maximum likelihood-ML) phylogenetic tree were determined by ultra-fast bootstrapping with 1000 iterations in IQ-TREE version 1.6.12 and analyses were performed in CIPRES Science Gateway V 3.3. The nucleotide sequence of the ITS region of *R. engelbrechti* Kuzmin, Halajian, Tavakol, Luus-Powell & Tkach, 2017 (Accession number: MG428406) was included as an outgroup to facilitate the production of consensus trees. The resulting phylogenetic tree was analyzed and edited in iTOL v6.6 software.

## 3. Results and discussion

*Molecular genetic analysis:* According to the results of the conducted molecular-genetic research, the rDNA of the *R. engelbrechti_uz* species found in the lungs of the *P. terentievi* frog distributed in the southern regions of our republic, belonging to the ITS-1+5.8S+ITS-2 region, with a length of 665 pairs of nucleotides was isolated and *R. engelbrechti* (Accession number: MG428406) and *R. bufonis* (Accession number: KF999609) of the same genus from the *National Center for Biotechnology Information* (NCBI) were used to compare these species (Fig. 1).

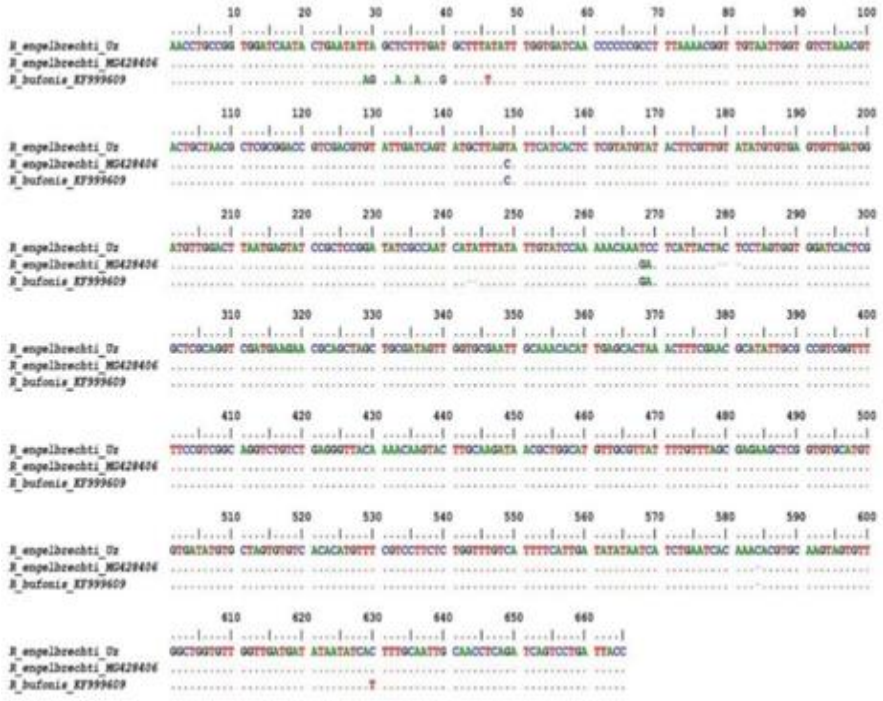

**Fig. 1.** Nucleotide sequence comparison of the rDNA ITS region of species belonging to the genus *Rhabdias* based on sequence material

As can be seen from Fig. 1, there is a difference in 3 nucleotides between the *engelbrechti_uz* sample from the *Rhabdias* genus collected from the southern regions of our republic and *the R. engelbrechti* sample obtained from the NCBI database, C-cytosine in the *R. engelbrechti* sample in the NCBI sample, T-thymine in the *R. engelbrechti* sample in the 268th nucleotide, G-guanine in the *R. engelbrechti* sample in the NCBI sample, C-cytosine in the *R. engelbrechti* sample in the 269th nucleotide. The sample taken from za found that A-adenine nucleotides were exchanged in the *R. engelbrechti* sample, and the difference between the total nucleotides was 0.4%.

There is a difference of 10 nucleotides between the nucleotides of *R.engelbrechti* belonging to the genus *Rhabdias* and *R. bufonis* from the NCBI database. Type T-thymine, 30th nucleotide A-adenine in *R. engelbrechti* type, G- guanine in *R. bufonis* type obtained from NCBI database, T-thymine in *R.engelbrechti* type at 40 nucleotides, G-guanine in *R. bufonis* type obtained from NCBI database, 149 nucleotide T-thymine in *R. engelbrechti* type, *S-cytosine* in

*R. bufonis* type obtained from NCBI database, *T-thymine* in *R. engelbrechti* type at nucleotide 268, G-guanine in *R. bufonis* type obtained from NCBI database, C- in nucleotide 269 in *R. engelbrechti* sample cytosine, and A-adenine nucleotide exchange was found in the *R. bufonis* strain obtained from the NCBI database. It was 1.5% of the total nucleotides. *Phylogenetic analysis:* According to the results of molecular genetic research, it was found that the representatives of this genus are grouped into 7 clades (groups) according to the analysis of nucleotide sequences belonging to the ITS region of rDNA of the studied species belonging to the genus *Rhabdias* and nucleotide sequences obtained from the GenBank database (Fig. 2).

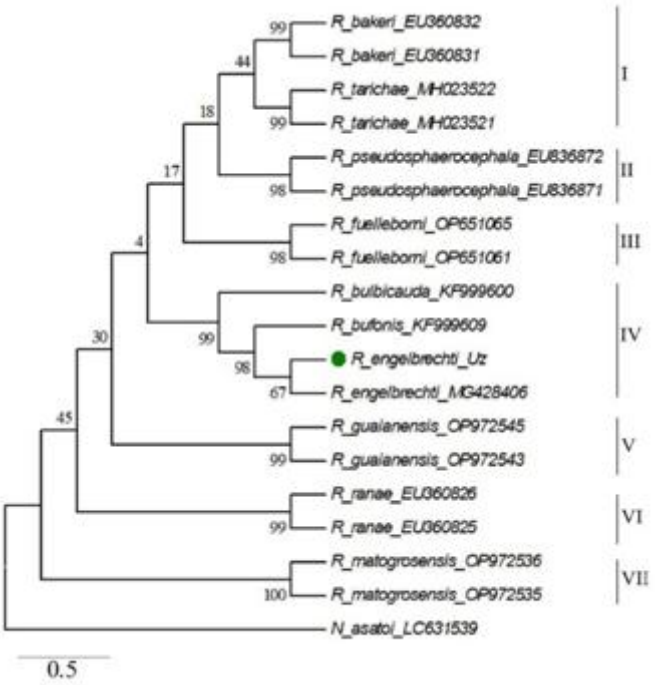


**Fig. 2**. A phylogenetic family tree of *Rhabdias* genus helminths developed based on the maximum likelihood-ML method

The first group includes *R. bakeri* and *R. tarichae* species with 44% relative to the main joint, and 99% within the species bootstrap support, and the second group, *R. pseudosphaerocephala* species with 18% relative to the main joint, within the species and 98%, and in the third group, the species *R. fuelleborni* has 17% relative to the main group, and 98% support within the species, and in the fourth group, the species *R. bulbicauda* has compared to the main group 4%, and within the species 99%, this group formed 3 more subgroups, 98% of *R. bufonis* species samples, 67% of *R. engelbrechti_uz* and *R. engelbrechti* species samples, in the fifth group *R. guaianensis* species is the main joint relative to 30%, and within the species, 99% bootstrap support, in the sixth group *R. ranae* type compared to the main joint is 45%, and within the species, 99% bootstrap support, in the seventh group *R. matogrosensis* species is the main joint 100% bootstrap has combined to form support (Fig. 2).

## 4. Conclusions

The difference between the *engelbrechti_uz* sample belonging to the *Rhabdias* genus and the *R. engelbrechti* sample obtained from the NCBI database is 0.4%, and the difference between these nucleotides is that the species of *R. engelbrechti* was found in the frog *P. terentievi*. Thus, it can be explained that the difference is related to environmental factors of the host.

The similarity between the nucleotide sequence of the rDNA 5.8S-ITS2 region between *R. engelbrechti* (PQ219671) from southern Uzbekistan and the nematode accession number MG428406 in the *National Center for Biotechnology Information* is 98.62%. Therefore, *R. engelbrechti* can be included among the helminth species that are widespread worldwide and have diverse characteristics with some morph-anatomical and genetic factors.

The nucleotide sequence obtained as a result of the molecular-genetic study of *R. engelbrechti* belonging to the *Rhabdias* genus was placed in the *National Center for Biotechnology Information*. To the best of our knowledge, this species was recorded for the first time in the territory of the Republic of Uzbekistan.

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## References

**[1]** Ikromov E.E., Ikromov E.F., Yildirimhan H.S., Azimov Dj.A., Amirov O.O. Biodiversity of

Helminths in genera of Bufotes and Pelophylax, Uzbekistan // Biharean Biologist, – Oradea, 2023. №17(1): pp.22 – 38. http://biozoojournals.ro/bihbiol/index.html.

**[2]** Kuzmin Y., du Preez L.H., Nel T., Svitin R. Three new species of Rhabdias Stiles & Hassall, 1905 (Nematoda: Rhabd.iasidae) parasitic in *Ptychadena spp*. (Amphibia: Anura: Ptychadenidae) and an identification key to *Rhabdias spp*. From Afrotropical anurans. Parasitology International, 2022. №:91, e102649.

**[3]** Kuzmin Y., Halajian A., Tavakol S., Luus-Powell W.J., Tkach V.V. Description and phylogenetic position of a new species of Rhabdias Stiles & Hassall, 1905 (Nematoda: Rhabdiasidae) from the banded rubber frog, *Phrynomantis bifasciatus* (Smith) (Amphibia: Microhylidae) in South Africa. Folia Parasitologica, 2017. pp.35-64.

**[4]** Kuchboev A.E., Shakarboev E.B., Kucharova I.Sh. Helminths of amphibians of Uzbekistan. Uzbek Biology Journal -Toshkent, 2002. №:1: pp.39-43 [In Russian].

**[5]** Tkach V.V., Kuzmin Y.I., Snyder S.D. Molecular insight into systematics, host associations, life cycles, and geographic distribution of the nematode family Rhabdiasidae. International Journal for Parasitology, 2014. №:44, pp.273–284.

**[6]** Boom R., Sol C.J.A. Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol., Mar, 1990. pp.495-503.

**[7]** Marko M.A., Chipperfield R. and Birnboim H.C. A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder, Anal. Biochem., 1982. №:121, pp.382-387.

**[8]** Vogelstein B. and Gillespie D. Preparative and analytical purification of DNA from agarose, Proc. Natl. Acad. Sci. USA, 1979. №:76, pp.615-619.

**[9]** Ikromov E.F., Akmuradova L.G., Ikromov E.E., Aliyev Sh.T. Helmint Fauna Of Pevtsov's Toad *Bufotes Pewzowi* (BEDRIAGA, 1898) In Uzbekistan // International scientific and practical conference "Modern trends in teaching natural sciences: practice and innovation", -Pavlodar, 2024, pp.296-297

**[10]** National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov