Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(6): 245 – 252 (2025)
www.ejabf.journals.ekb.eg

Morphological and Molecular-Genetic Characterization of the Nematode *Oswaldocruzia filiformis* Goeze, 1782 (Nematoda: Molineoidea) in the Amphibians of Southern and Central Uzbekistan

Aliyev Shohjahon T.[1*], Amirov Oybek O.[1], Kadirov Ilhomjon T.[2], Muminova Ra'nokhon N.[3], Xujamqulova Iroda F.[4], Abdunazarova Zebiniso Kh.[5]

[1] Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan, Tashkent
[2] Department of Biotechnology of Namangan State University, Namangan
[3] Department of Biology of Kokand State University, Kokand
[4] Termez University of Economics and Service, Termez
[5] Termiz State Pedagogical Institute, Termez

[*]**Corresponding Author:** biocandidatlotus@gmail.com

ARTICLE INFO
Article History:
Received: July 27, 2025
Accepted: Oct. 20, 2025
Online: Nov. 8, 2025

Keywords:
Oswaldocruzia filiformis,
Nematode,
Caudal bursa,
Spicula,
mt-dna, NCBI,
South and Central
Uzbekistan

ABSTRACT

In this article, the morphological and molecular-genetic analysis of the nematode *Oswaldocruzia filiformis* Goeze, 1782, found in amphibians of Southern and Central Uzbekistan, was conducted. As a result of molecular-genetic analyses, 419 nucleotide pairs of the mitochondrial DNA *coi* region were isolated and sequenced using PCR amplification. Consequently, the nematode *O. filiformis*_uz was compared with NCBI data and confirmed to match 99.5% with *O. filiformis* (MT300275). The obtained data and nucleotide analysis of species belonging to this genus in the NCBI database demonstrated that this species is a morphologically and molecular-genetically stable single species. Morphological and morphometric analyses revealed that the body of the nematode *O. filiformis* is thin, elongated, with a medium-width body, and the cephalic vesicles are located in the vesicle at the anterior end. The body length of male nematodes ranges from 6.3 to 8.2mm, with a width of 0.16 to 0.17mm, and their oral structure does not include lips. The esophagus length is 0.39 to 0.47mm, and the caudal bursa consists of three lobes and their ribs. The length of the spicules is 0.19 to 0.25mm, and the gubernaculum is composed of several parts. Female nematodes are slightly larger, with a body length of 14.2 to 18.6mm, a width of 0.21 to 0.25mm, and an esophagus length of 0.46 to 0.49mm. The ovary was found to form rings in the anterior part of the uterus.

INTRODUCTION

The herpetofauna of Uzbekistan is ancient and genetically complex, with endemic species of significant importance for Central Asia and our republic. Therefore, studying the factors affecting the biological diversity, population density, and distribution of these species across the region is of practical significance. Specifically, parasitological diseases are considered one of these factors.

Nematodes of the genus *Oswaldocruzia* Travassos, 1917 are parasites primarily inhabiting the small intestines of amphibians and reptiles. They are widespread among amphibians but also parasitize lizards and occasionally snakes. Representatives of this genus are distributed worldwide and include about 90 species **(Ben Slimane *et al.*, 1995, 1996a, b, 2016; Durette-Desset *et al.*, 2006; Bursey *et al.*, 2007; Schotthoefer *et al.*, 2009; Bursey & Goldberg, 2011; Svitin &**

Kuzmin, 2012; Svitin & Gorobchishin, 2015; Svitin, 2017). *Oswaldocruzia filiformis* is widely distributed across Eurasia and is often found in amphibians belonging to the genera *Bufotes* and *Rana* **(Petter & Quentin, 1976)**. Based on its life cycle, *O. filiformis* is a geonematode parasite. Its invasive larvae are found in soil or on plants **(Tarasovskaya, 2009; Svitin & Gorobchishin, 2015)**. Amphibians and lizards may become infected with these nematodes when they accidentally ingest the parasitic larvae along with food. Many researchers suggest that the samples of *O. filiformis* found in snakes may represent cases of post-cyclic parasitism **(Kirillov, 2000, 2010; Svitin & Kuzmin, 2012; Svitin & Gorobchishin, 2015)**.

Due to the scarcity of initial information about *O. filiformis* and the morphological similarity of the *Oswaldocruzia* genus species in the Palearctic, most nematodes found in amphibians and reptiles in Russia and European countries have been recorded as *O. filiformis*. These nematode specimens may actually belong to several different species. The initial descriptions of *Oswaldocruzia* spp. were based on differences in spicules and the structure of the male caudal bursa **(Travassos, 1937; Ryzhikov *et al.*, 1980; Vashetko & Siddikov, 1999; Kuchboev *et al.*, 2002; Popiołek *et al.*, 2004, 2011; Okulewicz *et al.*, 2014; Ikromov *et al.*, 2023)**. Although nematodes belonging to the *Oswaldocruzia* genus share close morphological characteristics, they differ in molecular-genetic aspects **(Anderson, 2000; Kuzmin *et al.*, 2003; Nadler *et al.*, 2011)**.

At present, numerous molecular-genetic studies are being carried out on vertebrate and invertebrate animals within the fauna of our republic, including nematodes **(Amirov *et al.*, 2021; Mirzaev, 2024)**, fish **(Quvatov *et al.*, 2023; Ubaydullayev *et al.*, 2025)** and insects **(Kadirov *et al.*, 2024; Kimyonazarov *et al.*, 2024)**, which have all been studied at the molecular level.

The purpose of this study is to identify and describe the morphological and molecular-genetic characteristics of the nematode *O. filiformis* found in amphibians living in the southern and central regions of Uzbekistan.

MATERIALS AND METHODS

Sample collection

To carry out these morphological and molecular-genetic research works, amphibians (*B. pewzowi, B. turanensis, P. terentievi*) living in the southern (Kashkadarya, Surkhandarya) and central (Bukhara, Navoi) regions of Uzbekistan were collected during 2024-2025. Their internal organs, especially intestines, were examined under a microscope for helminthological analysis **(Skryabin, 1928)**. The collected nematodes were fixed in 70% alcohol flasks. Permanent and temporary preparations were made from the collected samples for morphological analysis.

Morphological research method

Fixed nematodes were measured using a microscope for morphological dimensions (body length, oral capsule, spicules, tail shape) and identified based on diagnostic features. The morphological and morphometric analysis of nematode samples was conducted based on the literature **(Travassos, 1937; Ryzhikov *et al.*, 1980; Ben Slimane *et al.*, 1995; Anderson, 2000; Kirillov, 2000, 2010; Kuzmin *et al.*, 2003; Durette-Desset *et al.*, 2006; Bursey C.R. *et al.*, 2007; Popiołek *et al.*, 2011; Svitin & Gorobchishin, 2015; Okulewicz *et al.*, 2016)**.

Molecular phylogenetic research method

For molecular-genetic research, fragments of the *coi* region of mitochondrial DNA (*mt-dna*) were isolated from the nematode species mentioned above. For this purpose, 3 samples of *O. filiformis* nematodes were taken, and genomic DNA was extracted using the GeneJET Genomic DNA reagent kit.

The nucleotide fragments of the *coi* region of mitochondrial DNA (*mt-dna*) of nematodes were isolated using LCO forward (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO reverse (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers, which are commonly used in molecular taxonomy. To perform polymerase chain reaction (PCR), a master-mix was prepared as follows: 13.2µL of bidistilled water, 2µL of 10× PCR buffer, 0.4µL of dNTP, 1.0µL of LCO-F, 1.0µL of HCO-R, 2µL of DNA, and 0.4µL of Taq polymerase were added. The PCR amplification sequence for the *coi* region fragments of *mt-dna* was carried out according to the following procedure (Table 1).

Table 1. Sequence of PCR reaction for the *coi* region fragments of mitochondrial DNA

Reaction	Step	Temperature (°C), step, cycle		Time
PCR	Initial denaturation	94 - 95		2-5 minutes
	Denaturation	94°	35 cycles	30 seconds
	Annealing	45-50°		30 seconds
	Elongation	72°		60 seconds
	Final elongation	72°		5-10 minutes
	Storage	4°		∞

To determine the presence of DNA in the samples obtained from PCR, gel electrophoresis was performed at 100 V, 80-100 mA for 15-20 minutes on a 1.5% agarose gel. To purify the DNA in the PCR products, the BioSpin Gel Extraction kit was used. DNA sequencing was carried out using the ABI PRISM® BigDye™ Terminator v. 3.1 reagent kit, and the reaction products were recorded on an ABI PRISM 3100-Avant automated sequencer (Moscow, Russia). Analysis of the obtained nucleotide sequences was performed using specialized computer software such as Bioedit and Clustal W.

RESULTS AND DISCUSSION

Morphological research results

The body of *O. filiformis* nematodes is thin, elongated, with an average width, and the cephalic vesicles are located in the anterior end vesicle. The shape of the cephalic vesicle is variable, being either integral (undivided) or bipartite, consisting of an anterior (wider) and posterior (narrower) part. The posterior part can be smooth or can have transverse folds. The shape of the vesicle also varies among individual nematodes. The cuticle starts from the posterior part of the cephalic vesicle and extends along the entire body, forming continuous longitudinal folds. In some nematodes, the folds on the ventral side of the body in the anterior transverse sections of the esophagus are not distinctly visible (Fig. 1a, b).

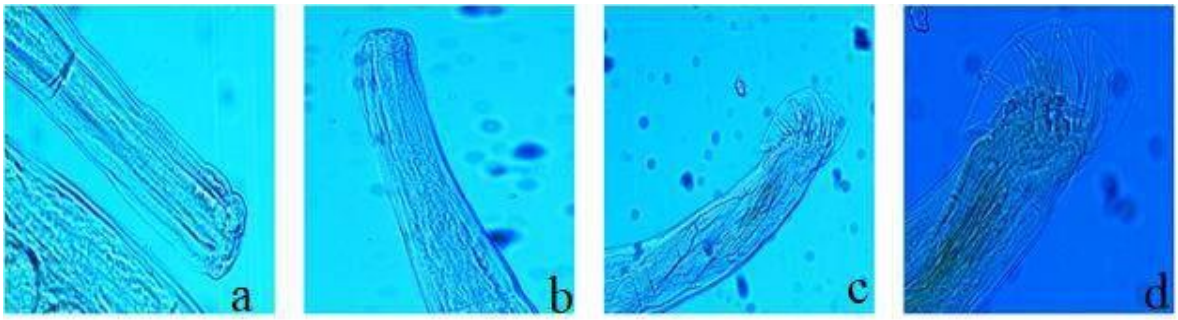

Fig. 1. *Oswaldocruzia filiformis* Goeze, 1787 (original) nematode: a – Head vesicle; b – Anterior end of the body; c – Caudal bursa; d – Bursa ribs (dorsal arches)

The body length of male nematodes is 6.3- 8.2mm, width 0.16- 0.17mm, and the mouth structure does not involve lips. The shape of the mouth is round, surrounded by 4 cuticular projections. The esophagus length is 0.39- 0.47mm, and the caudal bursa consists of three lobes and their ribs. The length of the spicules is 0.19- 0.25mm, and the gubernaculum is found to consist of several parts (Fig. 1).

The body length of female nematodes is 14.2- 18.6mm, body width 0.21- 0.25mm, and esophagus length 0.46- 0.49mm. The ovary is located at the anterior part of the uterus, forming rings. The tip of the ovary is positioned at the posterior part of the uterus, situated before the vulva opening. The ovary length is 6.5-8.1 mm, and the uterus length is 1.5-2.8 mm. The egg circumference is 0.01-0.012 × 0.07 mm. Eggs at the anterior end of the uterus mature earlier than those in the remaining parts.

Molecular phylogenetic research results

According to the results of molecular-genetic research, a part of the *mt-dna coi* region, with a length of 419 base pairs, belonging to the nematode *O. filiformis*_uz identified in the small intestine of amphibians (*B. pewzowi, B. turanensis, P. terentievi*), was isolated. For a comparative study of this species, *O. filiformis* (MT300275) and *O. ukrainae* (OQ351736) species belonging to the same genus were used from the *NCBI* database (https://blast.ncbi.nlm.nih.gov) (Fig. 2).

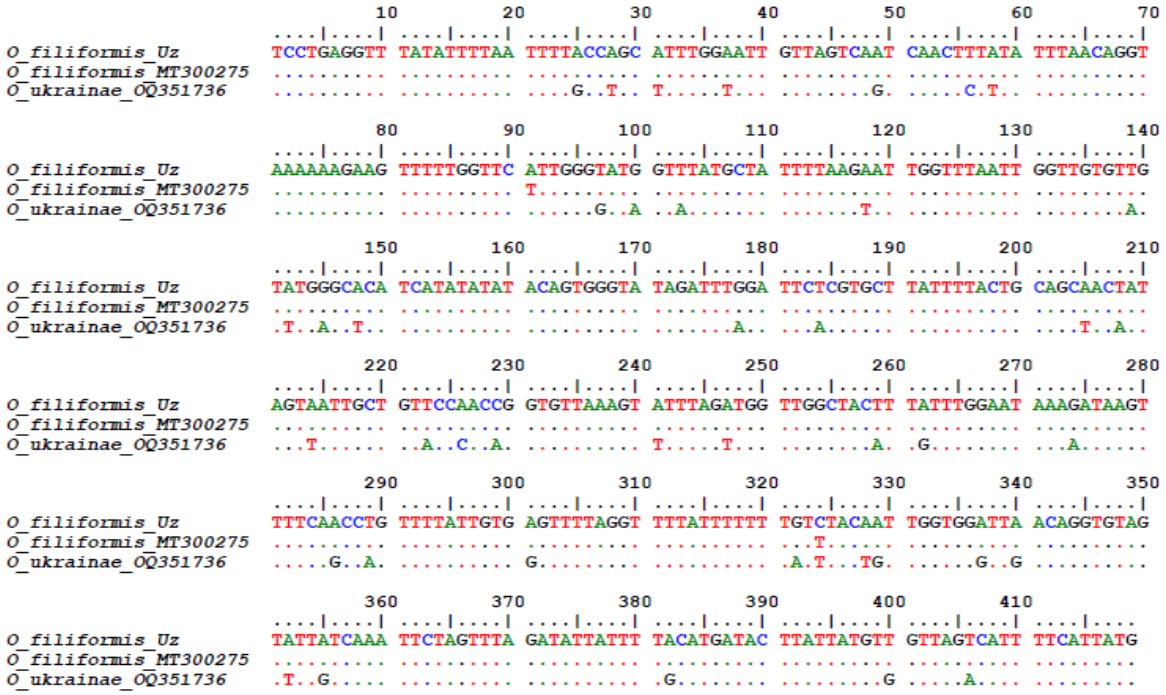

Fig. 2. In the samples of species belonging to the genus Oswaldocruzia, the nucleotide sequence of the *mt-dna coi* region fragment (from 5' to 3' direction – toward the end)

For comparison purposes, the nucleotides of the *O. filiformis*_uz sample belonging to the genus *Oswaldocruzia* were compared with those of the *O. filiformis* (MT300275) sample obtained from the NCBI database. As a result, 2 nucleotide differences were identified between them: at the 91st nucleotide, the *O. filiformis*_uz sample had A-adenine, while the *O. filiformis* (MT300275) sample from the NCBI database had T-thymine; at the 324th nucleotide, the *O. filiformis*_uz sample had C-cytosine, while the *O. filiformis* (MT300275) sample from the NCBI database had T-thymine. The overall nucleotide difference was determined to be 0.5%.

Additionally, between the *O. filiformis*_uz species and the *O. ukrainae* (OQ351736) species obtained from the NCBI database, 42 nucleotide differences were identified. These differences were as follows: at the 25th, 49th, 262nd, 286th, 301st, 329th, 337th, 340th, 355th, and 382nd nucleotides, the *O. filiformis*_uz species had A-adenine, while the *O. ukrainae* (OQ351736) species from the NCBI database had G-guanine; at the 28th, 31st, 37th, 58th, 118th, 142nd, 148th, 205th, 214th, 241st, 247th, 329th, and 352nd nucleotides, the *O. filiformis*_uz species had A-adenine, while the *O. ukrainae* (OQ351736) species from the NCBI database had T-thymine; at the 56th nucleotide, the *O. filiformis*_uz species had T-thymine, while the *O. ukrainae* (OQ351736) species from the NCBI database had C-cytosine; at the 97th and 400th nucleotides, the *O. filiformis*_uz species had T-thymine, while the *O. ukrainae* (OQ351736) species from the NCBI database had G-guanine; at the 100th, 145th, 178th, 274th, and 322nd nucleotides, the *O. filiformis*_uz species had G-guanine, while the *O. ukrainae* (OQ351736) species from the NCBI database had A-adenine; at the 103rd, 139th, 184th, 203rd, 223rd, 259th, 289th, and 406th nucleotides, the *O. filiformis*_uz species had T-thymine, while the *O. ukrainae* (OQ351736) species from the NCBI database had A-adenine; at the 226th nucleotide, the *O. filiformis*_uz species had A-adenine, while the *O. ukrainae* (OQ351736) species

from the NCBI database had C-cytosine; at the 229th nucleotide, the *O. filiformis*_uz species had C-cytosine, while the *O. ukrainae* (OQ351736) species from the NCBI database had A-adenine; at the 324th nucleotide, the *O. filiformis*_uz sample had C-cytosine, while the *O. ukrainae* (OQ351736) species from the NCBI database had T-thymine. The overall nucleotide difference was determined to be 10%.

This study confirmed the presence of the *O. filiformis* parasite in Uzbekistan with morphological and genetic evidence and also accurately demonstrated the host range and distribution area of this species. Molecular-genetic methods reinforced morphological differences, making diagnostic and monitoring tasks in parasitology more reliable.

Furthermore, molecular-genetic methods stand out as essential tools for animal identification in cases where the diagnostic accuracy of morphological characteristics is low **(Kuzmin *et al.*, 2003; Nadler *et al.*, 2011; Aliyev & Amirov, 2024; Aliyev *et al.*, 2024, 2025)**.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of conducted morphological and morphometric analyses, the body of the *O. filiformis* nematode was found to be thin, elongated, and of an average width, and its cephalic vesicles were noted to be located in the vesicle at the anterior end. The body length of male nematodes ranges from 6.3 to 8.2mm, with a width of 0.16- 0.17mm, and the lips do not participate in the structure of the mouth. The esophagus length is 0.39- 0.47mm, and the caudal bursa consists of three lobes and their ribs. The spicule length is 0.19- 0.25mm, and the gubernaculum is composed of several parts. Female nematodes are slightly larger, with a body length of 14.2- 18.6mm, a width of 0.21- 0.25mm, and an esophagus length of 0.46- 0.49mm. The ovary was found to form rings in the anterior part of the uterus.

As a result of molecular-genetic studies, the nucleotide sequence of the *mt-dna coi* region gene of the *O. filiformis* nematode in Uzbekistan was analyzed and sequenced. The nucleotide sequence of *O. filiformis* nematodes identified in the small intestines of amphibians (*B. pewzowi, B. turanensis, P. terentievi*) living in the southern (Kashkadarya, Surkhandarya) and central (Bukhara, Navoi) regions of Uzbekistan was found to match 99.5% with the *O. filiformis* species in the international NCBI database. This variation can be explained by the ecological factors of the host's living conditions. Thus, *O. filiformis* nematodes can be classified as widely distributed helminth species with polymorphic characteristics based on certain morpho-anatomical and genetic features.

GRATITUDE

We express our gratitude to the scientific team of the “Molecular Zoology” laboratory at the Zoology Institute of the Academy of Sciences of the Republic of Uzbekistan and the leadership of the scientific project “Molecular Genetic Classification of Wild Vertebrate Species of Bukhara and Navoi Regions” for their assistance in molecular-phylogenetic research.

NOVELTY STATEMENT

The molecular identification of *O. filiformis*_uz nematode was carried out for the first time in Uzbekistan. According to the results of molecular genetic studies, the nucleotide sequence

belonging to the *coi* region of the *mt-dna* of *O. filiformis*_uz nematode was sequenced and uploaded to the National Center for Biotechnology Information (NCBI).

REFERENCES

Aliyev, S.T. and Amirov, O.O. (2024). Taxonomy and epizootology of the helminths of the *Pelophylax terentievi* (Mezhzherin, 1992) frog in the southern regions of Uzbekistan. *Bulletin of the Khorezm Mamun Academy*, 9-13.

Aliyev, S.T.; Amirov, O.O.; Egamberdiyev, M.K. and Akhmadjonova, S.S. (2024). Morphological and Molecular-Genetic Classification of the Nematode *Rhabdias engelbrechti* Found in the Amphibian *Pelophylax terentievi* in the Aquatic Basins of South Uzbekistan. *Egyptian Journal of Aquatic Biology & Fisheries*, *28*(5), 831-841.

Aliyev, S.T.; Amirov, O.O.; Kuchboev, A.E.; Borzée, A.; Wang, M.; Yo'ldoshxonov, A.A.; Donayeva, S.A. and Norqobilova, R.D. (2025). Phylogenetic Relationships Classification of *Bufotes pewzowi* (Bedriaga, 1898) Inhabiting Near Aquatic Basins of Central and South Uzbekistan. *Egyptian Journal of Aquatic Biology & Fisheries*, *29*(3), 1953–1966.

Amirov, O.O.; Kuchboev, A.E.; Sobirova, H.G.; Karimova, R.R. and Omonov, S.N. (2021). Effect of plant extracts on the gastrointestinal nematodes of ruminants in Uzbekistan. *Advances in Animal and Veterinary Sciences*, *9*(9), 1396-1400.

Anderson, R.C. (2000). *Nematode Parasites of Vertebrates: Their Development and Transmission* (2nd ed.). CABI Publishing.

Ben Slimane, B.; Châabane-Banaoues, R.; Bâ, C.T. and Durette-Desset, M.C. (2016). Molecular and morphological evidence for the specific status of *Oswaldocruzia biolata* (Nematoda: Molineidae). *Journal of Helminthology*, *90*(4), 510–519.

Ben Slimane, B.; Luch, J. and Durette-Desset, M.C. (1995). Two new species of the genus *Oswaldocruzia* Travassos, 1917 (Nematode: Trichostrongylina: Molineidae) parasitizing Spanish amphibians. *Research and Review in Parasitology*, *55*(4), 209–215.

Ben Slimane, B.; Chabaud, A.G. and Durette-Desset, M.C. (1996a). The Trichostrongylina nematodes, parasites of amphibians and reptiles: problems taxonomies, phylogenetics and biogeographical. *Systematic Parasitology*, *35*, 179–206.

Ben Slimane, B.; Guerrero, R. and Durette-Desset, M.C. (1996b). *Oswaldocruzia venezuelensis* sp. n. (Nematoda: Trichostrongylina, Molineoidea), a parasite of *Bufo marinus* from Venezuela. *Folia Parasitologica*, *43*(4), 297–300.

Bursey, C.R. and Goldberg, S.R. (2011). New species of *Oswaldocruzia* (Nematoda: Molineidae) and other helminths in *Bolitoglossa subpalmata* (Caudata: Plethodontidae) from Costa Rica. *Journal of Parasitology*, *97*(2), 286–292.

Bursey, C.R.; Goldberg, S.R. and Telford, S.R. (2007). Gastrointestinal Helminths of 14 Species of Lizards from Panama with Descriptions of Five New Species. *Comparative Parasitology*, *74*(1), 108–140.

Durette-Desset, M.C.; Alves dos Anjos, L. and Vrcibradic, D. (2006). Three new species of the genus *Oswaldocruzia* Travassos, 1917 (Nematoda, Trichostrongylina, Molineoidea) parasites of *Enyalius* spp. (Iguanidae) from Brazil. *Parasite*, *13*(2), 115–125.

Ikromov, E.E.; Ikromov, E.F.; Yildirimhan, H.S.; Azimov, D.A. and Amirov, O.O. (2023). Biodiversity of Helminths in genera of *Bufotes* and *Pelophylax*, Uzbekistan. *Biharean Biologist*, *17*(1), 22-38.

Kadirov, T.I.; Akhmedova, Y.Z.; Khudoyberdieva, O.M. and Amirov, O.O. (2024). Diagnostics of two species of *Ammophila* Kirby from Uzbekistan. *Indian Journal of Entomology*, *86*(4), 1076–1080.

Kimyonazarov, S.Q.; Embergenov, M.A.; Akhmedova, Z.Y.; Kholmatov, B.R.; Gandjaeva, L.A.; Abdullaev, I.I.; Amirov, O.O. and Doniyorov, A.N. (2024). First record of *Trirogma caerulea* from Uzbekistan. *Zoosystematica Rossica*, *33*(1), 92–94.

Kirillov, A.A. (2000). Helminth fauna of reptiles from the Samara region. *Proceedings of Samara Scientific Centre RAS*, *2*(2), 324–329.

Kirillov, A.A. (2010). Helminths of reptiles from the Volga region are peculiar to other animals. *Vestnik of Samara University*, *80*(6), 196–205.

Kuchboev, A.E.; Shakarboev, E.B. and Kucharova, I.S. (2002). Helminths of amphibians of Uzbekistan. *Uzbek Biology Journal*, *1*, 39-43.

Kuzmin, Y.; Tkach, V.V. and Snyder, S.D. (2003). Helminths of amphibians and reptiles from northern Vietnam. *Comparative Parasitology*, *70*(2), 145–156.

Mirzaev, U.N.; Kuchboev, A.E.; Mavlyanov, O.; Amirov, O.O. and Narzullayev, S.B. (2024). Morphological and molecular characterization of root-knot nematodes. *Biosystems Diversity*, *32*(1), 135–141.

Nadler, S.A. and Pérez-Ponce de León, G. (2011). Integrating molecular and morphological approaches for characterizing parasite cryptic species. *Journal of Parasitology*, *97*(6), 1007–1015.

Okulewicz, A.; Hildebrand, J.; Łysowski, R.; Buńkowska, K. and Perec-Matysiak, A. (2014). Helminth Communities of Green and Brown Frogs from Poland (Lower Silesia Region). *Journal of Herpetology*, *48*(1), 34–37.

Petter, A.J. and Quentin, J.C. (1976). Keys to genera of the Oxyuroidea. *Systematic Parasitology*, *20*(3), 163–179.

Popiołek, M.; Witkowski, A.; Kotusz, J.; Kusznierz, J. and Baldy, K. (2004). Intestinal parasites of brown trout (*Salmo trutta fario* L.) from streams of the Stołowe Mountains National Park. *Parki Narodowe i Rezerwaty Przyrody*, *23*, 121–127.

Popiołek, M.; Rozenblut-Kościsty, B.; Kot, M.; Nosal, W. and Ogielska, M. (2011). Endoparasitic helminths of water frog complex in Poland: do differences exist between the parental species *Pelophylax ridibundus* and *Pelophylax lessonae*, and their natural hybrid *Pelophylax esculentus*? *Helminthologia*, *48*(2), 108–115.