

## Morphological and Molecular-Genetic Characterization of the Nematode *Aplectana acuminata* Schrank, 1788 (Nematoda: Cosmocercidae) in Uzbekistan

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### ABSTRACT

In this article, the partial nucleotides of the ribosomal DNA (rDNA) ITS region gene of *A. acuminata* nematodes were studied molecular-genetically. To conduct morphological and molecular-genetic research, 264 amphibian specimens were helminthologically examined from southern (Qashqadaryo and Surxondaryo regions) and central (Bukhara and Navoi regions) territories of Uzbekistan during 2023-2025. Morphological analyses revealed that the body length of male *A. acuminata* nematodes is 1.5- 3.6mm, while that of females is 3.3- 5.2mm; the muscular part of the esophagus forms a bulb, with the excretory pore located around it and opening after 0.26-0.37mm from the anterior part of the body. Comparative studies of our data and those of other authors determined that the width of the esophagus-intestine junction and the morphometric dimensions of the bulb circumference of *A. acuminata* nematodes are slightly larger. According to the results of molecular-genetic studies based on Kimura's K2P model, transition substitutions (A↔G and C↔T) showed high frequencies, with an average distance of 13.6233. Transversion substitutions (A↔C, A↔T, G↔C, G↔T) demonstrated lower values, with an average result of 5.6883. The transition/transversion (Ti/Tv) ratio was ≈2.4. Neutrality test results based on 42 ITS sequences identified a total of 648 polymorphic sites. The calculated Tajima's D value was 0.659444, indicating no significant deviation from neutral evolution. The phylogenetic tree of *A. acuminata\_uzb* specimens belonging to the genus *Aplectana* Railliet & Henry, 1916, along with closely related representatives available in the NCBI database, formed 5 monophyletic groups with bootstrap values ranging from 51.2 to 100%. For the first time in Uzbekistan, the nucleotide sequence of the ITS region of rDNA belonging to *A. acuminata\_uzb* nematodes was extracted and uploaded to the National Center for Biotechnology Information (NCBI) database by us. Based on morphological and molecular-genetic research results, this nematode species was confirmed to be a distinct, stable species.

### INTRODUCTION

The family *Cosmocercidae* Railliet, 1916 is known to include more than 200 species worldwide, primarily parasitizing the digestive systems of various amphibians and reptiles (González & Hamann, 2010; Sou & Nandi, 2015). The most widespread genera in this family are *Cosmocerca* Diesing, 1861, and *Aplectana* Railliet & Henry, 1916 with the genus *Aplectana* being

the most species-rich among amphibians (Bursey *et al.*, 2011, 2018; Campião *et al.*, 2014; González *et al.*, 2021).

Currently, there are 57 well-described species of the genus *Aplectana* Railliet & Henry, 1916, with 28 of them distributed across the Neotropical region (Ramallo *et al.*, 2007; Sou *et al.*, 2018; Alcantara *et al.*, 2021). These species have been recorded in various regions of the world, including the Neotropical (South and Central America, Brazil, Nicaragua, Argentina), Afrotropical, Palearctic (Germany, Denmark, England, Finland, France, Turkey, Saudi Arabia, Yemen, Central Asia), Indomalaya (India, China, Nepal, Japan), and other regions (Travassos, 1925; Holmes *et al.*, 2008; Yildirimhan & Öz, 2008; Mohammad *et al.*, 2015; Chen *et al.*, 2021; Frost, 2023; Ikromov *et al.*, 2023).

The amphibian parasite *Aplectana acuminata* (Schrunk, 1788) Railliet & Henry, 1916 nematode was studied as a species after Baker reclassified *Ascaris acuminata* Schrunk, 1788 as a representative of the genus *Aplectana* Railliet and Henry, 1916 (Baker, 1980). Morphological descriptions of *Aplectana acuminata* (Schrunk, 1788) Railliet & Henry, 1916 nematode recorded in Turkey have been thoroughly detailed (Yildirimhan & Öz, 2008).

In Uzbekistan, two species belonging to the genus *Aplectana* Railliet & Henry, 1916 (*A. multipapilosa*, *A. acuminata*) have been identified, with these species recorded in the intestines of *Bufotes* and *Pelophylax* genus representatives (Ikromov & Ikromov, 2019; Ikromov *et al.*, 2023). Among these, *Aplectana acuminata* (Schrunk, 1788) Railliet & Henry, 1916 is the most widespread and frequently observed species. These studies contribute to the scientific understanding of amphibian nematode fauna and its distribution within Uzbekistan.

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 (Kimyonazarov *et al.*, 2024; Kadirov *et al.*, 2024), which have all been studied at the molecular level.

However, the identification of cosmocercoid nematodes, including species of the genus *Aplectana* Railliet & Henry, 1916, often relies on micromorphology, and there is currently a greater need for molecular-genetic data (Aliyev *et al.*, 2024). Therefore, previous materials need to be re-examined.

The current study aimed to conduct the morphological and molecular-genetic characterization of the widely distributed amphibian nematode *Aplectana acuminata* (Schrunk, 1788) Railliet & Henry, 1916 in Uzbekistan.

## MATERIALS AND METHODS

### Sample collection

To conduct morphological and molecular-genetic research, amphibian samples were collected from the southern [Kashkadarya (Mirishkor 38°57'13.58"N, 64°42'46.42"E; Shakhrisabz 39°5'31.73"N, 66°52'37.59"E) and Surkhandarya (Termiz 37°13'46.33"N, 67°19'14.90"E; Boysun 38°14'38.89"N, 67°14'23.55"E) regions] and central [Bukhara (Korovulbazar 39°22'26.37"N, 64°29'1.04"E) and Navoi (Koshkuduk 40°59'56.22"N, 65°54'45.85"E) regions] parts of Uzbekistan during 2023–2025. Amphibian specimens were collected from water reservoirs, lakes, riverbanks,

and water storage basins using route and stationary methods for helminthological examination. A total of 264 amphibian specimens were studied, including 139 specimens of *Pelophylax terentievi* (Mezhzherin, 1992) (90 specimens from Kashkadarya and 49 specimens from Surkhandarya) (Mazepa, 2013; Showler, 2018; Ualiyeva *et al.*, 2022), and 125 specimens of *Bufo peskovyi* (Bedriaga, 1898) (73 specimens from Kashkadarya and 52 specimens from Surkhandarya) (Dufresnes *et al.*, 2019; Litvinchuk *et al.*, 2021; Aliyev *et al.*, 2025). Additionally, the identification of amphibian species composition was carried out with the collaboration of scientific staff from the Zoology Institute's Rare Animal Species Accounting and Cadaster Laboratory.

For conducting helminthological studies on amphibians, euthanasia was performed by injecting 20% benzocaine hydrochloride into the abdominal cavity in accordance with AVMA Euthanasia Guidelines (AVMA Guidelines for the Euthanasia, 2020). The body cavity was opened with a longitudinal ventral incision (Skryabin, 1928). The digestive system was dissected into sections, including the stomach, small intestine, large intestine, and cloaca. Each section and other organs (lungs, liver, gallbladder, kidneys, and urinary bladder) were placed into Petri dishes containing NaCl solution (0.9%) and examined under a stereomicroscope NSZ-818 (Nexcope, China). Identified nematodes were fixed in vials using a graded ethanol series (30% to 70%).

### **Morphological research method**

In the morphological and morphometric analysis of nematodes, specimens were soaked in distilled water and clarified with lactophenol. More than 200 permanent and temporary preparations were made under laboratory conditions. In determining the morphological accuracy of species, numerous literature sources were utilized (Travassos, 1925; Baker, 1980; Ryzhikov, 1980; Skryabin *et al.*, 1991; Ramallo *et al.*, 2007; Holmes *et al.*, 2008; Yildirimhan & Öz, 2008; Anderson *et al.*, 2009; Gibbons, 2010; Bursey *et al.*, 2011, 2019; Campião *et al.*, 2014; Mohammad *et al.*, 2015; Sou *et al.*, 2018; Ikromov & Ikromov, 2019; Alcantara *et al.*, 2021; Chen *et al.*, 2021; González *et al.*, 2021; Khrustalev & Moskvina, 2021; Frost, 2023; Ikromov *et al.*, 2023; Santos *et al.*, 2023; Aliyev *et al.*, 2024). The original microphotographs of species in the prepared specimens were captured using ML-2000 (Meiji Techno CO., Ltd., Japan) and NE930-FL (Nexcope, China) microscopes available at the Molecular Zoology Laboratory of the Institute of Zoology, Academy of Sciences of Uzbekistan. Drawings were made using a microprojector, and measurements were conducted using an optical micrometer. Additional indicators of amphibians' nematode infestation, such as invasion extensiveness (IE), invasion intensity (II), and dominance index (DI) values, were presented (Bush *et al.*, 1997; Reiczigel *et al.*, 2019). Collected voucher samples were fixed in 70% ethanol and labeled for storage in the collection at the Institute of Zoology, Academy of Sciences of Uzbekistan (for *Aplectana acuminata* (Schrank, 1788) Raillet & Henry, 1916 under № 212\_Uzb).

### **Molecular phylogenetic research method**

For molecular-phylogenetic analysis, *ITS* fragments of ribosomal DNA from the nematode species *A. acuminata* were isolated. Initially, genomic DNA was extracted from nematode tissues using the DNeasy Blood and Tissue Kit (Qiagen Inc., November 2023) reagents. The DNA concentration in each sample was determined using a Thermo Fisher Scientific (China) spectrophotometer and stored at  $-20^{\circ}\text{C}$  until the PCR process.

During the polymerase chain reaction (PCR) process, AV28 (ata tgc tta agt tca gcg ggt) forward and TW81 (ggt tcc gta ggt gaa cct gc) reverse primers were used for the amplification of the *ITS* region of ribosomal DNA. The reaction conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 9 cycles – denaturation (94°C, 1 minute), annealing (54°C, 1.5 minutes), and elongation (72°C, 1.5 minutes); the subsequent 24 cycles were conducted in the order of denaturation (94°C, 45 seconds), annealing (54°C, 45 seconds), and elongation (72°C, 2 minutes). Final elongation was carried out at 72°C for 5 minutes. PCR products were confirmed via electrophoresis on a 1% agarose gel (100 V, 80-100 mA, 30-40 minutes).

Sequencing was performed using ABI PRISM® BigDye™ Terminator v. 3.1 reagents, with results recorded on an ABI PRISM 3100-Avant automated sequencer (Moscow, Russia). The obtained nucleotide sequences were analyzed using Bioedit, Clustal W, and DNASTAR™ software.

For phylogenetic tree construction, *ITS* sequences obtained from the genus *Aplectana* and additional DNA sequences from the NCBI database (<https://www.ncbi.nlm.nih.gov/>) were used. Sequencing data were initially manually edited in the Genius Prime software, and consensus sequences were calculated using the MEGA 12 software. Additionally, alignment and verification were conducted using MAFFT v.7 (Kato *et al.*, 2005) and Clustal Omega 1.2.2 software. The phylogenetic tree was constructed using the Maximum Likelihood (ML) method with 1000 bootstrap repetitions, performed using IQ-TREE 1.6.12 software on the CIPRES Science Gateway V 3.3 platform.

The visualization and editing of the obtained tree were carried out using iTOL v6.6 software (Letunic & Bork, 2021). During the study, the *A. acuminata\_uzb* sample was included as an outgroup, enabling the formation of consensus trees based on the *ITS* region.

To evaluate the degree of nucleotide variation, the Kimura 2-parameter (K2P) model was applied (Kimura, 1980). This model calculates evolutionary distances between nucleotides by distinguishing between transition (A↔G, C↔T) and transversion (A↔C, A↔T, G↔C, G↔T) frequencies. Evolutionary distance was calculated using the following formula:

$$d = -\frac{1}{2} \ln(1 - 2P - Q) - \frac{1}{4} \ln(1 - 2Q)$$

Where, P – transition probability, Q – transversion probability.

Calculations were performed using MEGA 12 software (Kumar *et al.*, 2018), and the results were presented in the form of a pairwise distance matrix.

## RESULTS AND DISCUSSION

### Morphological research results

During the years 2023-2025, 264 specimens of amphibians, *Pelophylax terentievi* (Mezhzherin, 1992) and *Bufo peszowi* (Bedriaga, 1898) were examined helminthologically. As a result of morphological analysis of nematodes, the nematode *Aplectana acuminata* (Schrunk, 1788) Railliet & Henry, 1916 was identified in 53 amphibian specimens.

**Order:** Rhabditida

**Family:** Cosmocercidae Railliet, 1925

**Genus:** *Aplectana* Railliet & Henry, 1916

**Species:** *Aplectana acuminata* (Schrank, 1788) Railiet & Henry, 1916

Host species: *Pelophylax terentievi* (Mezhzherin, 1992) and *Bufo peszowi* (Bedriaga, 1898) (Amphibia: Ranidae; Bufonidae)

**Identified locations:** Mirishkor district (38°57'13.58"N, 64°42'46.42"E) and Shahrisabz district (39°5'31.73"N, 66°52'37.59"E) of Kashkadarya region, Termiz district (37°13'46.33"N, 67°19'14.90"E) and Boysun district (38°14'38.89"N, 67°14'23.55"E) of Surkhandarya region, Korovulbazar district of Bukhara region (39°22'26.37"N, 64°29'1.04"E), and Koshkuduk neighborhood of Navoi region (40°59'56.22"N, 65°54'45.85"E).

**Deposited sample:** № 212\_Uzb (original females)

**Localization:** intestine.

**Extensive invasion (EI):** *P. terentievi* – 22.3%

*B. peszowi* – 17.6%

**Intensive invasion (II):** *P. terentievi* – 3–8

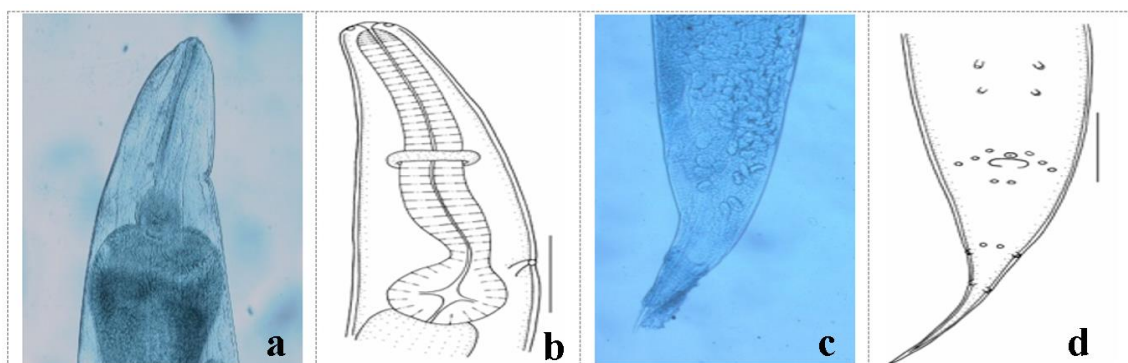
*B. peszowi* – 1–4

**Density index (DI):** *P. terentievi* – 1.32%

*B. peszowi* – 0.84%

*Aplectana acuminata* (Schrank, 1788) Railiet & Henry, 1916 are small-sized nematodes, with males having a body length of 1.5–3.6 mm and females 3.3–5.2 mm. The body is covered with a thin, transverse-lined cuticle. Sexual dimorphism is pronounced, with males being smaller than females. A lateral chain, starting from the anterior part of the body and ending near the tail tip, is present in both sexes. The mouth is formed by three small lips, with a pair of papillae on the dorsal lip, while each subventral lip has one papilla and one amphid (Fig. 1a, b).

The muscular part of the esophagus forms a bulb, with the excretory pore located around the bulb, opening 0.26–0.37 mm posterior to the anterior part of the body. Numerous cuticular processes are present on the surface of the cuticle; they are clearly visible on the conical tail of females (Fig. 1c, d).



**Fig. 1.** *Aplectana acuminata* (Schrank, 1788) Railiet & Henry, 1916 (♀) microphotographs and illustrations (original: a, c; illustration: b, d). a- anterior part of the body; b- lateral view of the anterior part; c- posterior part; d- lateral view of the posterior part

According to our data and those of other authors, when the main morphometric measurements of *A. acuminata* female nematodes were studied, the width at the esophagus-intestine junction and the circumference of the bulb differed slightly in size (Table 1).



**Table 1.** Comparative morphometric measurements of *Aplectana acuminata* (Schränk, 1788) Raillet & Henry, 1916 (♀) (n=60; mm)

Organs	Travassos (1931)	Santos <i>et al.</i> (2023)	Ikromov <i>et al.</i> (2023)	Our data (2023-2025)
Body length	4.0-4.8	3.5 (2.3-4.3)	3.9-5.1	3.3-5.2
Width at the esophagus-intestinal junction	0.089-0.118	0.242 (0.178-0.306)	0.43-0.68	0.4-0.71
The distance from the front part of the body to the excretory opening	-	0.408 (0.349-0.453)	-	0.26-0.37
Esophagus length	-	0.592 (0.529-0.594)	0.5-0.65	0.48-0.83
Bulbous circle	-	0.112 (0.096-0.117) × 0.133 (0.114-0.144)	0.17-0.19 x 0.16-0.18	0.13-0.2 x 0.19-0.24
The distance from the anterior end of the body to the nerve ring	-	0.202 (0.178-0.245)	-	-
The distance from the front end of the body to the vulva	1.9-2.4	1.6 (0.6-2)	-	1.4-2.9
Tail length	0.51-0.92	0.332 (0.321-0.394)	0.58-0.72	0.54-0.93

### Molecular phylogenetic research results

Genetic distance analysis based on the Kimura 2-parameter (K2P) model allowed the identification of the evolutionary pattern of nucleotide substitutions in *ITS* sequences. The results showed that transition substitutions (A↔G, C↔T) occur at a significantly higher frequency compared to transversion substitutions (A↔C, A↔T, G↔C, G↔T). The average transition distance was 13.6233, which is more than twice the transversion distance (5.6883). Consequently, the transition/transversion (Ti/Tv) ratio was approximately 2.4, indicating that mutational processes in the *ITS* region are not random and are influenced by specific molecular mechanisms and evolutionary constraints.

The observed Ti/Tv ratio corresponds to evolutionary patterns typical for ribosomal DNA markers. Transition substitutions generally have less negative impact on the structural and functional stability of the DNA molecule compared to transversions (Kimura, 1980; Kumar *et al.*, 2018). Therefore, the predominance of transitions enhances the reliability of the phylogenetic signal of the *ITS* region, confirming its effectiveness in systematic studies. This result aligns with previous molecular studies that reported the high frequency of transitions in both nuclear and mitochondrial markers (Yang & Rannala, 2012).

**Table 2.** Pairwise distance matrix based on the K2P model

	A	T	C	G
A	-	5.6883	5.6883	13.6233
T	5.6883	-	13.6233	5.6883
C	5.6883	13.6233	-	5.6883
G	13.6233	5.6883	5.6883	-

The obtained K2P pairwise distance matrix accurately reflected genetic differences between nucleotides and was subsequently used in the construction of the phylogenetic tree. This tree enabled the identification of inter-genus and intra-species differentiation. Particularly, the dominance of transitions over transversions confirms the methodological appropriateness of using the K2P model, as this model accounts for the unequal rates of transitions and transversions (Posada & Crandall, 2001) (Table 2).

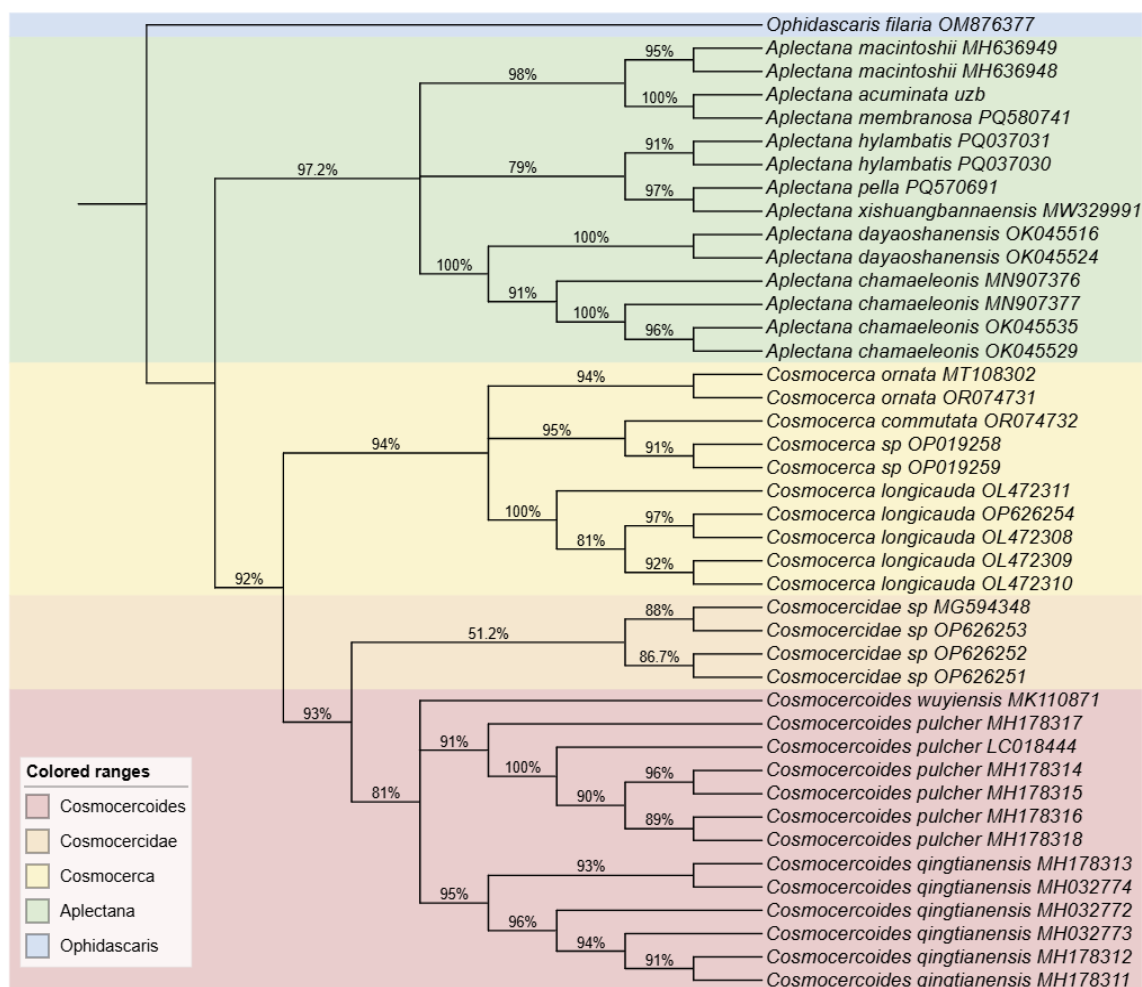
Overall, the nucleotide substitution pattern observed in *A. acuminata* demonstrates that the ribosomal *ITS* region aligns with evolutionary traits expected for phylogenetic and taxonomic studies. These findings further validate *ITS* sequences as highly reliable markers for determining genetic relationships within Nematoda. Consequently, this marker has been confirmed as an effective tool for resolving systematic issues at both species and genus levels.

#### Tajima's D analysis

Based on *ITS* markers, nucleotide diversity analysis identified a total of 648 polymorphic sites across 42 sequences. The segregation frequency ( $p_s$ ) was 0.964286, indicating a high level of variability among the analyzed samples. Nucleotide diversity ( $\pi = 0.263740$ ) reflects the genetic richness formed within the population, while Watterson's  $W$  value ( $W = 0.224100$ ) represents the expected level of diversity based on mutational processes. The proximity of these two indicators suggests that genetic diversity in the studied *ITS* region has formed at a relatively stable level (Workentine *et al.*, 2020).

Tajima's  $D$  statistic ( $D = 0.659444$ ) did not show significant deviation from the neutral evolution model. This indicates that the polymorphisms observed in *ITS* sequences are primarily formed as a result of neutral mutations and genetic drift (Teterina *et al.*, 2023). Although the positive value of  $D$  does not exclude the possibility of balancing selection or population substructure, its low level suggests the weakness of these probabilities. Therefore, these results should be interpreted cautiously (Rogers *et al.*, 2023).

The obtained data confirm that the *ITS* marker reliably reflects population-genetic signals based on the neutral model. These findings further demonstrate the effectiveness of the *ITS* region in phylogenetic and population studies. Overall, the detected high level of polymorphism and evolutionary pattern consistent with neutrality indicate that genetic diversity in *A. acuminata* populations is stably formed, confirming the utility of the *ITS* marker as a valuable tool for deeper analysis of population historical processes in this species.



**Fig. 2.** Phylogenetic tree of *Aplectana acuminata\_uzb* and closely related species based on the Maximum Likelihood (ML) method

The results of phylogenetic analysis based on the Maximum Likelihood (ML) method clearly illustrate the internal structure of the Cosmocercidae family (Fig. 2). The tree was rooted using the sample of *Ophidascaris filaria* (OM876377) and formed five main clusters: *Aplectana*, *Cosmocerca*, *Cosmocercidae* sp., *Cosmocercoides*, and *Ophidascaris* as the outgroup. The formation of these clusters further clarified the systematic composition of the family and the phylogenetic boundaries between different genera.

The *Aplectana* cluster (green) emerged as a robust monophyletic group with a high bootstrap value (97.2%). Within the cluster, the species *A. macintoshii* (98%) and *A. chamaeleonis* (100%) formed strongly supported units, demonstrating the phylogenetic stability of the representatives of this genus. The sample of *A. acuminata\_uzb* collected from Uzbekistan was also confidently placed within this genus, forming a common node with *A. macintoshii*, which molecular-genetic evidence confirms as proof of the species' presence in the region. This finding suggests that representatives of the genus *Aplectana* in Uzbekistan's nematode fauna are yet to be fully studied, indicating the potential for discovering new species.

The *Cosmocerca* cluster (yellow) was also supported as a monophyletic group with a high bootstrap value (92%). Notably, samples of *C. ornata* (94-95%) and *C. commutata* and *Cosmocerca*



sp. (100%) exhibited high reliability. However, samples of *C. longicauda* showed a lower bootstrap value (81%), which may be explained by the species' internal genetic diversity or incomplete taxonomic information.

The phylogenetic placement of *Cosmocercidae* sp. (orange) samples was recorded at relatively low-supported nodes (51.2-86.7%). This indicates the possibility that these samples belong to unidentified or unclassified taxa. Therefore, it is essential to reevaluate these group representatives through more detailed molecular-genetic and morphological studies.

The *Cosmocercoides* cluster (pink) was strongly supported with a high bootstrap value (93%). High levels of phylogenetic stability were observed within internal clades, particularly in *C. pulcher* (90-100%) and *C. qingtianensis* (91-100%), which formed robust units. These findings confirm the phylogenetic integrity of the *Cosmocercoides* genus.

Overall, the results of the ML analysis reliably demonstrated the monophyly of the *Aplectana*, *Cosmocerca*, and *Cosmocercoides* genera. The sample of *A. acuminata* collected from Uzbekistan was confidently assigned to the *Aplectana* genus, confirming the species' presence in the region based on molecular genetic evidence. This discovery expands the diversity of nematodes in Uzbekistan and highlights the need to further develop molecular-genetic studies in the region.

## CONCLUSIONS AND RECOMMENDATIONS

To date, *A. acuminata* has been frequently recorded in Uzbekistan based on its morphological characteristics. This study represents the first molecular-phylogenetic characterization of *A. acuminata* in Uzbekistan. For the first time, the nucleotide sequences of the *ITS* region of rDNA for the *A. acuminata\_uzb* sample have been uploaded to the NCBI database.

It was determined that representatives of the genus *Aplectana* Railliet & Henry, 1916, along with closely related genera, formed five monophyletic groups in the phylogenetic tree, with bootstrap support values ranging from 51.2 to 100%. The low bootstrap support observed in some monophyletic groups indicates that the evolutionary divergence of species within these groups occurred relatively recently.

*A. acuminata\_uzb* was positioned at the base of the *Aplectana* cluster, and its high genetic similarity to other local species, such as *Aplectana macintoshii* (95%) and *Aplectana membranosa* (100%), was confirmed with strong bootstrap support. This analysis contributed to clarifying the taxonomic structure of the genus *Aplectana* Railliet & Henry, 1916, and provided deeper insights into its evolutionary history.

The results of the molecular phylogenetic analysis confirmed that *A. acuminata\_uzb* is an independent species. Future genomic studies of this species will further aid in understanding its phylogeny and ecological adaptations. This study demonstrated the advantage of PCR-based sequencing methods in identifying the ecological and biogeographic significance, as well as the species composition, of *A. acuminata\_uzb*.

## 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, as well as to Associate Professor of the Department of Biotechnology of Namangan State University, Ph.D. E.F. Ikromov, and Senior Lecturer E.E. Ikromov for their support in the morphological analysis of nematode samples.

## NOVELTY STATEMENT

The molecular identification of *A. acuminata\_uzb* nematode was carried out for the first time in Uzbekistan. According to the results of molecular genetic studies, the nucleotide sequence related to the *ITS* region of the rDNA of *A. acuminata\_uzb* nematode was extracted and, for the first time, uploaded by us to the National Center for Biotechnology Information (NCBI).

## REFERENCES

- Aliyev, S.T.; Amirov, O.O.; Egamberdiyev, M.Kh. and Akhmadjonova, S.Sh. (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. <https://doi.org/10.21608/EJABF.2024.380435>
- 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. <https://doi.org/10.21608/ejabf.2025.432151>
- Alcantara, E.P.; Ferreira-Silva, C.; Forti, L.R.; Morales, D.H. and Silva, R.J. (2021). A new species of *Aplectana* (Nematoda: Cosmocercidae) in the marsupial frog *Gastrotheca microdiscus* (Amphibia: Hemiphractidae) from Brazil. *Zootaxa*, 4908(3), 426–434. <https://doi.org/10.11646/zootaxa.4908.3.7>
- 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. <https://doi.org/10.17582/journal.aavs/2021/9.9.1396.1399>
- Anderson, R.C.; Chabaud, A.G. and Willmott, S. (2009). *Keys to the Nematode Parasites of Vertebrates: An Archival Volume*. CABI International. <https://doi.org/10.1079/9781845935726.0000>

- AVMA Panel on Euthanasia. (2020).** *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*. American Veterinary Medical Association.
- Baker, M.R. (1980).** Revision of Old World species of the genus *Aplectana* Railliet & Henry, 1916 (Nematoda, Cosmocercidae). *Bulletin du Muséum National d'Histoire Naturelle*, 4(2), 955–998.
- Bursey, C.R.; Goldberg, S.R. and Kraus, F. (2011).** New species of *Aplectana* (Nematoda: Cosmocercidae) in *Sphenomorphus pratti* from Papua New Guinea. *Journal of Parasitology*, 97(4), 654–660. <https://doi.org/10.1645/GE-2720.1>
- Bursey, C.R.; Goldberg, S.R. and Grismer, L.L. (2018).** A new species of *Aplectana* (Nematoda, Cosmocercidae) in *Goniurosaurus bawanglingensis* (Squamata, Eublepharidae), from Hainan Province, China. *Acta Parasitologica*, 63(1), 190–197. <https://doi.org/10.1515/ap-2018-0022>
- Bush, A.O.; Lafferty, K.D.; Lotz, J.M. and Shostak, A.W. (1997).** Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, 83(4), 575–583. <https://doi.org/10.2307/3284227>
- Campião, K.M.; Morales, D.H.; Dias, O.T.; Aguiar, A.; Toledo, G.M. and Tavares, L.E.R. (2014).** Checklist of helminth parasites of amphibians from South America. *Zootaxa*, 3843(1), 1–93. <https://doi.org/10.11646/zootaxa.3843.1.1>
- Chen, H.X.; Gu, X.H.; Ni, X.F. and Li, L. (2021).** Description of a new species of *Aplectana* (Nematoda: Ascaridomorpha: Cosmocercidae) using an integrative approach and preliminary phylogenetic study of Cosmocercidae and related taxa. *Parasites & Vectors*, 14, 189. <https://doi.org/10.1186/s13071-021-04667-9>
- Dufresnes, C.; Mazepa, G.; Jablonski, D.; Caliari Oliveira, R.; Wenseleers, T.; Shabanov, D.A.; Auer, M.; Ernst, R.; Koch, C.; Ramírez-Chaves, H.E.; Mulder, K.P.; Simonov, E.; Tiutenko, A.; Kryvokhyzha, D.; Wennekes, P.L.; Zinenko, O.I.; Korshunov, O.V.; Al-Johany, A.M.; Peregontsev, E.A.; Masroor, R.; Betto-Colliard, C.; Denoël, M.; Borkin, L.J.; Skorinov, D.V.; Pasyukova, R.A.; Mazanaeva, L.F.; Rosanov, J.M.; Dubey, S. and Litvinchuk, S.N. (2019).** Fifteen shades of green: The evolution of *Bufotes* toads revisited. *Molecular Phylogenetics and Evolution*, 141, 106615. <https://doi.org/10.1016/j.ympev.2019.106615>
- Frost, D.R. (2023).** *Amphibian Species of the World: An Online Reference. Version 6.1*. American Museum of Natural History. Retrieved from <https://amphibiansoftheworld.amnh.org>
- Gibbons, L.M. (2010).** *Keys to the Nematode Parasites of Vertebrates: Supplementary Volume*. CABI Publishing.
- González, C.E. and Hamann, M.I. (2010).** First report of nematode parasites of *Physalaemus santafecinus* (Anura: Leiuperidae) from Corrientes, Argentina. *Revista Mexicana de Biodiversidad*, 81(3), 677–687. <https://doi.org/10.22201/ib.20078706e.2010.003.666>
- González, C.E.; Hamann, M.I. and Duré, M.I. (2021).** Nematodes of amphibians from the South American Chaco: distribution, host specificity and ecological aspects. *Diversity*, 13(7), 321. <https://doi.org/10.3390/d13070321>
- Holmes, R.M.; Bochiglieri, A.; Araújo, F.R.R.C. and Silva, R.J. (2008).** New records of endoparasites infecting *Hypsiboas albopunctatus* (Anura: Hylidae) in a savanna area in

- Brasília, Brazil. *Parasitology Research*, 102(4), 621–623. <https://doi.org/10.1007/s00436-007-0797-z>
- Ikromov, E.F. and Ikromov, E.E. (2019).** Species and ecological diversity of nematode amphibians of Uzbekistan. *International Journal of Science and Research*, 8(9), 1073–1075. <https://doi.org/10.21275/ART20201218>
- Ikromov, E.E.; Ikromov, E.F.; Yildirimhan, H.S.; Azimov, Dj.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, Yu.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.
- Katoh, K.; Kuma, K.; Toh, H. and Miyata, T. (2005).** MAFFT version 5: Improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, 33(2), 511–518. <https://doi.org/10.1093/nar/gki198>
- Khrustalev, A.V. and Moskvina, A.S. (2021).** *Annotated Catalog of the Typical Collection of Helminths*. Nauka. [In Russian]
- Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16(2), 111–120. <https://doi.org/10.1007/BF01731581>
- 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 *Tirogma caerulea* from Uzbekistan. *Zoosystematica Rossica*, 33(1), 92–94.
- Kumar, S.; Stecher, G.; Li, M.; Knyaz, C. and Tamura, K. (2018).** MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Letunic, I. and Bork, P. (2021).** Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 49(W1), W293–W296. <https://doi.org/10.1093/nar/gkab301>
- Litvinchuk, S.N.; Svinin, A.O. and Dujsebajeva, T.N. (2021).** Morphological differentiation between diploid and polyploid species of green toads (Anura: Bufonidae: *Bufotes*) in Central Asia. *Bonn Zoological Bulletin*, 70(2), 361–371. <https://doi.org/10.20363/BZB-2021.70.2.361>
- Mazepa, G. (2013).** *Evolution of Water Frogs Pelophylax in Central Asia: How Hybridization and Mitochondrial Introgression among Ecologically Divergent Species Promote Occupation of Novel Environment* [Master's Thesis]. Uppsala University.
- 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.
- Mohammad, M.K.; Habeeb, W.K.; Shubber, B.M.; Ali, B.M. and Al-Waaly, A.A. (2015).** Intestinal helminth parasites of the Eurasian marsh frog *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) collected in Al Diwaniya city, the middle of Iraq. *Bulletin of the Iraq Natural History Museum*, 13(4), 11–20.



- Posada, D. and Crandall, K.A. (2001). Selecting the best-fit model of nucleotide substitution. *Systematic Biology*, 50(4), 580–601. <https://doi.org/10.1080/106351501750435121>
- Quvatov, A.Q.; Kuchboev, A.E.; Mirzayev, U.T.; Amirov, O.O.; Atamuratova, M.Sh. and Narboev, Z.U. (2023). Morphometric and molecular characteristics of *Cottus jaxartensis*. *Egyptian Journal of Aquatic Biology & Fisheries*, 27(6), 215–223.
- Ramallo, G.; Bursey, C.R. and Goldberg, S.R. (2007). Two new species of cosmocercids (Ascaridida) in the toad *Chaunus arenarum* (Anura: Bufonidae) from Argentina. *Journal of Parasitology*, 93(4), 910–916. <https://doi.org/10.1645/GE-1131R.1>
- Reiczigel, J.; Marozzi, M.; Fábíán, I. and Rozsa, L. (2019). Biostatistics for parasitologists – a primer to quantitative parasitology. *Trends in Parasitology*, 35(4), 277–281. <https://doi.org/10.1016/j.pt.2019.01.003>
- Rogers, R.L.; Grizzard, S.L. and Garner, J.T. (2023). Strong, recent selective sweeps reshape genetic diversity. *Molecular Biology and Evolution*, 40(2), msad024. <https://doi.org/10.1093/molbev/msad024>
- Ryzhikov, K.M.; Sharpilo, V.P. and Shevchenko, N.N. (1980). *Helminths of Amphibians of the Fauna of the USSR*. Nauka. [In Russian]
- Santos, A.N.; Borges, E.S.; Wilkens, Y.; Santos, J.N.; Costa-Campos, C.E. and Melo, F.T.V. (2023). A new species of *Aplectana* Railliet & Henry, 1916 (Nematoda: Cosmocercidae) in the Brazilian Amazon and the taxonomic status of *Aplectana longa*. *Brazilian Journal of Veterinary Parasitology*, 32(4), e014023. <https://doi.org/10.1590/S1984-29612023074>
- Showler, D.A. (2018). *A Checklist of the Amphibians and Reptiles of the Republic of Uzbekistan with a Review and Summary of Species Distribution*. Sustainable Houbara Management.
- Skryabin, K.I. (1928). *The Method of Complete Helminthological Dissections of Vertebrates, Including Humans*. Moscow State University Press. [In Russian]
- Skryabin, K.I.; Shikhobalova, N.P. and Mozgovoy, A.A. (1991). *Key to Parasitic Nematodes. Volume 2: Oxyurids and Ascarids*. Academy of Sciences of the USSR. [In Russian]
- Sou, S.K. and Nandi, A.P. (2015). On a new species of *Cosmocerca* (Nematoda; Cosmocercidae) from *Microhyla rubra* (Anura: Microhylidae) from West Bengal, India. *Acta Parasitologica*, 60(2), 261–265. <https://doi.org/10.1515/ap-2015-0037>
- Sou, S.K.; Sow, K.K. and Nandi, A.P. (2018). *Aplectana hoplobatrachus* sp. nov. (Nematoda: Cosmocercidae) in *Hoplobatrachus crassus* (Jerdon, 1853) (Anura: Dicroglossidae) from Birbhum District, West Bengal, India. *Zootaxa*, 4472(1), 194–200. <https://doi.org/10.11646/zootaxa.4472.1.12>
- Teterina, A.A.; Willis, J.H.; Lukac, M.; Jovelín, R.; Cutter, A.D. and Phillips, P.C. (2023). Genomic diversity landscapes in outcrossing and selfing *Caenorhabditis* nematodes. *PLOS Genetics*, 19(8), e1010879. <https://doi.org/10.1371/journal.pgen.1010879>
- Travassos, L. (1931). Pesquisas helminthológicas realizadas em Hamburgo: IX. Ensaio monographico da família Cosmocercidae Trav., 1925 (Nematoda). *Memórias do Instituto Oswaldo Cruz*, 25(3), 237–298. <https://doi.org/10.1590/S0074-02761931000300003>
- Ualiyeva, D.; Ermakov, O.A.; Litvinchuk, S.N.; Guo, X.; Ivanov, A.Yu.; Xu, R.; Li, J.; Xu, F.; Arifulova, I.I.; Kaptyonkina, A.G.; Khromov, V.A.; Krainyuk, V.N.; Sarzhanov, F. and Dujsebayaeva, T.N. (2022). Diversity, phylogenetic relationships, and distribution of marsh



- frogs (the *Pelophylax ridibundus* complex) from Kazakhstan and Northwest China. *Diversity*, 14(10), 869. <https://doi.org/10.3390/d14100869>
- Ubaydullayev, O.Kh.; Amirov, O.O.; Quvatov, A.Q.; Yusupov, A.P.; Narboev, Z.U.; Donayeva, Sh.A. and Nomonov, J.N. (2025).** Molecular-genetic analysis of *Channa argus* (Cantor, 1842) (Teleostei: Channidae) distributed in the Kashkadarya River, Uzbekistan. *Egyptian Journal of Aquatic Biology & Fisheries*, 29(1), 1171–1180.
- Workentine, M.L.; Chen, R.; Zhu, S.; Gavriliuc, S.; Shaw, N.; de Rijke, J.; Redman, E.M.; Avramenko, R.W.; Wit, J.; Poissant, J. and Gilleard, J.S. (2020).** A database for ITS2 sequences from nematodes. *BMC Genetics*, 21(1), 74. <https://doi.org/10.1186/s12863-020-00880-0>
- Yang, Z. and Rannala, B. (2012).** Molecular phylogenetics: principles and practice. *Nature Reviews Genetics*, 13(5), 303–314. <https://doi.org/10.1038/nrg3186>
- Yıldırımhan, H.S. and Öz, M. (2008).** Helminth fauna of *Lyciasalamandra billae* (Franzen & Klewen) (Luschan salamander) collected from Antalya. *Türkiye Parazitoloji Dergisi*, 32(4), 390–392.