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Morphological and molecular characteristics of the species *Cosmocerca commutata* and *C. ornata* (Nematoda: Cosmocercidae) in Uzbekistan

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Abstract. *Ikromov EE, Kuchboev AE, Ikromov EF, Sümer N, Yildirimhan HS, Amirov OO, Zhumabekova B. 2023. Morphological and molecular characteristics of the species* Cosmocerca commutata *and* C. ornata *(Nematoda: Cosmocercidae) in Uzbekistan. Biodiversitas 24: 4609-4616.* In studies on the helminth fauna of amphibians conducted by numerous researchers, two species of the genus *Cosmocerca,* namely *Cosmocerca ornata* (Dujardin, 1845) Diesing, 1861 and *C. commutata* (Diesing, 1851), have emerged as the most common and extensively studied. This paper elucidates the morphology and molecular characteristics of two nematode species, *Cosmocerca commutata* and *C. ornata,* found in the intestines of *Pelophylax* sp. and *Bufotes pewzowi* in the Ferghana Valley of Uzbekistan. The captured amphibians were individually kept in plastic bags with water and vegetation until the examination. It is the first record of *B. pewzowi* as a host for both species *C. commutata* and *C. ornata*. Standard methods were used for the fixation and processing of nematodes. These nematodes мorphologically differ in body size, relative length of spicules and gubernaculum (*C. commutata* has twice the length of gubernaculum and spicules compared to *C. ornata*), shape and number of plectanes (*C. commutata* has 8 pairs of plectans and 4 pairs of caudal papillae, while *C. ornata* has 5 pairs of plectanes and 3 pairs of caudal papillae), and tail morphology and length. The morphological evidence supports that *C. commutata* and *C. ornata* commonly parasitize anurans in Uzbekistan. This study provides molecular data of both species and their sequences differed by 1.5% (6 bp) in the ITS1+5.8S+ITS2 region. Each species of them formed a well-supported clade in the phylogenetic tree. Our findings contribute to the comprehension of species and genetic diversity of the genus *Cosmocerca* in Uzbekistan.

Keywords: *Cosmocerca*, ITS, morphology, Nematoda, ribosomal DNA

INTRODUCTION

The superfamily Cosmocercoidea belongs to the class of nematodes and it encompasses three families: Atractidae Railliet, 1917, Cosmocercidae Railliet, 1916, and Katlaniidae Lane, 1914 (Hodda 2011; Zhang 2011). Among these families, Cosmocercidae stands out with over 200 known species, predominantly parasitizing the digestive system of various amphibians and reptile species (González and Hamann 2010; Sou and Nandi 2015). Specifically, within amphibians, approximately 30 species of the genus *Cosmocerca* (Diesing 1861) have been documented in their digestive systems (Bursey et al. 2015; Mohammad et al. 2015).

In studies on the helminthfauna of amphibians conducted by numerous researchers, two species of the genus *Cosmocerca*, namely *Cosmocerca ornata* (Dujardin, 1845) Diesing, 1861 and *C. commutata* (Diesing, 1851), have emerged as the most common and extensively studied. These species have been frequently reported in various regions across the globe, including Europe (Ukraine, France, Italy), Asia (Asian part of Russia, Iran, Turkey, Iraq, India), Latin America (Brazil), and Africa (León-Règagnon 2019; Sou et al. 2019; Kuzmin et al. 2020). *Cosmocerca ornata* is a widely distributed nematode parasite found in the Palearctic region. Morphologically, males of *C. ornata* are characterized by the presence of plectanes and a small granular spot (Baker and Vaucher 1984). The experimental life cycle of the amphibian parasite nematode *C. ornata* was studied for the first time (Kirillova and Kirillov 2021). Two species *C. commutata* and *C. ornata* described from India, are now considered valid (Sou et al. 2018). In Uzbekistan, studies on the helminth fauna of amphibians have identified two species of nematodes belonging to the genus *Cosmocerca* (Ikromov and Ikromov 2019; Ikromov et al. 2020). Among these species, *C. commutata* and *C. ornata* have been found to be the most prevalent and commonly observed. These findings contribute to the scientific understanding of nematode diversity and distribution within the region of Uzbekistan.

Shchepina and Baldanova (2010) investigated the helminth fauna of *Bufo raddei* in Transbaikalia and identified eleven species of parasitic nematodes while also examining the morphometric parameters of *C. commutata* and *C. ornata*. Chikhlyaev et al. (2014) studied the helminth fauna of *Rana temporaria* in the Volga basin, providing taxonomic information and localization details of *C. ornata*. Kuzmin et al. (2020) conducted helminthological studies on *Pelophylax ridibundus* and *P. esculentus* in northern Ukraine, recording 27 helminth species, including *C. ornata*. Yildirimhan et al. (1999) found *C. commutata* in the intestines of *Bufo viridis* collected in the swamps of Bursa and Edirne (Turkey). Additionally, Mohammad et al. (2015) detected a small number of *C. commutata* parasites in the intestines of *Pelophylax ridibundus* captured in Al Diwaniya (Iraq). Amphibian helminths, including *Cosmocerca* species, are widely distributed. In a study by Leon-Regagnon in Shiraz, Iran, the nematode *C. ornata* was found to be the most prevalent among the helminths infecting the Eurasian marsh frog, *Pelophylax ridibundus* (León-Règagnon 2019). These findings highlight the occurrence and importance of *C. ornata* in amphibian populations and contribute to our understanding of helminth diversity. In the region of Antalya, both mountain frogs (*Rana camerani* and *Rana macrocnemis*) were found to be infected with the nematode *C. ornata*, as reported by another researcher (Düşen 2011). Additionally, Saeed et al. (2007) conducted studies on the infestation and invasion intensity of three amphibian species, namely *Rana ridibunda*, *B. viridis*, and *Hyla arborea*, in Iraq. According to the study findings, *R. ridibunda* exhibited a lower level of infection and parasitic load with the nematodes *C. commutata* and *C. ornata*. These observations suggest the presence of *C. commutata* in amphibian populations in that particular area. The research was conducted in the southern continent of the world, specifically in the southern state of Bahia, Brazil. A total of 119 samples from the microhylid frog *Chiasmocleis capixaba* (Microhylidae) were examined, revealing that 66.4% of the specimens were infected with the intestinal nematode *C. ornata* (Van Sluys et al. 2006). This finding indicates a high prevalence of *C. ornata* in the studied population of *C. capixaba* frogs.

Furthermore, during the investigation of helminth fauna in amphibians commonly found on the African continent, the presence of the species was documented, highlighting its wide distribution (Halajian et al. 2013). These observations suggest that *C. ornata* has a broad geographic range and can be found in diverse amphibian populations across different regions. It should be noted that so far, as synonyms of *C. ornata*, scientists have represented the species *C. minuscula*, *Paracosmocerca mucronata*, *Cosmocercella polissensis*, *C. indica*, *Paracosmocerca spinocerca*, and *C. macrogubernaculum* (Sou and Nandi 2015). Similarly, *C. kashmirensis*, *C. indica*, *C. macrogubernaculum* and *C. ornata* have been reported as synonyms of *C. commutata* (Sou and Nandi 2015).

In recent years, molecular techniques have played a crucial role in the identification and taxonomic studies of parasitic nematodes, such as Ascaridid (Li et al. 2014; Sato et al. 2015; Zhang et al. 2018; Chen et al. 2020; Kuchboev et al. 2020; Soatov et al. 2023). Currently, only 23 species belonging to the subfamily Cosmocercinae have been genetically characterized in the GenBank database, with sequences available for markers such as 18S, 28S, ITS, 12S, cox1, and cox2. Specifically, only 10 sequences representing different *Cosmocerca* spp. are accessible in the database (Chen et al. 2020; Alcantara et al. 2022). However, the molecular identification of cosmocercoid nematodes, including the species *C. commutata*, remains limited due to the scarcity of genetic data.

The present study aims to elucidate the morphological and molecular characteristics of the common anuran nematodes, *C. commutata* and *C. ornata*, in Uzbekistan.

MATERIALS AND METHODS

Sampling and microphotography

During the period from 2019 to 2022, a total of 278 individuals from two amphibian species, *Pelophylax* sp. (Mazepa 2013; Ualiyeva et al. 2022) and Pewzow's toad *Bufotes pewzowi* (Bedriaga, 1898) (Dufresnes et al. 2019; Litvinchuk et al. 2021), were examined to collect parasitic helminths in the Namangan, Ferghana, and Andijan regions of the Fergana Valley, Tyura-Kurgan (41.028310"N, 71.500459"E), Mingbulak (40.867500"N, 71.382963"E), Pap (40.795729"N, 70.962605"E), Chust (40.958541"N, 71.305649"E), Uchkurgan (41.025507"N, 71.967785"E), Altyaryk (40.495897"N, 71.388120"E), Dangara (40.588714"N, 70.888522"E), Ulugnor (40.821684"N, 71.597074"E) and Bustan (40.703123"N, 71.890094"E). The captured amphibians were individually kept in plastic bags with water and vegetation until the examination. To determine the species of amphibians used in our study, we referred to the research work conducted by Mazepa (2013), Ualiyeva et al. (2022), Dufresnes et al. (2019), and Litvinchuk et al. (2021).

Collected anurans were euthanized by injection of 20% benzocaine hydrochloride into the abdominal cavity following the AVMA Euthanasia Guidelines (AVMA Guidelines for the Euthanasia 2020). The collected fixed nematodes were immersed in distilled water for approximately 15 min, cleared in glycerol for 5 min, and observed under a microscope MBC 10 (LOMO). The collection, fixation, and processing of the nematodes followed with Skrjabin (1928). The drawings of the nematodes were produced using an ML 2000 microscope attachment (Meiji) and documented using a digital camera. The specific diagnostic criteria for helminths were based on Ryzhikov et al. (1980). We applied standard indices as follows: extensiveness of infection (EI, %), intensity of infection (II, individuals), and index of helminth abundance (IA, individuals).

The collected voucher specimens were fixed in 70% ethanol and labeled for storing in the collection (# 356 Uz for *C. commutata* and # 357 Uz for *C. ornata*) at the Institute of Zoology Academy of Sciences of the Republic of Uzbekistan.

Ethical statement: Fieldwork conducted in the inland waters of Uzbekistan adhered to the current Uzbek environmental legislation, specifically the Law of the Republic of Uzbekistan 'On the protection and use of wildlife' (No.545-I 26.12.1997; https://lex.uz/docs/-31719). The research also followed the ethical guidelines outlined in the 'Guidelines for the Use of Live Amphibians and Reptiles in Field and Laboratory Research' (2004) (Section 6) published by the American Society of Ichthyologists and Herpetologists (ASIH) (https://asih.org/animal-care-guidelines/), ensuring the ethical treatment of the studied animals.

DNA extraction, polymerase chain reaction amplification and sequencing

The genomic DNA was extracted from three males of each nematode species by DNeasy Tissue Kit (Thermofisher.com). We amplified the internal transcribed spacers (ITS) 1+5.8S+ITS2 fragments in the nuclear DNA by a polymerase chain reaction (PCR) using the primer set AB28 (5′-ATATGCTTAAGTTCAGCGGGT-3′) and TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) (Subbotin et al. 2001). PCR was performed in 20 µL PCR reaction mixture containing 40 ng of each template, 1.5 µL of each primer, 0.6 µL of dNTP, 0.6 µL of Taq polymerase, 2 µL of PCR buffer (Sileks M (Moscow, Russia)), and 13.8 µL of distilled water with ProFlex PCR system (Applied Biosystems). The thermal conditions consisted of an initial step of 3 min at 95°C followed by 35 cycles of 20 s at 93°C, 30 s at 55°C, and 2 min at 72°C, followed by a final extension of 10 min at 72°C (Kuchboev and Krücken 2022). PCR products were separated by electrophoresis on a 1% agarose gel with edithium bromide and refined by Sileks M (Moscow, Russia). DNA sequencing was performed using the ABI PRISM® BigDye™ Terminator v. 3.1, the reaction products were recorded on an ABI PRISM 3100-Avant automatic sequencer (Moscow, Russia). The obtained sequences were aligned by Clustal W (Larkin et al. 2007), visualized Bioedid 7.0 (Hall et al. 1999), and analyzed with DNAstar ™ software program. A maximum-likelihood phylogram was calculated using PhyML 3.0.1 (Anisimova et al. 2011).

The sequences list from Genbank (National Center for Biotechnology Information - https://www.ncbi.nlm.nih.gov/) used in phylogenetic analysis in this study (Table 1). Species of *Cosmocercoides pulcher* and *C. wuyiensis* were considered an outgroup in the phylogram.

The taxonomy of parasites is available on the Global Biodiversity Information Facility website (https://www.gbif.org/). Statistical analysis was conducted using the Biostat 2007 and Microsoft Office Excel 2003.

RESULTS AND DISCUSSION

As a result of the research, *C. commutata* and *C. ornata* were found in the rectum of the Pewzow's toad and marsh frog.

Order Spirurida
Family Cosmocercidae
Subfamily Cosmocercinae
Genus *Cosmocerca* Diesing, 1861

Cosmocerca commutata

Cosmocerca commutata (Diesing, 1851) (Figure 1)

Host: Pewzow's toad - *Bufotes pewzowi* (Bedriaga, 1898)

Localization: rectum.

Infection indicators: EI - 13.2%, II - 1 - 4 specimens, IA- 0.21 specimens.

Sampling places: Tyura-Kurgan, Mingbulak, Pap, Chust, Uchkurgan, Altyaryk, Dangara, Ulugnor and Bustan regions.

Description. In terms of morphometrics in this study, the male specimens exhibit a body length ranging from 3.1 to 4.5 mm and a width of 0.35 to 0.46 mm. The length of the spicules measures between 0.21 and 0.23 mm, while the gubernaculum ranges from 0.17 to 0.19 mm. A total of eight plectanes can be observed, and following the anus, there are four pairs of papillae. For the female specimens, the body length ranges from 5.4 to 6.7 mm, with a width of 0.51 to 0.72 mm. The lateral fins display a width between 0.018 and 0.021 mm (Table 2).

Cosmocerca ornata

Cosmocerca ornata (Dujardin, 1845) Diesing, 1861 (Figure 2).

Host: *Pelophylax* sp. and Pewzow's toad - *Bufotes pewzowi* (Bedriaga, 1898).

Localization: rectum.

Infection indicators: in the lake frog, EI - 11.4%, II - 1 - 8 specimens, IA - 1.06 specimens; in toad Pewzowi, EI - 30.6%, II: 2-16 specimens, IA: 2.49 specimens.

Sampling sites: collected throughout the Ferghana Valley.

Based on our detailed descriptions, the morphology of *C. ornata* is as follows: Male individuals exhibit a body length ranging from 1.1 to 1.8 mm and a width of 0.1 to 0.16 mm (Figure 2). The spicule is partially rudimentary, while the gubernaculum measures between 0.091 and 0.11 mm. A total of 5 plectans can be observed, and within the caudal region, there are 5 pairs of papillae. In females, the body length ranges from 2.9 to 3.8 mm, with a width of 0.33 to 0.46 mm. The lateral fins are relatively thick, measuring 0.09 mm in width (Table 2).

Thus, *C. commutata* can be easily distinguished from *C. ornata* by the shape and presence of plectans and caudal papillae in males. *C. commutata* has 8 pairs of plectanes and 4 pairs of caudal papillae, while *C. ornata* has 5 pairs of plectanes and 3 pairs of caudal papillae, and tail morphology and length

In the course of scientific research, we noted that the amphibian *B. pewzowi* was first noted by us as a new definitive host for the nematodes *C. commutata* and *C. ornata*. Pewzow's toad has a rather high infestation with both species of *Cosmocerca* (*C. ornata* - EI - 30.6%, AI - 2-16 specimens; *C. commutata* - EI - 13.2%, AI - 1-4).

The main morphometric parameters of *C. commutata* and *C. ornata* according to our data and other authors (Table 2).

In the study of the morphometric parameters of the female and male of the studied nematodes *C. commutata* and *C. ornata*, the specimens were any more in length and width of the body, along the length of the esophagus and gubernaculum.

Table 1. Cosmocercidae species used in the phylogenetic tree construction from the GenBank database (NCBI)

Parasites	GenBank accession	Location
Cosmoserca commutata	OR074732	Uzbekistan
C. ornata	OR074731	Uzbekistan
C. japonica	LC052780	Japan
C. simile	MN839761	China
C. daly	OM248663	South Africa
C. monicae	OM248661	South Africa
C. makhadoensis	OM248662	South Africa
Cosmocercoides pulcher	MH178315	China
C. wuyiensis	MK110871	China

Molecular data analysis

Following the morphological identification, we conducted molecular studies on the nematodes *C. commutata* and *C. ornata* by analyzing their rDNA nucleotide sequences. Specifically, we obtained the sequences of 408 bp of ITS1+5.8S+ITS2 fragment in both *C. commutata* and *C. ornata* (Figure 3). The sequences differed 6 bp (positions 109, 218, 242, 243, 278 and 307) between *C. commutata* and *C. ornata* (Figure 3). 1.5% dissimilarity, we can confirm that these two species are phylogenetically distinct species. In addition, *C. japonica* and *Aplectana chamaeleonis* species from the GenBank International Database (NCBI) were used to compare nucleotide differences.

The interspecific divergences between *C. commutata* and other species were as follows: 10.1% (41 bp) in *Cosmocerca japonica* Yamaguti, 1938 (LC052776; Sato et al. 2015) and 11.2% (45 bp) in *Aplectana chamaeleonis* (Baylis, 1929) (OK045529; Chen et al. 2020). In the case of *C. ornata*, the following interspecific divergences were detected: 11.6% (47 bp) in *C. japonica* and 11.5% (46 bp) in *A. chamaeleonis*. These results underscore the substantial genetic divergence among these species.

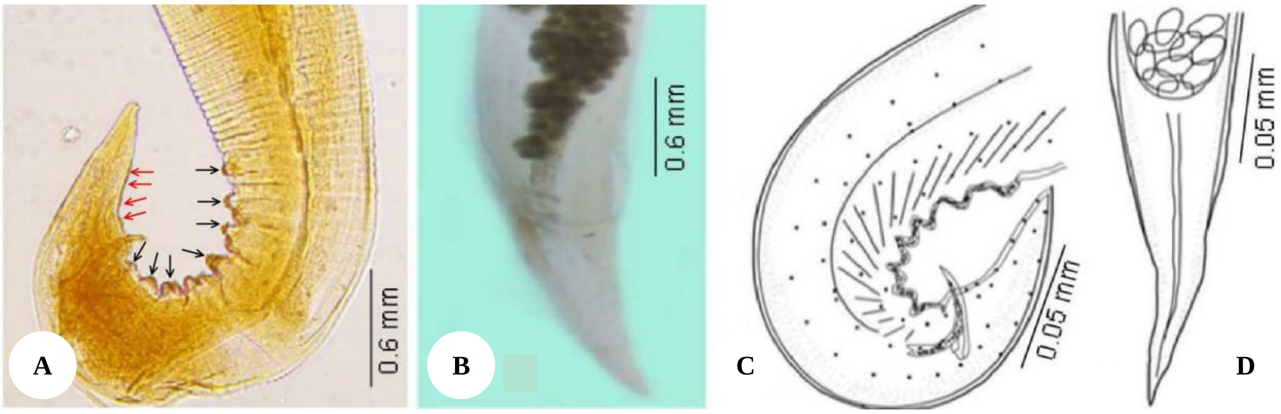


Figure. 1. Microphotograph and drawings of *Cosmocerca commutata* (Diesing, 1851). A. Tail end of the male; B. Tail end of the female (original); C. Tail end of the male; d, tail end of the female (Ryzhikov et al. 1980)

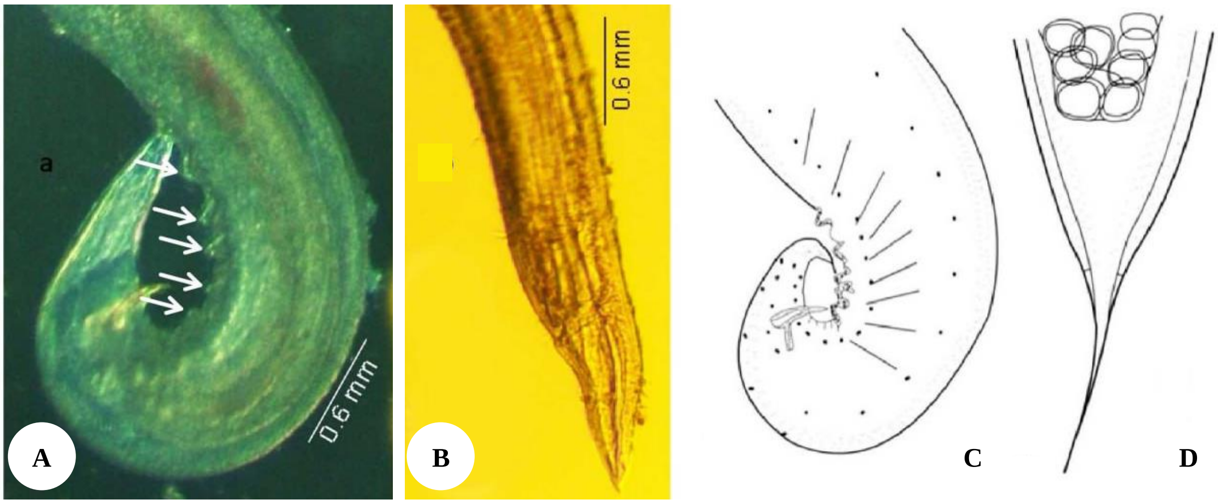
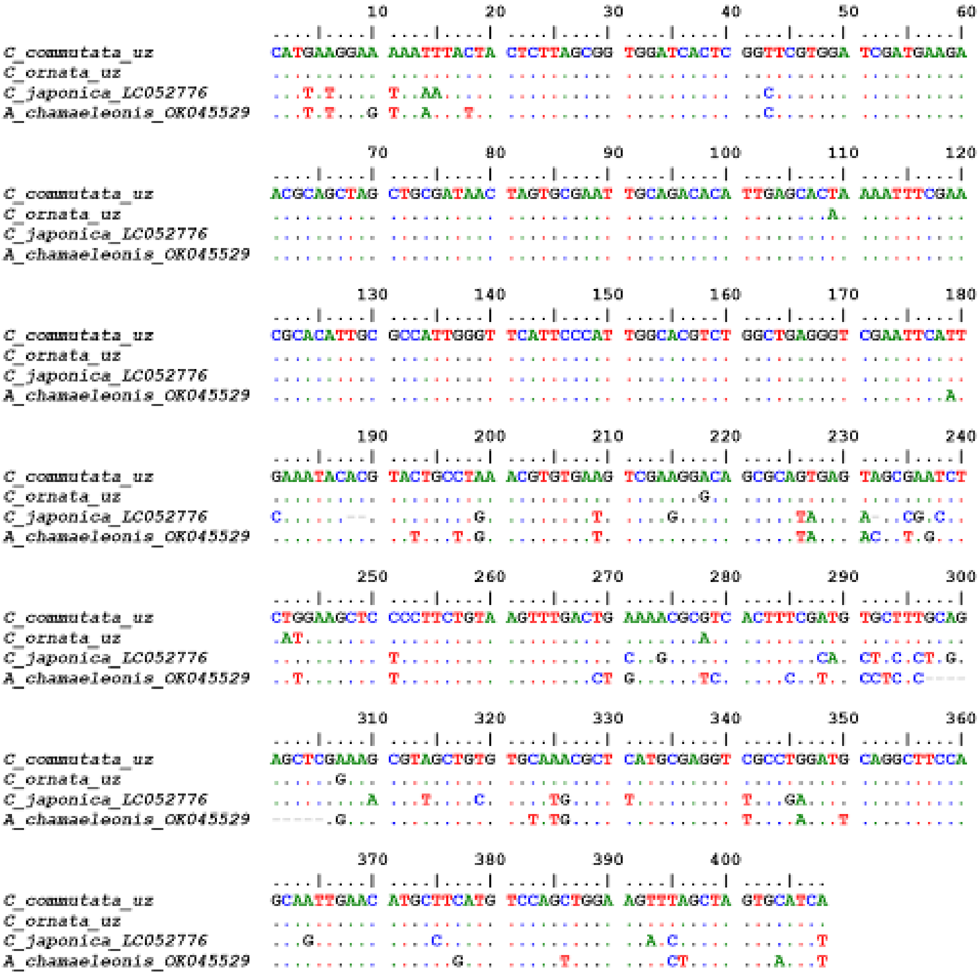


Figure 2. Microphotograph and drawings of *Cosmocerca ornata* (Dujardin, 1845). A. Tail part of the male; B. Tail part of the female (original); C. Tail part of the male; D. Tail part of the female (Ryzhikov et al. 1980)

Table 2. Comparative morphometric parameters of *Cosmocerca commutata* and *C. ornata* (mm)

Organs		**Our data**				**Yildirimhan (1999)**		**Sou et al. (2019)**	
		C. commutata **(male)**	***C. commutata*** **(female)**	***C. ornata*** **(male)**	***C. ornata*** **(female)**	***C. commutata*** **(male)**	***C. commutata*** **(female)**	***C. ornata*** **(male)**	***C. ornata*** **(female)**
Body length		3.1-4.5	5.4-6.7	1.1-1.8	2.9-3.8	4.3-6.1	4.5-6.9	1.60-2.20	2.75-5.11
Body width		0.35-0.46	0.51-0.72	0.10-0.16	0.24-0.36	0.34-0.53	0.39-0.80	0.11-0.18.	0.21-0.33
Esophagus length		0.47-0.56	0.48-0.61	0.24-0.31	0.40-0.55	0.42-0.58	0.49-0.64	0.29-0.35	0.47-0.52
Pharynx length		-	-	-	-	0.016-0.04	0.024-0.032	0.025-0.027	0.032-0.039
Bulbul length		0.11-0.14	0.12-0.16	0.047-0.061	0.053-0.091	0.114-0.136	0.10-0.16	0.06-0.07	0.07-0.08
Bulbuls width		0.086-0.103	0.95-0.107	0.041-0.051	0.098-0.109	-	-	0.052-0.059	0.09-0.10
From the forelimb	To the nerve ring	0.175-0.181	0.194-0.223	0.118-0.174	0.147-0.182	-	-	0.15-0.19	0.12-0.17
	To exhaust port	0.193-0.201	0.221-0.26	0.194-0.246	0.26-0.32	-	-	0.20-0.23	0.23-0.31
	Before the opening of the vulva	-	4.34-4.74	-	1.46-1.96	-	-	-	1.39-2.62
Number of plectanes		8	-	5	-	rosette papilla	-	5	-
Gubernaculum length		0.17-0.22	-	0.091-0.101	-	0.20-0.25	-	0.082-0.086	-
Spicule length		0.21-0.23	-	0.101-0.106	-	-	-	0.072-0.077	-
Tail length		0.108-0.158	0.537-0.567	0.128-0.164	0.146-0.366	conical shape	conical shape	0.13-0.14	0.19-0.21
Egg diameter		-	0.55 x 0.86	-	0.083x0.090	-	0.48-0.58 x 0.75-0.96	-	-



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                                   10         20         30         40         50         60
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             CATGAAGGAA AAATTTACTA CTCTTAGCGG TGGATCACTC GGTTCGTGGA TCGATGAAGA
C_ornata_uz                .......... .......... .......... .......... .......... ..........
C_japonica_LC052776        ...T.T.... T..AA..... .......... .......... ..C....... ..........
A_chamaeleonis_OK045529    ...T.T...G T..A...T.. .......... .......... ..C....... ..........

                                   70         80         90        100        110        120
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             ACGCAGCTAG CTGCGATAAC TAGTGCGAAT TGCAGACACA TTGAGCACTA AAATTTCGAA
C_ornata_uz                .......... .......... .......... .......... ........A. ..........
C_japonica_LC052776        .......... .......... .......... .......... .......... ..........
A_chamaeleonis_OK045529    .......... .......... .......... .......... .......... ..........

                                  130        140        150        160        170        180
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             CGCACATTGC GCCATTGGGT TCATTCCCAT TGGCACGTCT GGCTGAGGGT CGAATTCATT
C_ornata_uz                .......... .......... .......... .......... .......... ..........
C_japonica_LC052776        .......... .......... .......... .......... .......... ..........
A_chamaeleonis_OK045529    .......... .......... .......... .......... .......... ........A.

                                  190        200        210        220        230        240
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             GAAATACACG TACTGCCTAA ACGTGTGAAG TCGAAGGACA GCGCAGTGAG TAGCGAATCT
C_ornata_uz                .......... .......... .......... .......G.. .......... ..........
C_japonica_LC052776        C......--. ........G. ........T. ....G..... .....TA... A-..CG.C..
A_chamaeleonis_OK045529    .......... ..T...T.G. ........T. .......... .....TA... AC..T.G...

                                  250        260        270        280        290        300
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             CTGGAAGCTC CCCTTCTGTA AGTTTGACTG AAAACGCGTC ACTTTCGATG TGCTTTGCAG
C_ornata_uz                .AT....... .......... .......... .......A.. .......... ..........
C_japonica_LC052776        .......... T......... .......... C..G...... .......CA. CT.C.CT.G.
A_chamaeleonis_OK045529    ..T....... T......... ........CT G.....TC.. ....C..T.. CCTC.C----

                                  310        320        330        340        350        360
                           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
C_commutata_uz             AGCTCGAAAG CGTAGCTGTG TGCAAACGCT CATGCGAGGT CGCCTGGATG CAGGCTTCCA
C_ornata_uz                ......G... .......... .......... .......... .......... ..........
C_japonica_LC052776        .........A ...T....C. ....TG.... T......... T...GA.... ..........
A_chamaeleonis_OK045529    -----.G... .......... ..T.TG.... .......... T....A...T ..........

                                  370        380        390        400
                           ....|....| ....|....| ....|....| ....|....| ....|...
C_commutata_uz             GCAATTGAAC ATGCTTCATG TCCAGCTGGA AGTTTAGCTA GTGCATCA
C_ornata_uz                .......... .......... .......... .......... ........
C_japonica_LC052776        ...G...... ....C..... .......... ..A.C..... .......T
A_chamaeleonis_OK045529    .......... ......G... .....T.... ....CT.... ...A...T
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Figure 3. Comparison of the nucleotide sequence of the ITS region of representatives of the genus *Cosmocerca* based on the sequence materials. Fragments of ITS1+5.8S+ITS2 rDNA of species *C. commutata*, *C. ornata* and *C. japonica* in the direction from 5' to the end 3', identical nucleotide bases are marked with dots

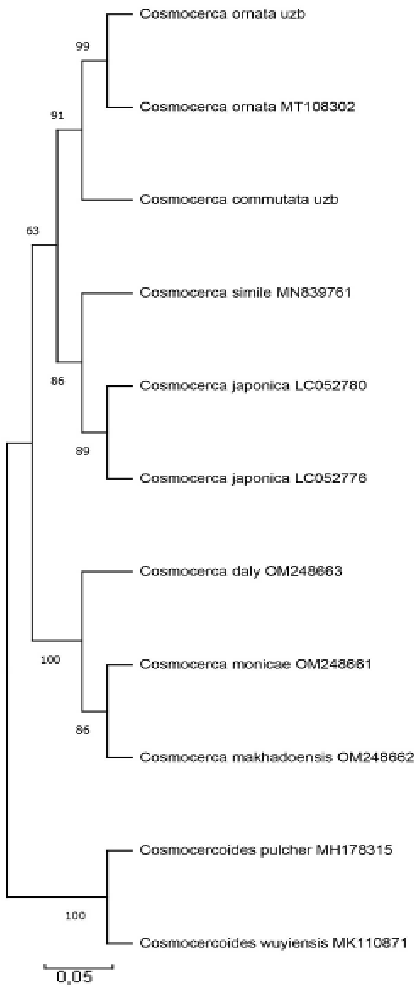

Figure 4. Maximum likelihood (ML) trees on partial ITS+5.8S+ITS2 sequence data showing the phylogenetic relationships of members of the genus *Cosmocerca*. The species identified by us are *Cosmocerca ornata* uzb and *Cosmocerca commutata* uzb. Species *Cosmocercoides pulcher* and *C. wuyiensis* were considered an outgroup

The phylogenetic tree by Maximum likelihood (ML) trees based utilizing partial sequences of ITS-1+5.8S+ITS-2 (Figure 4), revealed a distinct clade consisting of seven *Cosmocerca* species. Within this clade, *C. ornata* occupied a basal position, while a subclade comprising six other species emerged *C. commutata*, isolated in Uzbekistan, and formed a separate branch within the tree clade. Additionally, distinct lineages were observed for the species *C. pulcher* and *C. wuyiensis*, which appeared as basal to the broader diversity of the genus *Cosmocerca*. These findings provide insights into the phylogenetic relationships among these nematode species.

Discussion

The spicules of adult males of *C. gymnophthalmicola* n. sp., newly identified from eastern Brazil, differ from those of *C. commutata* by being longer (0.26 mm) but shorter (2.2 mm) in body length (Ávila and da Silva 2019).

When comparing the morphometric parameters of the female and male, the studied nematodes *C. commutata*, with Turkish samples (Yildirimhan et al. 1999), our specimens were smaller in length and width of the body, along the length of the esophagus and gubernaculum. For male nematodes *C. ornata*, morphometric parameters were slightly low compared to the material obtained in the described territory of India (Sou et al. 2019) in the following parameters: by the length and width of the body, by the length of the esophagus and bulbus, by the width of the bulb and more by the proximity of the nerve ring from the anterior end of the body, and in the female, the nerve ring is located at a slightly greater distance. The low body values of both nematode species indicate different geographic and ecological habitats of their hosts, as well as population characteristics.

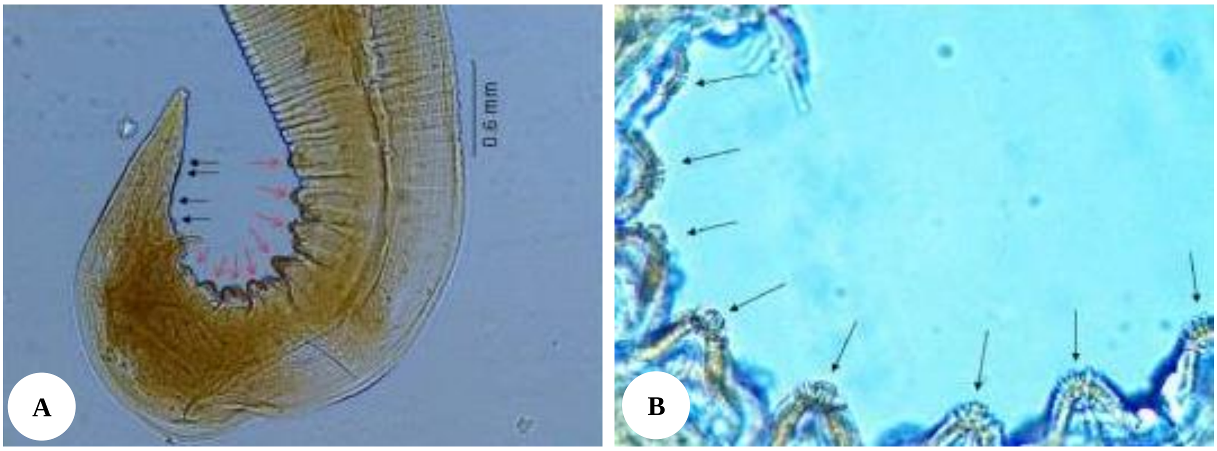

Figure 5. The figure shows the caudal papillae and plectans of *Cosmocerca commutata*. A. Caudal papillae are indicated by black arrows, plectanes are indicated by red arrows; B. Plectanes

Plectans are cuticular outgrowths that serve as mixed receptors, and the number of plectans varies among different species. For instance, species like *C. brasiliense*, *C. tyleri*, and *C. ishaqui* typically have 9 to 11 pairs of plectans (Bursey et al. 2015; Sou et al. 2018). In the case of *C. gymnophthalmicola* n. sp. and *C. banyulensis* research recorded 6 pairs of plectans. On the other hand, species such as *C. chilensis*, *C. parva* and *C. rara* usually have 5 pairs of plectans. However, there is variability in the number of plectans in *C. parva* and *C. rara*, with some individuals having up to 5-7 pairs (Ávila and da Silva 2019). Nevertheless, our research did not observe such variability in either of the nematode representatives identified. Overall, the spicule characteristics and the number of plectans provide distinguishing features for different species within the genera *Cosmocerca*.

The *Cosmocerca* species under investigation are widely distributed nematodes that parasitize amphibians. The amphibian *B. pewzowi* is reported here for the first time as a new definitive host for the nematodes *C. commutata* and *C. ornata*. These two species can be easily differentiated based on several characteristics, including body size, relative length of spicules (*C. commutata* has twice the length of gubernaculum and spicules compared to *C. ornata*), shape and number of plectans (*C. commutata* has 8 pairs of plectans and 4 pairs of caudal papillae, while *C. ornata* has 5 pairs of plectans and 3 pairs of caudal papillae), as well as the morphology and length of the tail Figure 5.

A comparison of the nucleotide sequences of *C. commutata* and *C. ornata* revealed differences in 6 nucleotides, representing a 1.5 % variation. A phylogenetic tree of partial ITS-1+5.8S+ITS-2 rDNA sequences grouped these nematodes into a monophyletic clade with other *Cosmocerca* sequences from the GenBank. These findings support the notion that *C. commutata* and *C. ornata* are distinct species. To finally resolve the issue, we propose to conduct additional studies on both species of another gene, such as mtDNA. This would further enhance our understanding of the species diversity and genetic characteristics of the genus *Cosmocerca* in Uzbekistan.

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