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Molecular-Genetic Analysis of *Channa argus* (Cantor, 1842) (Teleostei: Channidae) Distributed in the Kashkadarya River, Uzbekistan

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ABSTRACT

The Amur snakehead (*Channa argus*) was accidentally brought to the territory of Uzbekistan in the 1960s, and now it is widespread in all water bodies of the republic from sea level to 800 meters. The current article presents the results of research on the molecular genetic analysis of *Channa argus* caught from the middle reaches of the Kashkadarya River. In the current study, *Channa argus* species was located in the phylogenetic tree with *Channa bankanensis*, which is distributed in the territory of Indonesia, on adjacent branches and combined to form a 95% bootstrap. The genetic distance between them was 18.4%. The nucleotide sequence of this species was included in the Genbank and accession numbers were obtained.

INTRODUCTION

Until now, animals of the fauna of our republic, including fish **(Quvatov *et al*., 2023)** and insects **(Kimyonazarov *et al*., 2024)**, have been studied at the molecular level.

According to the last research, species belonging to the genus Snakehead (*Channa*) are widespread in all water sources of Asian and African continents and tropical and subtropical regions **(Zhou *et al*., 2019)**.

The Amur snakehead is distributed mainly in the Amur River and Chinese water basins **(Kamilov, 1973; Amanov *et al*., 1990)**. L.S. Berg mentioned the occurrence of 2 subspecies of *Channa argus, Channa argus argus* and *Channa argus warpachowskii*, in the territory of the USSR **(Berg, 1949)**. Currently, synonyms of this species exist, such as

Ophicephalus pekinensis (Basilewsky, 1855), *Ophicephalus argus warpachowskii* (Berg, 1909), and *Ophiocephalus argus kimurai* (Shih, 1936) **(Courtenay & Williams, 2004)**.

Channa argus species was accidentally brought from the Amur basin to the "Akkorgon" fish farm in 1961 together with herbivorous fish. Nowadays, it is widespread in all the water basins (without mountain part) of the Republic **(Kamilov, 1973; Amanov *et al.*, 1990; Amanov & Mirzaev, 1993)**. Last decade, researches have been conducted on the molecular genetic analysis of representatives of the *Chana* genus, of which *Channa argus* species has been recorded in the researches of some scientists **(Serrao *et al.*, 2014; Zhou *et al.*, 2019)**.

MATERIALS AND METHODS

Channa argus samples were collected from the middle reaches of the Kashkadarya River (38°53'03"N 66°15'36"E) during 2023-2024 (Fig. 1). A 5-meter-long Braden net was used to collect ichthyological materials from the river. Scientific observations on the samples were carried out in field conditions and were analyzed using generally accepted methods in ichthyology **(Pravdin, 1966)**.

During the extraction of genomic DNA from *Channa argus* species, 50- 100mg of tissue was taken from the muscle of the sample and stored in 96% ethanol. PureLinkTM genomic DNA kit (Invitrogen, USA) reagents were used to isolate the genomic DNA of this species.

After DNA extraction, the mtDNA *co1* region primer (Fish F2 5' - TCGACTAATCATAAAGATATCGGCAC-3', and Fish R2 5'- ACTTCAGGGTGACCGAAGAATCAGAA-3'), widely used in the molecular-genetic identification of vertebrates, especially fish, was used to perform the polymerase chain reaction (PCR) process **(Ward *et al.*, 2005)**.

The indicator of thermal cyclic processes in PCR was set as follows: 94°C – 3 minutes; 94°C – 20 seconds, 54°C – 45 seconds, 72°C – 1.1 min (35 times repeated) and the final process was 72°C – 7 minutes. After completing the polymerase chain reaction, gel electrophoresis was performed in a 2% agarose gel at a voltage of 100 V for 45 minutes in a horizontal direction gel electrophoresis. The PCR product was washed using Sileks M (Moscow, Russia). Prior to sequencing, PCR products were processed using the ABI PRISM® BigDye™ Terminator v.3.1 reagent kit, and the reaction products were sent to an ABI PRISM 3100-Avant automatic sequencer (Tashkent, Uzbekistan) for sequencing.

Analysis of the obtained nucleotide sequence was carried out using special computer programs MEGA11, BioEdit, and BLAST. In the formation of the phylogenetic tree, the nucleotide sequence of the *co1* gene, obtained as a result of the study, was used together with the nucleotide sequence of other species, belonging to the genus *Channa* in the NCBI international database (Table 1). *Parachanna africana* (MF496971.1) was selected as an outgroup **(Larkin *et al.*, 2007, Quvatov *et al.*, 2023)**. The obtained *co1*

gene nucleotide sequence was edited in the MEGA11 program based on the ClustalW algorithm. The genetic distance of the species was calculated using the Kimura-2-parameter (K2P) indicator MEGA 11. A phylogenetic tree was created using the neighbor-joining (NJ) method in the MEGA11 program. The current article aimed to perform the molecular genetic analysis of *Channa argus* caught from the middle reaches of the Kashkadarya River.

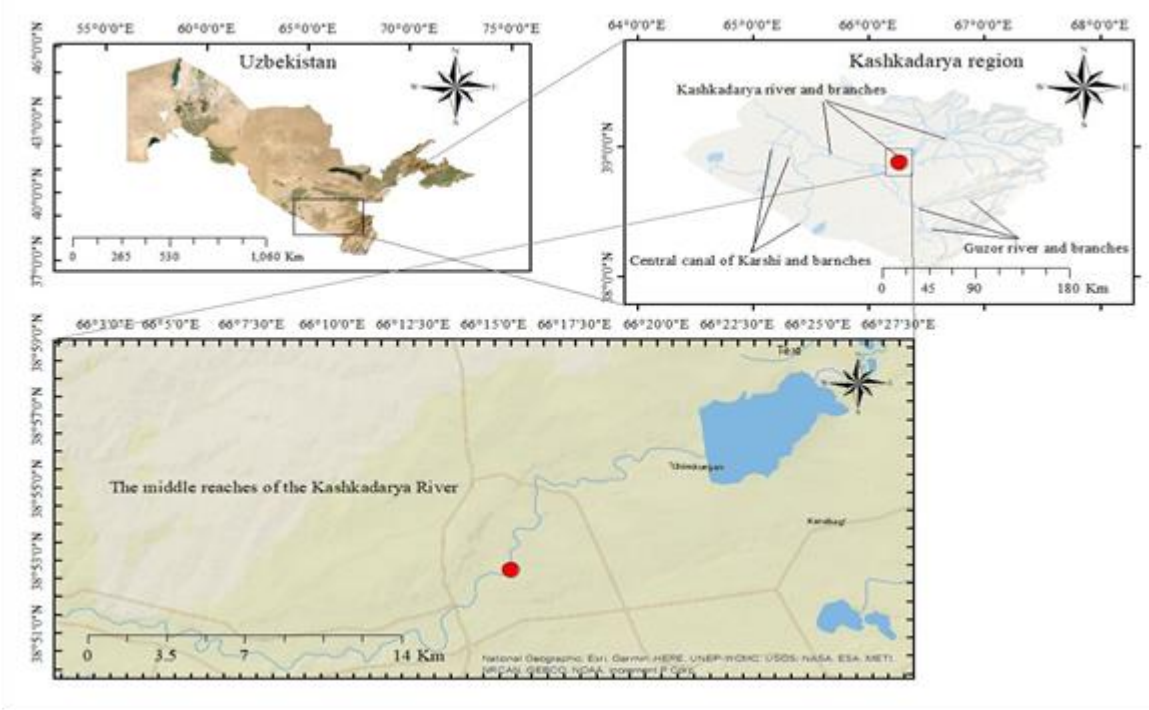

Fig. 1. Geoinformation system map of the ichthyological sample collection point

Table 1. Information about representatives of the genus *Channa* in the NCBI database

№	Species of fish	Input code ID	Regestration point
	*Ch_argus*_Qash_Uz	PQ187011	Uzbekistan: Kashkadarya Region, Kashkadarya River
1	*Ch_argus*_Chir_Uz	PQ192577	Uzbekistan: Tashkent Region, Chirchik River
3	*Ch_argus*	MW649187	Uzbekistan: Fergana Region
4	*Ch_andrao*	KY563773	India
5	*Ch_aristonei*	MN910263.1	India

6	*Ch_asiatica*	KC819604.1	China
7	*Ch_aurantimaculata*	OK035705.1	India
8	*Ch_auroflammea*	MK423216.1	Laos
9	*Ch_bankanensis*	MW020468.1	Indonesia
10	*Ch_baramensis*	MF496697.1	Malaysia
11	*Ch_barca*	OR780457.1	India
12	*Ch_bipuli*	OP418211.1	India
13	*Ch_bleheri*	MK471230.1	India: Dibru-Saikhowa, Assam
14	*Ch_brahmacharyi*	OP418201.1	India
15	*Ch_brunnea*	MK431774.1	India: West Bengal
16	*Ch_burmanica*	OR780458.1	Myanmar: Putao
17	*Ch_diplogramma*	OQ296141.1	India
18	*Ch_gachua*	OR492435.1	India

RESULTS

The results obtained from the BLAST program showed that the nucleotide sequence of the fish sample caught from the Kashkadarya River belongs to the genus *Channa*. Analyzing the results of molecular genetic studies, information on the nucleotide sequence of species belonging to the genus *Channa* was obtained from the National Center for Bioinformatics Information (NCBI) Genbank and compared (Table 2).

Table 2. Average K2P genetic distance between species belonging to the genus *Channa*

№	Species name	1	2	3	4	5	6	7	8	9
1	*Ch_argus*_Qash_Uz									
2	*Ch_argus*_Chir_Uz	0.61								
3	*Ch_argus*	0.61	0.61							
4	*Ch_asiatica*	17.1	17.3	16.5						

5	*Ch_bankanensis*	18.4	18.4	17.6	18.2					
6	*Ch_baramensis*	21.2	21.4	20.6	20.8	22.7				
7	*Ch_brunnea*	24	24.2	23.3	23.9	25.3	23.2			
8	*Ch_burmanica*	24.5	24.5	23.6	23.9	24.8	22.7	13.5		
9	*Ch_diplogramma*	20.6	21	20.2	20.7	19.8	21.2	25.4	24.2	
10	*Ch_gachua*	24.6	24.8	23.9	22.8	26.7	24.9	17	15.9	23.6

According to the results of the conducted molecular genetic research, 670 basepairs of the nucleotide sequence of the *co1* region of the mtDNA of the *Channa argus* species belonging to the genus *Channa* Scopoli; 1777 was extracted and submitted to the National Center for Bioinformatics Information (NCBI) (https:// www.ncbi.nlm.nih.gov) and access numbers were obtained. *Channa argus* (Input number: PQ187011).

The sample studied was compared with *Ch_argus*_Chir_Uz (Input number: PQ192577) and *Channa argus* (Input number: MW649187) samples of the same species in GenBank (Fig. 2). Accordingly, differences in 4 nucleotides were detected between the nucleotides of our sample and *Ch_argus*_Chir_Uz (Input number: PQ192577) obtained from the NCBI database, and this difference was 0.59%.

The results showed that G-guanine 195, 245, and 376 in our samples were exchanged with C-cytosine and A-adenine in *Ch_argus*_Chir_Uz (Input number: PQ192577). Also, C-cytosine at 253 nucleotide in our sample was exchanged with A-adenine in *Ch_argus*_Chir_Uz (Input number: PQ192577). Our sample and *Channa argus* (Input number: MW649187) obtained from the NCBI database were compared, and 4 nucleotide differences were found. G-guanine at nucleotides 195 and 245 in our sample were exchanged with C-cytosine at *Channa argus* (Input number: MW649187), C-cytosine at nucleotide 364 in our sample was exchanged with G-guanine in *Channa argus* (Input number: MW649187), and A-adenine at nucleotide 397 in our samples was exchanged with G-guanine at *Channa argus* (Input number: MW649187).

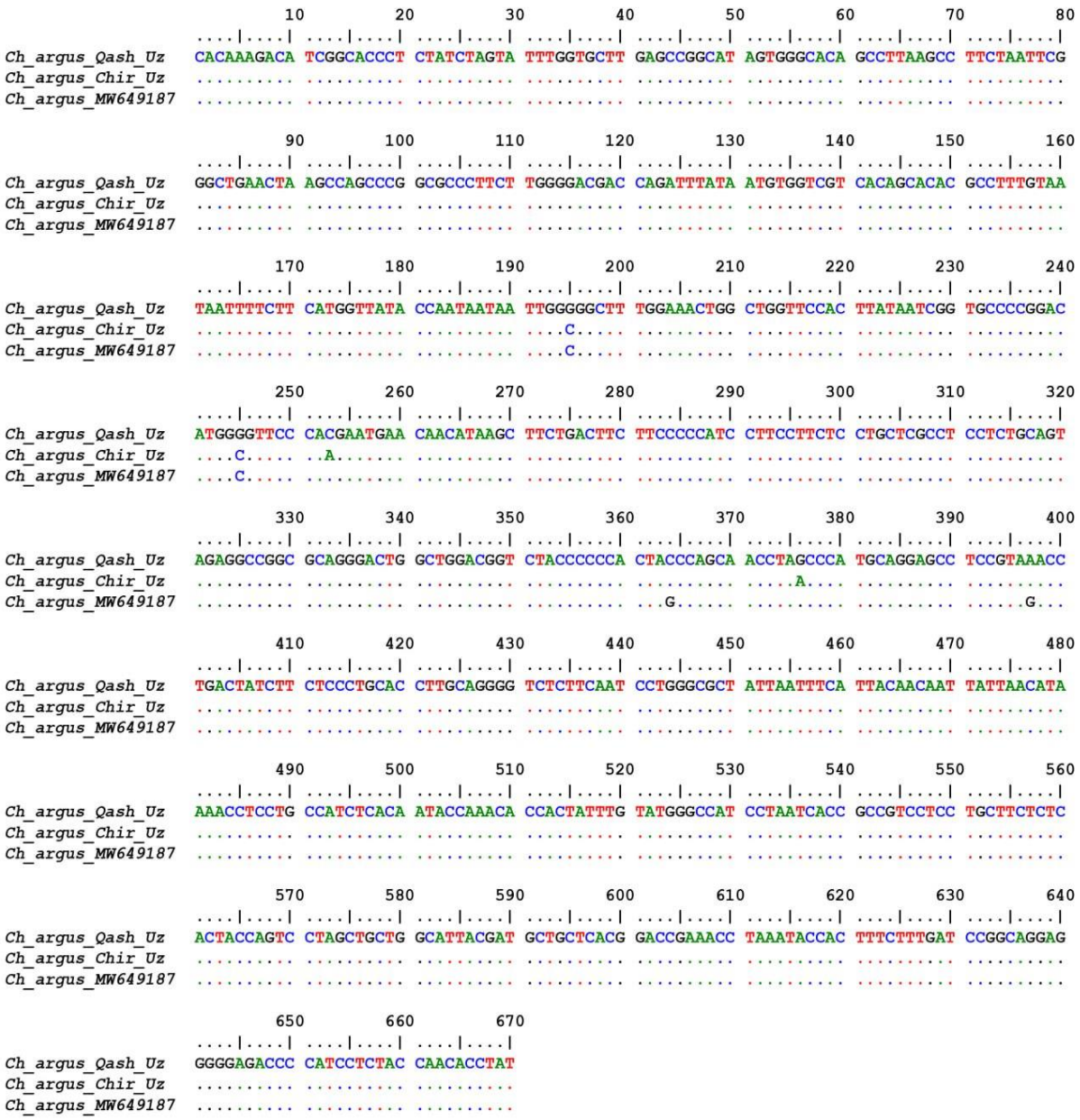

Fig. 2. Comparison of the nucleotide sequence of the mtDNA *co1* region of *Channa argus* species belong to the genus *Channa* based on sequence materials

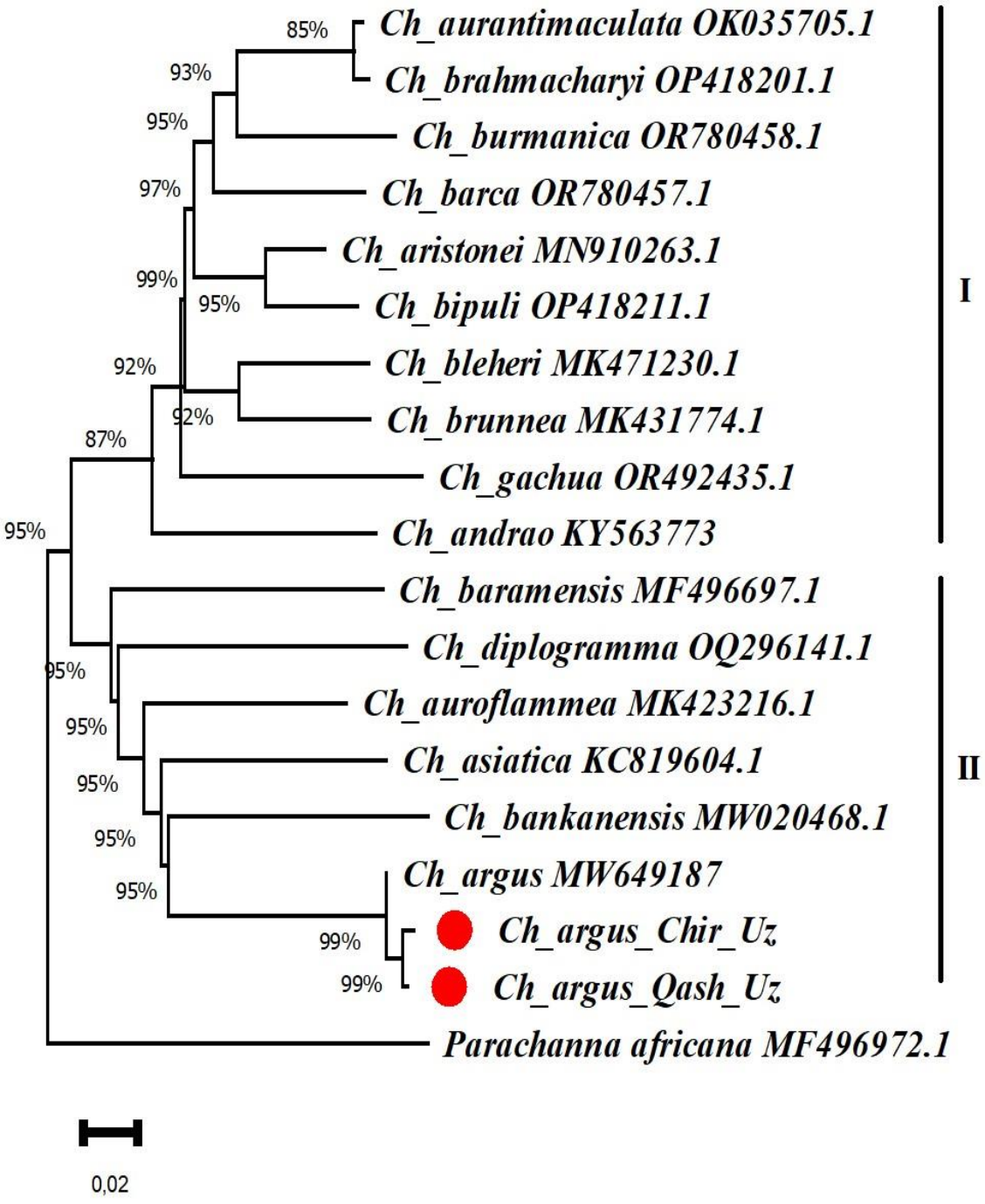

Fig. 3. Phylogenetic tree of *Channa argus* species and 15 species belong to genus *Chana*, based on NJ method

DISCUSSION

Molecular-genetic analysis of *Channa argus* species *co1* gene nucleotide sequence (NCBI) database 15 species belonging to this genus were phylogenetically analyzed. According to the results of the analysis, the species belonging to the genus *Channa* were divided into two large clades (groups) in the phylogenetic data. Representatives of both groups were combined to form a 95% bootstrap. The species *Channa argus* was placed in the phylogenetic tree with the species *Channa bankanensis* distributed in the territory of Indonesia on the side branches and were combined to form a 95% bootstrap (Fig. 3). The genetic distance between them was 18.4% (Table 2).

The natural range of Channa argus is eastern China and the Amur basin. This species accidentally arrived in Central Asia in 1960 during the acclimatization of herbivorous fish from the Far East. Currently, this species can be found in all river and plain water bodies of our Republic **(Amanov & Mirzayev, 1993)**.

This study represents the largest and most comprehensive global synthesis of sequence diversity within the family Channidae yet undertaken. In lieu of limited snakehead taxonomic expertise and inadequate morphological keys, molecular techniques provide a rapid method of identification. The substantial sequence diversity identified in this study within broadly defined taxa in both *Channa* genera highlights the need for comprehensive examination of the molecular and morphological systematics within the Channidae.

CONCLUSION

The nucleotide sequence of 670 base pairs of the mtDNA of *Channa argus* species *co1* was extracted and compared with organisms belonging to the same species, and it was found that there were 0.59% differences between nucleotides. Data were deposited in the NCBI, and input numbers were obtained: *Channa argus* (Input number: PQ187011).

In the phylogenetic tree of species belonging to the genus *Channa*, it was found that *C. argus* species is located closer to *C. bankanensis* species in the sameclade and creates a 95% bootstrap.

GRATITUDE

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CONTRIBUTION OF AUTHORS IN THE ARTICLE

Materials collection, molecular genetic experiments and statistical processing were carried out by O. Ubaydullayev and O. Amirov. Editing was done by A. Quvatov.

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