



Morphometric and molecular characteristics of *Cottus jaxartensis* (Scorpaeniformes: Cottidae)

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ABSTRACT

The article presents the results of morphometric and molecular genetic studies on the fish species *Cottus jaxartensis* collected from the Ugam River of the watershed of the Chirchik River (Uzbekistan). The material used was 6 fish samples, with 57- 84mm long (average 73.5). The numbers of rays are *ID* 7-8 and *IID* 17-18 on the dorsal fins, ventral (*V*) 5, pectoral (*P*) 14-15, anal (*A*) 12, caudal (*C*) 17-18; the number of pores on lateral lines are 34-36; the coefficient of variation of plastic features is varied up to 0.4-19.2% (average 7.8 %), of which signs *o*, *io*, *H*, *h*, *lca*, *hD*, *lP*, *io/c* have high variability, and the rest of the characters had medium and low variability. A comparative study of the nucleotide sequence of the 16S gene of mtDNA of the species *C. jaxartensis* with the nucleotide sequence of the species *C. ricei* was carried out from GenBank of the National Center for Biotechnology Information. Between the nucleotides of the 16S region of mtDNA in the species *C. jaxartensis* and the species *C. aleuticus* and *C. gobio*, there are differences of 6 nucleotides, and the difference with the total number of nucleotides is 1.8%; with species *C. asper*, *C. marginatus*, *C. perplexus*, *C. poecilopus*, *C. pollux* and *C. volki* between 9 nucleotides (2.7%); with species *C. bairdii*, *C. beldingii*, *C. gulosus* and *C. szanaga* between 12 nucleotides (3.6%); with species *C. cognatus*, *C. pitensis* and *C. reinii* between 11 nucleotides (3.5%). The 16S gene of the mtDNA nucleotide sequence data was first placed in the GenBank, and the accession number OR470689 was obtained.

INTRODUCTION

Currently, more than 600 species of the superfamily Cottoidea (cottoids) are known to be common in the marine environment, with about 100 species of sculpins living in freshwater habitats of the northern hemisphere. Comprising species from the genera *Cottus*, *Trachidermus*, *Mesocottus*, and *Myoxocephalus*, as well as the Baikal sculpins, these freshwater sculpins are probably originated polyphyletically from ancestral species of marine sculpins (Yabe, 2011; Yokoyama & Goto, 2011).

The freshwater sculpins of the genus *Cottus* Linnaeus, 1758 are bottom-dwelling fishes that comprise approximately 64 species from the subarctic areas to the temperate

northern hemisphere, including Europe, Siberia, Central and East Asia, and North America (Goto *et al.*, 2014; Watanabe *et al.*, 2014).

Most *Cottus* species have fluvial or lacustrine life histories, living in freshwater throughout their lives. However, several *Cottus* species that have diadromous life histories are distributed in East Asia and on the Pacific coast of North America. In East Asia including Japan, catadromous, freshwater amphidromous (hereafter, referred to as amphidromous), lacustrine, and fluvial life histories are common (Goto, 1990; Yokoyama & Goto, 2011).

When studying representatives of the genus *Cottus*, numerous molecular genetic studies were carried out. The mitochondrial DNA of these species with complete genomic nucleotides was studied, in particular, the genomic mDNA of the *Cottus asper* species (16511 base pairs), and the mDNA of the *C. perifretum* species (16523 base pairs), while the *C. rhenanus* strain is an mDNA with 16522 base pairs. The nucleotide sequence of these strains is included in some international bioinformatics databases (GenBank registration number: MF326939, MF326940, MF326941), while the control samples of the *C. asper* species are stored in the University of Los Angeles, and a control specimen of the species *C. rhenanus* is held at the University of AN Oldenburg (Kayla Fast *et al.*, 2017).

In Uzbekistan, there are 2 representatives of the genus *Cottus* – *Cottus spinulosus* Kessler, 1872 and *Cottus jaxartensis* Berg, 1916 (Berg, 1949; Turdakov, 1963; Mirzaev, 2001, 2015; Mirzaev & Kuvatov, 2018, 2020; Sideleva, 2021). This study presented a morphological and molecular genetic analysis, as well as phylogenetic relationships of the species *C. jaxartensis* belonging to the genus *Cottus*.

MATERIALS AND METHODS

Materials were collected in April 2023, and 6 ichthyological samples were taken from the lower reaches of the Ugam River, a tributary of the Chirchik River at the following coordinate points 41°41'59.34"N, 69°56'24.02"E (Fig. 1), and their morphometric and molecular biological analysis was carried out.

Collection, sorting, fixation, processing of fish samples, and calculated morphometric work were performed on the basis of the calculation scheme proposed by Pravdin (1966). Morphometric statistical analysis is presented according to Lakin (1990).



Fig. 1. Map of the geoinformation system of points where ichthyological samples were collected

On using morphometric designations, the following generally accepted abbreviations were used: l – fish body length (mm), c – head length, ao – snout length, o – eye diameter, po – back of the eye, hc – head height, io – forehead width, H – body height, h – height of the caudal axis, aD – antedorsal distance, pD – postdorsal distance, lca – length of the caudal axis, lD – length of the base of the first dorsal fin, $II lD$ – length of the base of the second dorsal fin, hD – height of the first dorsal fin, $II hD$ – height of the second dorsal fin, la – length of the base of the anal fin, hA – height of the anal fin, lP – length of the pectoral fin, lV – length of the pelvic fin, PV – distance between the pectoral and ventral fins, VA – distance between the pelvic and anal fins, ao/c – ratio of snout length to head length, o/c – ratio of eye diameter to head length, po/c – ratio of the length of the back of the eyes to the length of the head, hc/c – ratio of head height to head length, and io/c – the ratio of the width of the forehead to the length of the head.

DNA isolation, polymerase chain reaction (PCR), and sequencing. During the isolation of genomic DNA from the species *C. jaxartensis*, a 50- 100mg sample was taken from the muscle portion of the sample and stored in 96% ethanol. The PereLink™ Genomic DNA Kit (Invitrogen, USA) was used to isolate the genomic DNA of this species.

After extraction, we performed PCR using 16Scp primers mtDNA (forward primer 5'-CGAGGGCTTTACTGTCTCTT-3', and reverse primer 5'-CCTATTGTCG ATATGG ACTC T-3'), which are known to amplify vertebrate DNA (Caragiulo *et al.*, 2013; Wultsch *et al.*, 2016). PCR was performed with 21.3 μ l of water, 10 μ M forward primer, 10 μ M reverse primer, 0.3 μ l of BSA, and 2 μ l of template to illustrate PuReTaq Ready-To-Go PCR Beads with ProFlex PCR system (Applied Biosystems). The thermocycler profile was 94°C for 2

min, 10 cycles at 94°C for 15s, 52.5°C for 15s, 72°C for 45s, 15 cycles at 94°C for 15s, 52°C for 15s, 72°C for 45s, 20 cycles at 94°C for 15s, 51.5°C for 15s, 72°C for 45s and a final cycle at 68°C for 20min (Soatov *et al.*, 2023). 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 (Tashkent, Uzbekistan). The obtained sequences were aligned by Clustal W (Larkin *et al.* 2007). They were visualized by Bioedit 7.0 (Hall *et al.* 1999), and analyzed with the DNASTar™ software program.

RESULTS AND DISCUSSION

Cottus jaxartensis is a locally distributed endemic relict species of the western Tien Shan, inhabiting the Chatkal, Koxsu, Pskem, and Ugam tributaries of the Chirchik River basin. It inhabits the mountain and foothill parts of the rivers, with clear transparent waters, relatively slow flow, and stony pebble bottoms (Mirzaev, 2000).

Cottus jaxartensis populations in Uzbekistan have experienced recent sharp declines since they are susceptible to changes in water environments caused by anthropogenic pressure, such as agricultural run-off, destruction of the natural regime of the river flows as a result of hydro-building and pollution, recreation activity, and competition with invasive fish species (Mirzaev, 2000). Therefore, this species has been protected species 2(VU:D) – Vulnerable, declining wild species)) since 2003 (The Red Data Book of the Republic of Uzbekistan, 2019).

Morphological characteristics

The characteristics of *Cottus jaxartensis* are presented on the basis of 6 specimens caught from the Ugam River. The head is wide, fairly smooth, and has well-developed spines on the frontal part. The eyes are almost entirely directed upward. The body is naked, the pectoral fins are very wide and long. The shoulder part is dark brown (Fig. 2).

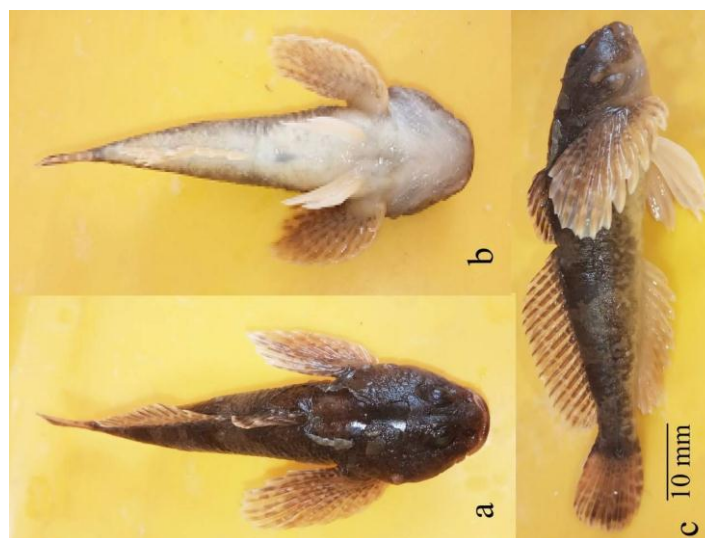


Fig. 2. Appearance of *Cottus jaxartensis* from dorsal (a), ventral (b) and lateral (c) sides (TL=91mm)
Photo: Kuvatov A.

Table 1. Morphometric characteristics of *Cottus jaxartensis*

Sign	Min.-Max. <i>n</i> = 6	<i>M</i> ± <i>m</i>	σ	<i>Cv</i> , %
<i>l</i> , mm	57 - 84	73.5 ± 4.9	12.1	16.5
Relative to body length (<i>l</i>), (%)				
<i>C</i>	26.8-28.6	27.3 ± 0.26	0.6	2.3
<i>Ao</i>	8.1-9.5	8.8 ± 0.22	0.5	6.1
<i>O</i>	4.9-6.3	5.6 ± 0.24	0.6	10.3
<i>Po</i>	13.1-14.3	13.6 ± 0.20	0.5	3.6
<i>Hc</i>	14.9-18.8	17.0 ± 0.66	1.6	9.5
<i>Io</i>	3.1-5.0	4.28 ± 0.34	0.8	19.2
<i>H</i>	15.1-20.0	17.1 ± 0.89	2.2	12.7
<i>H</i>	5.5-7.5	6.6 ± 0.33	0.8	12.2
<i>aD</i>	33.2-35.0	34.2 ± 0.28	0.7	2.0
<i>pD</i>	43.7-49.2	45.9 ± 1.02	2.5	5.4
<i>Lca</i>	11.7-16.3	14.4 ± 0.86	2.1	14.5
<i>ID</i>	16.1-18.6	17.4 ± 0.37	0.9	5.2
<i>II ID</i>	41.1-44.1	42.6 ± 0.52	1.3	3.0
<i>Hd</i>	6.1-8.5	7.6 ± 0.44	1.1	14.2
<i>II hD</i>	11.6-13.8	12.8 ± 0.36	0.9	7.0
<i>La</i>	25.1-27.5	26.5 ± 0.42	1.0	3.9
<i>hA</i>	13.1-15.5	14.5 ± 0.37	0.9	6.3
<i>IP</i>	26.8-33.9	29.8 ± 1.27	3.1	10.5
<i>IV</i>	22.1-25.4	23.7 ± 0.55	1.3	5.7
<i>PV</i>	5.0-6.3	5.7 ± 0.22	0.5	9.5
<i>VA</i>	25.9-27.5	26.8 ± 0.25	0.6	2.3
Relative to head length (<i>c</i>), (%)				
<i>ao/c</i>	31.0-33.0	32.0 ± 0.38	0.9	2.9
<i>o/c</i>	18.5-22.7	20.6 ± 0.75	1.8	8.9
<i>po/c</i>	50.1-50.2	50.3 ± 0.09	0.2	0.4
<i>hc/c</i>	55.9-68.2	62.1 ± 2.18	5.3	8.6
<i>io/c</i>	12.3-18.2	15.6 ± 1.07	2.6	16.8

As shown in Table (1), of the 26 plastic traits of *C. jaxartensis*, 8 traits are high and 5 traits are moderate in the coefficient of variation.

Molecular genetic analysis

A comparative study of the nucleotide sequence of 327 base pairs of the 16S gene of the mtDNA of the species *C. jaxartensis* with the nucleotide sequence of the species *C. ricei* was carried out from the Genbank National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) (registration number: MT539215) (**Fig. 3**).

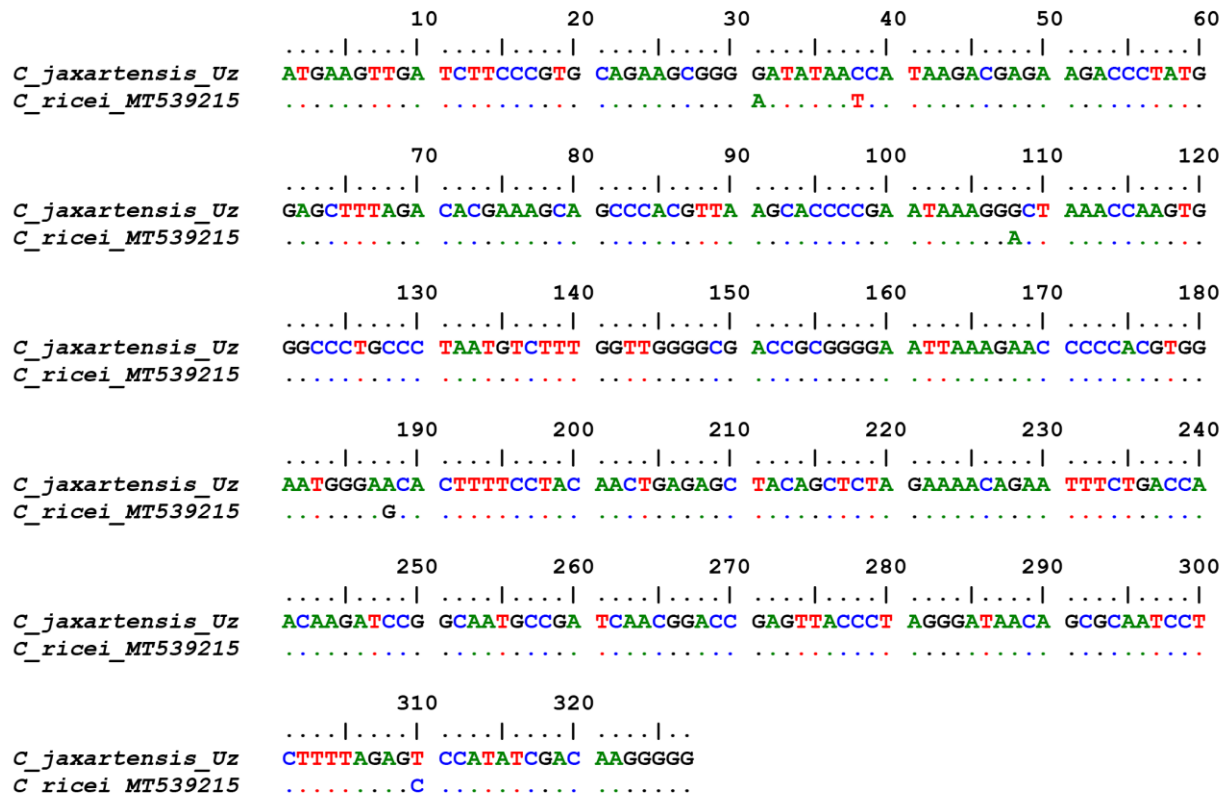


Fig. 3. Nucleotide sequence of the 16 S mtDNA region of *C. jaxartensis* and *C. ricei* species belonging to the genus *Cottus*

As can be seen from Fig. (3), differences in five nucleotides were noted among the nucleotide species *C. jaxartensis* and *C. ricei*. In particular, in nucleotides 31 and 108 in the species *C. jaxartensis*, there was G-guanine, and in the species *C. ricei* there was A-adenine, in nucleotide 38 in the species *C. jaxartensis*, there was C-cytosine, while in species *C. ricei*, T-thymine was detected. Moreover, in nucleotide 188 in the species *C. jaxartensis*, A-adenine was recorded, and in the species *C. ricei*, G-guanine was assessed. In addition, in the 310th nucleotide in the species *C. jaxartensis*, T-thymine was determined, and in the species *C. ricei*, the base was C -cytosine. The 16S mtDNA region of the species *C. jaxartensis* was comparatively studied with the nucleotide sequences of other species of this genus from the Genbank (Table 2).

Table (2) reveals the differences of 6 nucleotides between the nucleotides of the 16 S region of mtDNA in the species *C. jaxartensis* and the species *C. aleuticus* and *C. gobio* and the difference with the total number of nucleotides is 1.8%; however, with the species *C. asper*, *C. marginatus*, *C. perplexus*, *C. poecilopus*, *C. pollux*, and *C. volki* between 9 nucleotides, it was recorded with a percentage of 2.7, and with the species *C. bairdii*, *C. beldingii*, *C. gulosus* and *C. szanaga* meju 12 nucleotides, it was 3.6%. Whereas, with the species *C. cognatus*, *C. pitensis*, and *C. reinii*, the difference was detected between 11 nucleotides, recording a value of 3.5%. On the other hand, with the species *C. dzungaricus* between 7 nucleotides, the value was 2.13%, and with the species *C. klamathensis* between 13 nucleotides, the percentage was 3.9. Furthermore, with the species *C. koreanus* between 10 nucleotides, a percentage of 3 was recorded; while, with species *C. perifretum* and *C. ricei*

between 5 nucleotides, a value of 1.5% was administered; with *C. rhenanus* between 8 nucleotides, the value was 2.4%.

Table 2. Information about representatives of the genus *Cottus* from the GenBank (NCBI)

Representative and genus <i>Cottus</i> from the Genbank database	Registration number	Location of the study	Differences among nucleotides pieces/%
<i>C. jaxartensis</i>	OR470689	Uzbekistan	0/0
<i>C. aleuticus</i>	OM256460	USA	6/1.8
<i>C. asper</i>	EF458400	USA	9/2.7
<i>C. bairdii</i>	AY539018	USA	12/3.6
<i>C. beldingii</i>	OL688775	USA	12/3.6
<i>C. cognatus</i>	MT539221	USA	11/3.5
<i>C. dzungaricus</i>	NC024739	USA	7/2.13
<i>C. gobio</i>	KJ128752	Sweden	6/1.8
<i>C. gulosus</i>	OL404934	USA	12/3.6
<i>C. klamathensis</i>	OK945944	USA	13/3.9
<i>C. koreanus</i>	MW894645	Korea	10/3
<i>C. marginatus</i>	NC066924	USA	9/2.7
<i>C. perifretum</i>	NC036146	USA	5/1.5
<i>C. perplexus</i>	OL339397	USA	9/2.74
<i>C. pitensis</i>	OL825618	USA	11/3.3
<i>C. poecilopus</i>	NC014849	USA	9/2.7
<i>C. pollux</i>	LC097787	Japan	9/2.7
<i>C. reinii</i>	NC004404	USA	11/3.3
<i>C. rhenanus</i>	NC036147	USA	8/2.4
<i>C. ricei</i>	MT539215	USA	5/1.5
<i>C. szanaga</i>	NC032039	USA	12/3.6
<i>C. volki</i>	KY563344	USA	9/2.7

CONCLUSION

It was noted that the meristic characters of the species *C. jaxartensis* did not differ from the originally recorded specimens. The coefficient of variation of plastic features of *C. jaxartensis* ranges from 0.4- 19.2 % (average 7.8 %). Of these, characters *o*, *io*, *H*, *h*, *lca*, *hD*, *lP*, *io/c* have high variability; characters *hc*, *llhd*, *PV*, *o/c*, *hc/c* have medium variability, and the remaining characters have low variability.

As a result of molecular studies, we obtained sequences (327 bp) of 16S mtDNA gene fragments isolated of the species *C. jaxartensis*. These scores were close to *C. ricei* species (registration number: MT539215) from the International Bioinformatics Database, and the difference between the total nucleotides was five nucleotides and 1.5%. The resulting nucleotide sequence data was first placed in the Genbank of the National Center for Biotechnology Information, and the accession number OR470689 was obtained.

Primary specimens *C. jaxartensis* are kept in the Zoological Collection of the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan under the barcode number “ICIZ (UZZI) 2200254”.

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SHARE OF AUTHORS

The collection of ichthyological materials, the description of morphological and morphometric features, and the writing of the article were carried out by A.Q. Quvatov and M.Sh. Atamuratova, molecular genetic analysis, organization of the placement of the obtained nucleotide sequence in the international bioinformatics database was carried out by O.O. Amirov, and the article was analyzed by U.T. Mirzaev and A.E. Kuchboev.

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