

Distribution and Molecular Genetic Analysis of Biotopes of Bivalves of the Families Unionidae and Corbiculidae in the Syrdarya River

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Abstract A total of 9 species belonging to 2 families and 4 genera and 2 subspecies are distributed in the Syrdarya river. We found out that a total of 8 species are distributed in the upper part of Sirdaryo and Fergana Valley, 3 species from the Unionidae family and 2 subspecies, and 3 species from the Corbiculidae family. It was studied that 11 species are distributed in the middle part of Syrdarya, including 4 species from the Unionidae family and 2 subspecies, and 5 species from the Corbiculidae family. As a result of the molecular-genetic analysis of bivalve molluscs of the families Unionidae, Corbiculidae in the Syrdarya River from the Genbank database (Genebank, NCBI) *Corbicula fluminalis* (O.F. Muller, 1774) species (accession number PP256501), *Corbiculina ferghanensis* (Kursalova et Starobogatov, 1971) species (accession number PP239092) and *Sinanodonta gibba* (Benson, 1895) was taken as the type (accession number PQ059482).

Keywords Family Corbiculidae, *Corbiculina tibetensis*, *Corbiculina ferghanensis*, *Corbicula purpurea*, *Sinanodonta gibba*, *Sinanodonta puerorum*

1. Introduction

Today, the increase in droughts on a global scale is causing changes in the hydrological regime of large river basins and the transformation of biodiversity. Changes in the land use system of mankind, along with the reduction of wetland ecosystems, are ensuring the crisis of hydrofauna representatives. Accordingly, it is of scientific and practical importance to assess the current state of hydrofauna representatives of river basins with unstable hydrological regimes and identify the target factors causing their crisis.

Studies on the study of hydrobionts in water types were conducted by some authors [2,3].

2. Material and Methods

When collecting materials from the area, the methods were used [4]. Samples brought from the Syrdarya aquatic ecosystems were identified in laboratory conditions to the species level using the identifiers. In compiling the taxonomic system, "System of bivalve mollusks of the Zarafshan River Basin", "Fauna of mollusks of aquatic

ecosystems of Central Asia and adjacent areas" were formed according to the systematics [1,4,6,5].

Species were isolated from some samples of bivalve mollusks *Corbicula fluminalis*, *Corbiculina ferghanensis* and *Sinanodonta gibba* collected from the aquatic ecosystems of the Syrdarya coast for molecular genetic studies. GeneJET GENOMIC DNA reagents were widely used to extract DNA from the obtained samples [7].

3. Results and Discussion

The Syrdarya River is one of the longest rivers in Central Asia, second only to the Amu Darya River in terms of water content. Until now, the river has been called by various names, and in the works of ancient Greek historians it was mentioned as Yaksart, Danu, Saykhun, and Khasart. It takes its name from the confluence of the Naryn and Karadarya rivers in the area of the Balikchi village of the Fergana Valley. The river flows into the Aral Sea. The length of the Syrdarya is more than 2,272 km, and the basin area is approximately 462,000 km². [8,9].

To date, species of the Unionidae and Corbiculidae families have not been specifically studied in the riverine aquatic ecosystems. As a result of the studies, it was determined that 9 species and 2 subspecies belonging to 2 families and 4 genera are distributed in the aquatic ecosystems of the Syrdarya coast (Table 1). The studies were conducted in two areas on the river bank in a stationary state:

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In the Fergana Valley section of the river, it was found that *Corbicula fluminalis* from the Corbiculidae family *Corbicula* genus was distributed in sandy and rocky biotopes. However, *Corbicula cor*, *Corbicula purpurea* species were not found in the waters of this area, which may be due to the fluctuations in the water level and the hydrochemical composition of the waters. *Corbicula tibetensis* 1.1, *Corbicula ferghanensis* 1.2 from the Corbiculina genus, which are more densely distributed than other species, were found in

The distribution of the rare stenobiont species *Colletopterium bactrianum*, *Colletopterium cyreum sogdianum*, and *Corbicula fluminalis* in the waters of the region, as well as the distribution of the eurybiont species *Sinanodonta gibba*, *Sinanodonta puerorum*, *Sinanodonta orbicularis*, *Colletopterium ponderosum volgensis*, *Corbiculina tibetensis*, and *Corbiculina ferghanensis*, was analyzed. In the middle part of the Syrdarya River, 11 species are distributed in the Syrdarya region, of which 4 species and 2 subspecies from the Unionidae family, and 5 species from the Corbiculidae family were studied. A higher number and density of species were observed in the middle part of the river than in the upper part. The distribution and density of species in the middle part of the river were specifically analyzed, the depth is 1.5-2.8 m, the average water temperature is 14-20 °C, the water clarity is 0.20-0.28 cm, and the height above sea level is 340-489 m. In the parts at higher altitudes, the distribution of the Unionidae family *Sinanodonta* genus *Sinanodonta gibba* 1.4, *Sinanodonta puerorum* 1.2, *Sinanodonta orbicularis* 1.6, and the *Colletopterium* genus *Colletopterium bactrianum* 0.3, *Colletopterium cyreum sogdianum* 0.6, and *Colletopterium ponderosum volgensis* 0.9 was studied on average per grain [5,8,9].

№	Types	Syrdarya		Biotopes			Environmental groups
		Upper part (Fergana Valley)	Middle part (Syr Darya vil.)	Rocky	Sand	Clays	
(Bivalvia) class Unionidae family							
E.1	<i>Sinanodonta gibba</i>	0,8±0,1	1,4±0,2	-	-	+	Pelorheophile
E.2	<i>Sinanodonta ruerorum*</i>	0,9±0,1	1,2±0,1	-	+	-	Pelorheophile
E.3	<i>Sinanodonta orbicularis *</i>	0,7±0,1	1,6±0,3	-	-	+	Pelorheophile
S.4	<i>Solletopterum bactrianum*</i>	-	0,3±0,1	-	-	+	Reophile
S.5	<i>Colletopterum cureum sogdianum</i>	0,3±0,1	0,6±0,1	-	-	+	Reophile
E.6	<i>Colletopterum ponderosum volgensae</i>	0,4±0,1	0,9±0,1	-	-	+	Pelolimnofil
	Family Corbiculidae						
S.7	<i>Corbicula cor*</i>	-	0,6±0,1	-	+	-	Pelorheophile
S.8	<i>Corbicula fluminalis</i>	0,3±0,1	0,7±0,1	+	-	-	Pelorheophile
S.9	<i>Corbicula purpurea*</i>	-	0,6±0,1	-	+	-	Pelorheophile
E.10	<i>Corbiculina tibetensis</i>	1,1±0,2	1,7±0,2	+	-	-	Pelorheophile
E.11	<i>Corbiculina ferghanensis</i>	1,2±0,2	1,8±0,2	-	+	-	Pelorheophile
	Total number of species	8	11	2	4	5	
* Species recorded for the first time from the aquatic ecosystems of the Syrdarya River (5)							
S- Stenobiont species distributed in the aquatic ecosystems of the Syrdarya coast (5)							
E- Eurybiont species distributed in the aquatic ecosystems of the Syrdarya coast (6)							

The Syrdarya River differs from other rivers in its high turbidity. In the section of the Syrdarya near the city of Bekabad, an average of 2.17 kg of turbidity was observed per 1 m³ of water. The high content of turbidity in the river is of great importance in the formation of biotopes favorable for the spread of large bivalve mollusks of the genera *Sinanodonta* and *Colletopterum*. These genera live semi-buried in the mud. In the relatively fast-flowing sandy and rocky areas of the river, *Corbicula cor* 0.6, *Corbicula fluminalis* 0.7, *Corbicula purpurea* 0.6 from the *Corbicula* family are distributed, which are stenobiont species with low density. We can include species of the *Corbiculina* genus, which have a higher density and are widely adapted to the Syr Darya aquatic ecosystems than other species. The density of these species is *Corbiculina tibetensis* 1.7, *Corbiculina ferghanensis* 1.8, and they are species that have adapted to changes in important factors such as the hydrochemical composition of water and flow velocity. It was studied that the species *Colletopterum bactrianum*, *Colletopterum cyreum sogdianum* from the genus *Colletopterum* of the Unionidae family, *Corbicula cor*, *Corbicula fluminalis*, *Corbicula purpurea* from the genus *Corbicula* of the Corbiculidae family, which are distributed in the waters of this area of the river, are stenobionts, and the species *Sinanodonta gibba*, *Sinanodonta puerorum*, *Sinanodonta orbicularis*, *Colletopterum ponderosum volgense* from the genus *Sinanodonta* of the Unionidae family, *Corbiculina tibetensis*, *Corbiculina ferghanensis* from the genus *Corbiculina* of the Corbiculidae family are eurybionts.

When analyzing the ecological groups of species in the upper part of the Syr Darya River, the Fergana Valley, and the middle part of the Syr Darya River, the Syrdarya region, it was determined for the first time that 8 species of pelorheophiles accounted for 72.7% (*Sinanodonta orbicularis*, *Corbicula cor*, *Sinanodonta gibba*, *Sinanodonta puerorum*, *Corbicula fluminalis*, *Corbicula purpurea*, *Corbiculina tibetensis*, *Corbiculina ferghanensis*), 2 species of rheophiles accounted for 18% (*Colletopterum cyreum sogdianum*, *Colletopterum bactrianum*), and one species of pelolimnophiles accounted for 9.2% (*Colletopterum ponderosum volgense*). When analyzing the distribution in biotopes, it was found that 2 species were distributed in rocky soils (18.5%, *Corbicula fluminalis*, *Corbiculina tibetensis*), 4 species were distributed in sandy soils (36.5%, *Sinanodonta puerorum*, *Corbicula cor*, *Corbicula purpurea*, *Corbiculina ferghanensis*), and 5 species were distributed in clay soils (45%, *Sinanodonta gibba*, *Sinanodonta orbicularis*, *Colletopterum bactrianum*, *Colletopterum cyreum sogdianum*, *Colletopterum ponderosum volgense*).

We conducted molecular genetic analyses of the bivalve mollusks *Corbicula fluminalis*, *Corbicula ferghanensis*, and *Sinanodonta gibba*, which are distributed in relatively polluted waters on the coast of the Syrdarya River due to human factors. Molecular taxonomic analysis of *Corbicula*

fluminalis for molecular genetic analysis of the mollusk *Corbicula fluminalis*, belonging to the genus *Corbicula* Megerle von Mühlfeld, 1811, sample materials were collected from the Syrdarya River and Syrdarya region. The collected samples were fixed in 96% ethanol solution. Based on the morphological and morphometric characteristics of *C. fluminalis*, this species was subjected to molecular genetic analysis. The GeneJET GENOMIC DNA reagent kit was used to extract genomic DNA from the heel of *C. fluminalis*. Polymerase chain reaction (PCR) was performed using genomic DNA isolated from the *Corbicula fluminalis* species. In this reaction, primers LCO-1460: ggycacaaatcataagatattgg and HCO-219 taaacttcagggtgacaaaaaatca, which read the mitochondrial DNA SOI region, which is widely used in the identification of bivalve mollusks, were used. The results obtained from the PCR were run on gel electrophoresis. The products obtained from the PCR were purified and sent for sequencing. The initial results obtained from the sequencing were studied with bioinformatics programs (Figure 1). [1].

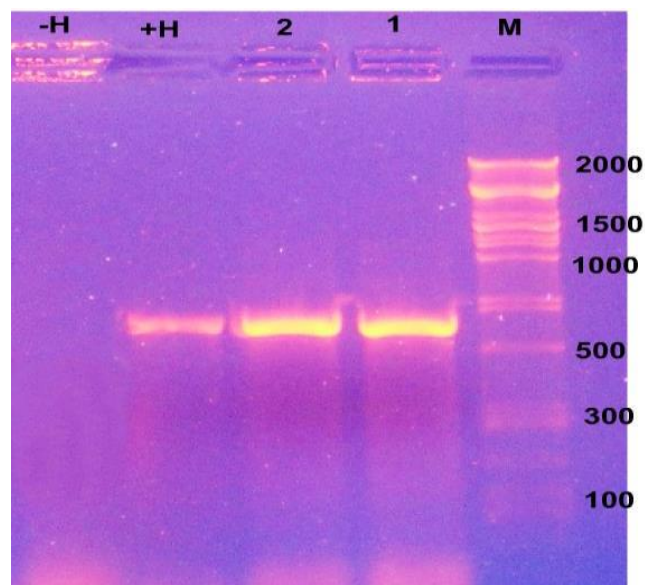


Figure 1. PCR result of *C. fluminalis*. (Note: M-marker. 1. *C. fluminalis*, +N-control, -N-control.)

Molecular genetic studies The nucleotide sequence of 610 base pairs belonging to the COI region of the mRNA of the species *Corbicula fluminalis*, belonging to the genus *Corbicula*, was isolated and compared with the nucleotide sequence of the species *C. fluminalis* (accession number: MH822472) from the National Center for Bioinformatics Information (NCBI) (<https://www.ncbi.nlm.nih.gov>) (Figure 2). 15 nucleotide differences were observed between the nucleotides of the species *Corbicula fluminalis* and *C. ferghanensis*. Next, the differences between the species *Corbicula fluminalis* and the nucleotides of the species *Corbicula fluminalis* (accession number: MH822472) obtained from the National Center for Bioinformatics Information (NCBI) (<https://www.ncbi.nlm.nih.gov>) were studied.

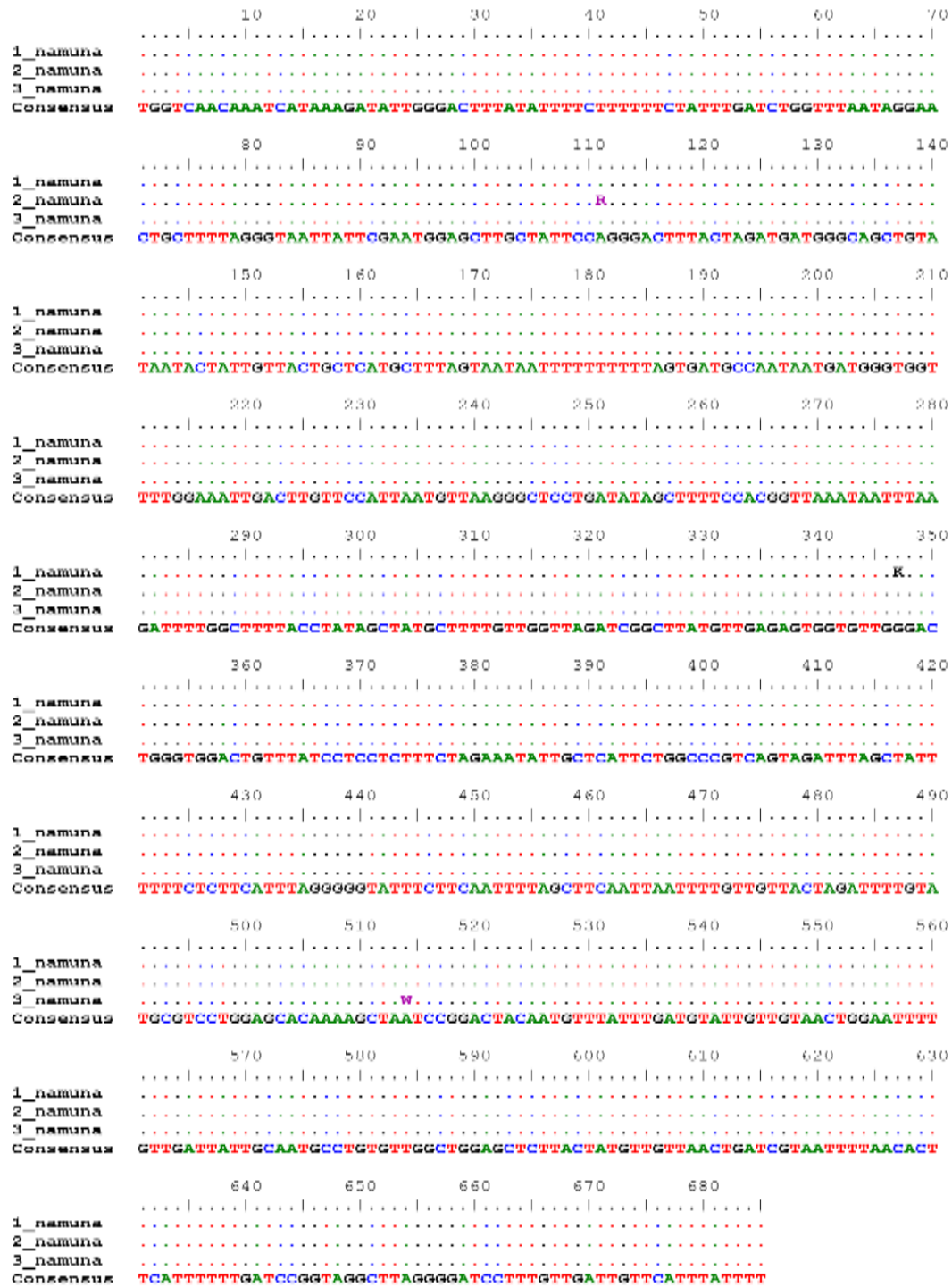


Figure 2. Comparison of the nucleotide sequences of the mRNA COI region of species belonging to the genus *Corbicula* based on sequence material. (Note: Nucleotide sequence of the species *C. fluminalis*)

Molecular genetic analysis of *Corbiculina ferghanensis*. During the research period, mollusks collected from the middle reaches of the Syrdarya River and the Dostlik canals were fixed in 70% ethanol solution to conduct molecular genetic analysis of the species *Corbicula Megerle von Mühlfeld*, 1811, which belongs to the genus *Corbicula*. The species *C. ferghanensis* was isolated for molecular genetic studies based on morphological and morphometric characteristics

[1,8]. The GeneJET GENOMIC DNA reagent kit was used to isolate genomic DNA from the tail of the species. The genomic DNA isolated from the species *C. ferghanensis* was subjected to polymerase chain reaction (PCR). The PCR product was run on gel electrophoresis (Figure 3).

PCR products were purified and sequenced. The data obtained from the sequence were analyzed using bioinformatics programs (Fig. 4).

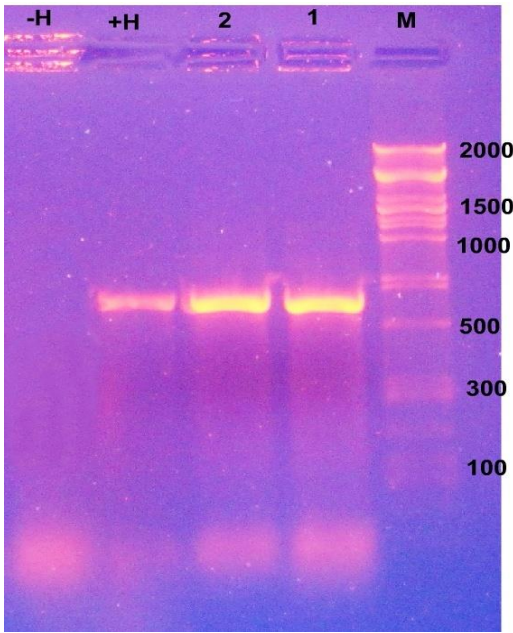


Figure 3. PCR result of *C. ferghanensis* species. (Note: M-marker. *C. ferghanensis*, +N-control, -N-control. Note: M-marker. *C. ferghanensis*, +N-control, -N-control.)

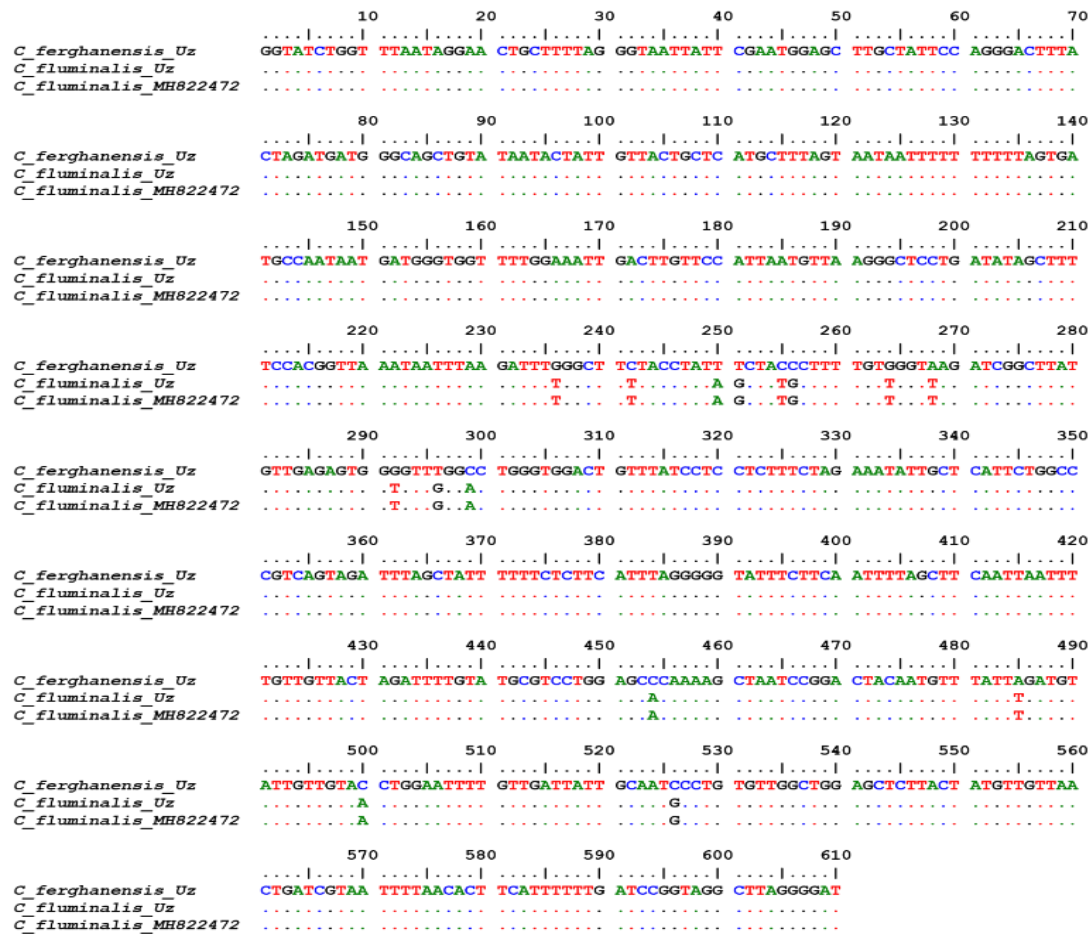


Figure 4. Comparison of nucleotide sequences of the mRNA COI region of species belonging to the genus *Corbicula* based on sequence material. (Note: Nucleotide sequence of *C. ferghanensis*. Sensensus – corrected nucleotide sequence (the sequence is in the 5' to 3' direction, with identical nucleotide bases marked with dots). According to the results of molecular genetic studies, a 610-base pair nucleotide sequence belonging to the COI region of the mRNA of *C. fluminalis* and *C. ferghanensis*, belonging to the *Corbicula* genus, was isolated and assigned an accession number in the Genebank database of the National Center for Bioinformatics Information (NCBI) (<https://www.ncbi.nlm.nih.gov>) (PP239092).)

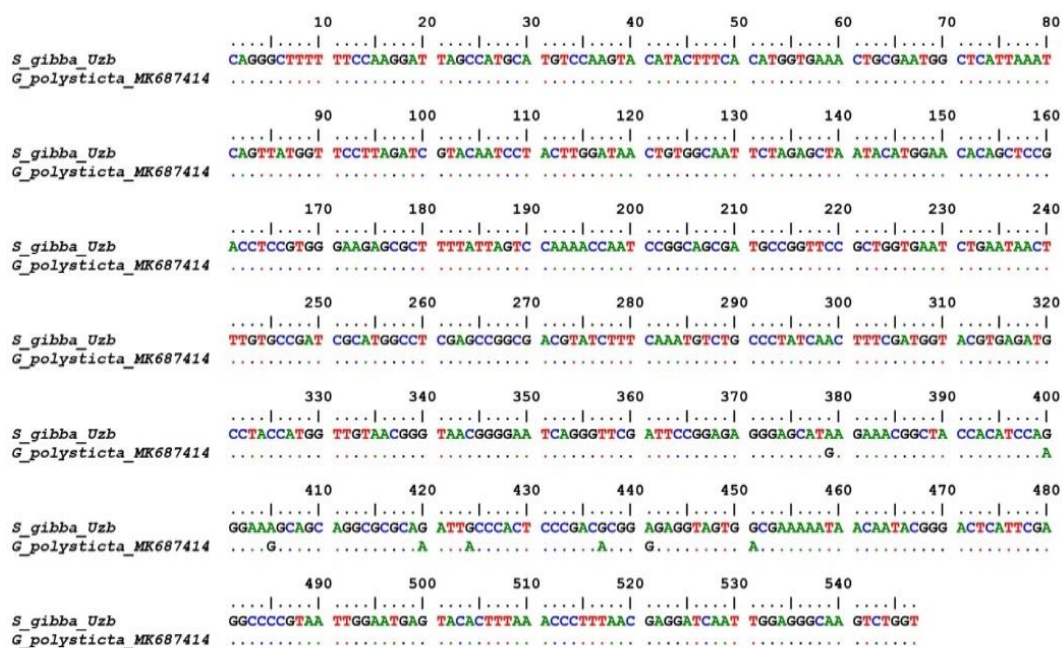


Figure 5. Comparison of nucleotide sequences of the species *Synanodonta gibba* with that of the species *Gibbosula polysticta* (accession number: MK687414) from the GenBank database

Table 2. Molecular genetic analysis of some bivalve mollusks distributed along the Syr Darya River (<http://ncbi.nlm.nih.gov>)

№	Bivalve mollusc families and genera	Type name	Species accession numbers in the Genebank database (Genebank, NCBI)	Type picture	Comparison of nucleotide sequences with a species obtained from the Genbank database
1	Family Corbiculidae (Gray, 1847), genus Corbicula (Meqerle, 1811)	<i>Corbicula fluminalis</i> (O.F. Muller, 1774)	PP256501		<i>Corbiculina ferghanensis</i>
2	Family Corbiculidae Gray, 1847 Genus Corbiculina Dall, 1903	<i>Corbiculina ferghanensis</i> (Kursalova et Starobogatov, 1971)	PP239092		<i>Corbicula fluminalis</i>
3	Family Unionidae Rafinesque, 1820 Genus Sinanodonta Modell, 1944	<i>Sinanodonta gibba</i> (Benson, 1895)	PQ059482		<i>Gibbosula polysticta</i>

Molecular genetic analysis of *Sinanodonta gibba* (Benson, 1895).

The morphological structure of species belonging to the Unionidae family has been studied in detail. Changes in the aquatic environment are observed in the morphology of the shells of bivalve mollusc species, indicating the need for molecular genetic analysis of these species. Based on the sequence chromatography obtained in molecular genetic studies of the species *Sinanodonta gibba* belonging to the Unionidae family, nucleotides with a length of 547 base pairs belonging to the 18S domain of the rDNA of *Sinanodonta gibba* were isolated using the species *Gibbosula polysticta* (accession number: MK687414) from the International Center for Bioinformatics Information (<https://blast.ncbi.nlm.nih.gov>) [5,7].

According to bioinformatic analysis, there were 8 nucleotide differences between the nucleotides of *G. polysticta* obtained from the *S. gibba* genebank database (Figure 5).

The nucleotides of the species *Sinanodonta gibba* were found to be A-adenine at nucleotides 379, 405, and 441, and the nucleotides of the species *Gibbosula polysticta* were found to be G-guanine at nucleotides 400, 420, 424, 437, and 451. The nucleotide sequence of the 18S domain of the rDNA of the species *Sinanodonta gibba* of the family Unionidae was studied bioinformatically. It was found that there were 8 nucleotide differences between the nucleotides of these species, and these differences amounted to 1.46% [1].

The genebank database (Genebank, NCBI) was used to identify the species *Corbicula fluminalis* (accession number PP256501), *Corbiculina ferghanensis* (Kursalova et Starobogatov, 1971) (accession number PP239092), and *Sinanodonta gibba* (Benson, 1895) (accession number PQ059482) (Table 2).

4. Conclusions

A total of 9 species and 2 subspecies belonging to 2 families and 4 genera are distributed in the Syrdarya River. We found that a total of 8 species are distributed in the upper part of the Syrdarya, the Fergana Valley, including 3 species and 2 subspecies from the Unionidae family, and 3 species from the Corbiculidae family. In the middle part of the Syrdarya, in the Syrdarya region, 11 species are distributed, including 4 species and 2 subspecies from the Unionidae family, and 5 species from the Corbiculidae family. When analyzing the ecological groups, it was found that pelorheophiles account for 72.7%, rheophiles for 18%, and pelorimnophiles for 9.2%, 18.5% in rocky biotopes, 36.5% in sandy biotopes, and 45% in muddy biotopes. The genebank database (Genebank, NCBI) was used to obtain the

species *Corbicula fluminalis*, *Corbiculina ferghanensis* (accession number PP239092), and *Sinanodonta gibba* (accession number PQ059482).

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