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***M.I. Bitner, N.V. Smolina, Y.A. Utkin***

## SEXUAL DIMORPHISM AND GENETIC DIVERSITY OF THE *CARASSIUS CARASSIUS* POPULATION IN THE EUTROPHIC WATER BODY OF THE TURA RIVER BASIN

***Битнер М.И., Смолина Н.В., Уткин Я.А.***

## ПОЛОВОЙ ДИМОРФИЗМ И ГЕНЕТИЧЕСКОЕ РАЗНООБРАЗИЕ ПОПУЛЯЦИИ *CARASSIUS CARASSIUS* В ЭВТРОФНОМ ВОДОЕМЕ БАССЕЙНА РЕКИ ТУРА

**Abstract.** The study aimed to identify sexual differences and genetic diversity of the crucian carp *Carassius carassius* (Linnaeus, 1758) inhabiting a eutrophic reservoir of the Tura river basin (Western Siberia). This species has been dynamically declining in numbers in Eurasian waters in recent decades [5; 15; 16; 19]. During the study, a biological analysis of 100 individuals of the crucian carp was conducted, including the determination of morphometric characteristics and the area of erythrocyte nuclei. The study showed that this population is characterized by a short dwarf form with a sex ratio of 1: 1, where all individuals in the sample had the fourth stage of gonadal maturity. Reliable sex differences in size-weight and meristic traits were revealed. Females were distinguished by a greater commercial length and body weight, and also had reliably higher values for 55.6% of the studied meristic traits. Analysis of the erythrocyte nuclear area showed that all individuals were diploids, while sex differences in this indicator were not significant. Genetic analysis of mtDNA revealed a single CCA2 haplotype, which indicates low genetic diversity of this population. The results of the study emphasize the need to monitor changes in the population structure of golden crucian carp and develop measures to preserve this species.

**Key words:** golden crucian carp; haplotype; population structure; eutrophic reservoir.

**About the authors:** Maria I .Bitner, ORCID: 0000-0002-6942-5838, junior research fellow, Nizhnevartovsk State University, Nizhnevartovsk, Russia, m.i.sid@yandex.ru; Natalia V. Smolina, ORCID:0000-0002-8066-9294, Candidate of Biological Sciences, Tyumen State University, Tyumen, Russia, natan11@mail.ru; Yaroslav A. Utkin, ORCID:

**Аннотация.** Исследование направлено на выявление половых различий и генетического разнообразия золотого карася *Carassius carassius* (Linnaeus, 1758), обитающего в эвтрофном водоеме бассейна реки Тура (Западная Сибирь). Данный вид в последние десятилетия динамично снижает численность в водоемах Евразии [5; 15; 16; 19]. В ходе работы проведён биологический анализ 100 особей золотого карася, включая определение морфометрических характеристик и площади ядер эритроцитов. Исследование показало, что данная популяция характеризуется низкотелой карликовой формой с соотношением полов 1:1, где все особи в выборке имели четвертую стадию зрелости гонад. Выявлены достоверные половые различия по размерно-весовым и меристическим признакам. Самки отличались большей длиной и массой тела, а также имели достоверно большие значения по 55,6% из исследованных меристических признаков. Анализ площади ядер эритроцитов показал, что все особи являются диплоидами, при этом половые различия по этому показателю оказались не значимыми. Генетический анализ мтДНК выявил единственный гаплотип CCA2, что указывает на низкое генетическое разнообразие в данной популяции. Результаты исследования подчеркивают необходимость мониторинга за изменениями в структуре популяций золотого карася и разработки мероприятий по сохранению данного вида.

**Ключевые слова:** золотой карась; гаплотип; структура популяции; эвтрофный водоем.

**Сведения об авторах:** Битнер Мария Ивановна, ORCID: 0000-0002-6942-5838, младший научный сотрудник, Нижневартовский государственный университет, г. Нижневартовск, Россия, m.i.sid@yandex.ru; Смолина Наталья Васильевна, ORCID: 0000-0002-8066-9294, канд. биол. наук, Тюменский государственный университет, г. Тюмень, Россия, natan11@mail.ru; Уткин Ярослав Александрович, ORCID: 0009-0008-7204-0665, млад.

0009-0008-7204-0665, junior research fellow, Institute of Molecular and Cellular Biology SB RAS, Novosibirsk, Russia, utkin.jaroslav@mcb.nsc.ru

науч. сотр., Институт молекулярной и клеточной биологии Сибирского отделения РАН, г. Новосибирск, Россия, utkin.jaroslav@mcb.nsc.ru.

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## Introduction

In the waterbodies of Northern Eurasia, the crucian carp (*Carassius carassius* L.) has historically been relatively abundant [1; 4; 8; 10; 15; 19].

The crucian carp is one of the most ecologically adaptable native fish species, resistant to various adverse environmental factors such as oxygen deficiency, extreme temperatures, and poor feeding conditions.

Under such circumstances, this species often forms a low-bodied dwarf form – *C. carassius* morpha *humilis* Heckel, 1840, which demonstrates a wide range of variability in several of its biological and morphological traits [4; 7; 12].

Since the late 20th century, in most waterbodies of Eurasia, as well as in lakes of the steppe and forest-steppe zones and in floodplain taiga reservoirs of Western Siberia, a disappearance or a rapid decline in the abundance of *C. carassius* has been observed [3; 6; 8; 9; 16; 17; 19].

Possible reasons for the reduction of its range include displacement due to the introduction of the silver crucian carp from the Amur river [3; 6; 8; 15; 16; 19; 23; 26], which has a higher growth rate [30]; a potential hybridization among cyprinid species [3; 6; 8; 15; 16; 19; 22]; and a low genetic diversity of the crucian carp, suggesting a reduced ability to adapt to changing environmental conditions [16; 23; 26; 27]. These factors determine growing scientific interest in the current state and biological characteristics of the crucian carp populations, both in Russia and abroad [15; 16; 19; 22; 23; 26; 31; 32].

Previously, sexual dimorphism in the crucian carp populations outside the breeding season was characterized as weak. For example, in the waterbodies of Kazakhstan, females and males differed only in the size of the pectoral and anal fins [8]. No sexual dimorphism was found in populations of the crucian carp in some waterbodies of the Udmurt Republic [12]. In the Middle Ob river basin, differences were recorded only in head length and maximum body height [3; 5; 8].

As a result of studies on the crucian carp population in Lake Igumenskoye (Tomsk), sexual dimorphism was revealed only in plastic (morphometric) traits: females had greater head length, anteventral, and antepectoral distances; males had larger ventroanal, ventrocaudal, anocaudal, dorsocaudal distances, head height, eye diameter, dorsal and anal fin heights, and the lengths of pectoral, pelvic, and lower caudal fin lobes [19].

A comparative analysis of how often certain countable morphological traits (meristic traits) appear is used to identify intra-population and sex-related features in bony fishes. This approach

is based on the fact that these traits have high heritability and show up early in ontogenesis [13]. However, studies focused on sexual differences in meristic traits that include the three sections of the vertebral column and the number of seismosensory canals on paired cranial bones are still insufficient for populations of the crucian carp.

It is known that *C. carassius* forms bisexual populations with a 1:1 female-to-male ratio, and individuals have a diploid (2n) set of chromosomes [3; 4; 5; 8; 16]. Research on the genetic diversity of the genus *Carassius* based on mitochondrial DNA (mtDNA) has shown that the crucian carp has at least two closely related haplotypes (CCA1 and CCA2). One of them was originally identified in the Czech Republic, and the other in Kazakhstan [26; 27]. It is also known that a comparative mtDNA analysis of the crucian carp and the silver crucian carp revealed a difference of about 6% in nucleotide substitutions, which support the relatedness of the two species [3]. Until now, mtDNA of the crucian carp from the study region has not been analysed.

The aim of this work is to examine sexual differences and genetic diversity in a population of the crucian carp inhibiting a eutrophic waterbody within the Tura river basin.

**Materials and Method**s

For this study, we selected a winterkill-prone body of water, Lake Srednee (coordinates: 57°38’15.9”N, 64°28’50.9”E), which belongs to the Tura river basin (Western Siberia) and is not connected to the main river channel. Lake Srednee is a small, roughly circular lake. Its shoreline is mostly surrounded by a mixed forest, while the western part is swampy. The lake’s surface area is 1.07 km², and the depth to the silty bottom is between 1.1 m and 1.6 m.

The fish community of the lake includes the following species: *C. carassius, Carassius gibelio* (Bloch, 1782), *Rhynchocypris percnurus* (Pallas, 1814), and *Perccottus glenii Dybowski,* 1877. During the study period, the lake showed an increased level of primary production throughout its area. The following species of higher aquatic plants were common: *Typha latifolia L.* (1753), *Nuphar lutea (L.) Smith., Lemna minor L.* (1753), and *Potamogeton natans L.* (1753). There was also an intensive growth of the cyanobacterium *Aphanizomenon flos-aquae*.

Sampling was carried out in July 2018. Fish were collected with a fishing seine (20.0 m long, 1.5 m high, 24-mm mesh size) and with telescopic rods equipped with two-hook rigs.

According to its hydrochemical characteristics during sampling, the water of Lake Srednee was classified as belonging to a hydrocarbonate class, a sodium group, and a low-mineralised type (type I) [6]. During the research period, the concentrations of ammonium nitrogen and total iron exceeded the maximum allowable limits. The increased amount of organic matter and high primary production during the summer growing season match the conditions typical of a eutrophic waterbody prone to winterkill.

The research object was the population of the crucian carp, historically long-established in this particular waterbody. The total sample size comprised 100 specimens. For all individuals, a biological analysis was conducted to determine: sex and age; gonadal maturity stages; commercial length measurements (L, cm) – from the tip of the snout to the base of the caudal fin, measured horizontally; maximum body height (qh) – from the highest dorsal point to the ventral side, measured vertically; total body mass (m, g); cytometric analysis of the erythrocyte nuclear area (ENA); and scoring of meristic traits considering bilateral distribution according to established

ichthyological guidelines [12; 15; 18]. For 30 individuals (15 females and 15 males) selected from the total sample, a sequence analysis of the mitochondrial DNA control region (460 bp) was performed to investigate the genetic diversity of the population.

For all individuals in the sample, there were analyzed 18 meristic traits, including: the number of spiny rays in the dorsal fin (Dк); the number of branched rays in the dorsal fin (Dв); spiny rays in the anal fin (Aк); branched rays in the anal fin (Aв); the number of gill rakers on the first gill arch (Sp.br.); perforated scales (l.l.); rays in the pectoral fin (Pв); rays in the pelvic fin (Vв); the total number of scales in the lateral line (l.l. total.); the number of scale rows above the lateral line (l.l. above) and below the lateral line (l.l. under); total vertebrae number in the vertebral column (Vo); vertebrae count in the abdominal region (Va), transitional region (Vi), and caudal region including urostyle (Vc + ct); as well as the number of sensory canal pores (ССК) located on three paired cranial bones – praeoperculum (pop), dentale (dn), and frontale (f) [12; 16; 18; 20].

To measure the erythrocyte nuclear area (ENA), arterial blood samples were taken from live specimens, followed by fixation and staining of erythrocyte nuclei using a fixative-stain solution composed of 0.3% dry eosin methylene blue dye according to May-Grünwald in methanol. Cytogenetic analysis was performed microscopically, and cytological measurements of erythrocyte nuclei were conducted using the licensed version of the Levenhuk Lite software. The erythrocyte nuclear area was calculated using the formula for the area of an ellipse, based on measurements of erythrocyte length and width [2; 6]. To ensure statistical reliability of the ENA determination, 20 randomly selected erythrocytes were measured per individual.

Statistical processing included the calculation of the mean value (Xmean), the standard error of the mean (mX), and the coefficient of variation (CV) [14].

The normality of the distribution of traits was tested using the Kolmogorov–Smirnov test. Since the indicators of body length, height, body mass, and meristic traits deviated from a normal distribution, the Mann–Whitney U-test was used to compare the mean values. The GSI in the sample followed a normal distribution; therefore, the Student’s t-test was applied to compare females and males for this parameter [14]. Statistical analysis was performed using Microsoft Excel 2016.

Fin clips of the fish, preserved in 80% ethanol, served as the biological material for the genetic analysis. DNA extraction was performed using the D-cells kit according to the manufacturer's protocol. A fragment of the mtDNA control region (D-loop) was amplified using the “BioMaster HS-Taq PCR-Color” kit (Biolabmix) with primers L15923 (5′-TTAAAGCATCGGTCTTGTAA-3′) and H16150 (5′-GCCCTGAAATAGGAACCAGA-3′), following the methodology of Takada et al., 2010 [29]. PCR products were purified from the reaction mixture using ExoSAP-IT reagent (Applied Biosystems). Sequencing reactions were carried out in a thermal cycler using BigDye™ Terminator v1.1 reagents (Applied Biosystems) with the following thermal profile: initial denaturation (1 cycle) – 96°C (4 min); 40 cycles (denaturation – 98°C (10 s), primer annealing – 57°C (10 s), extension – 60°C (4 min)); deactivation – 96°C (3 min); hold – 4°C (indefinite). Reaction products were purified using the BigDye XTerminator Purification Kit (Thermo Fisher Scientific). The sequencing of the purified DNA mixture was performed using a 3500 Genetic Analyzer (Applied Biosystems) capillary sequencer. The alignment of the nucleotide sequences of the mtDNA control region fragment (460

bp) of the crucian carp was performed using the MAFFT v7.245 algorithm in the Geneious v10.0.9 software. The obtained sequences were compared with the publicly available GenBank database.

Alignment of nucleotide sequences of the mitochondrial control-region fragment of the golden crucian carp was performed using the MAFFT v7.245 algorithm [24]. To confirm species identity, phylogenetic relationships of the obtained sequences with sequences of the golden and silver crucian carp from the GenBank database [21] were reconstructed using the maximum-likelihood method in RAxML v8.0 [28]. The outgroup comprised the closely related species of the Japanese silver crucian carp (Carassius cuvieri Temminck & Schlegel, 1846) (mtDNA: AP011237.1). The phylogenetic tree visualization was performed using the iTOL version 6 online tool [25].

The study was conducted in accordance with all applicable international, national and institutional guidelines for the care and use of animals.

**Results and Discussion**

The studied sample of *C. carassius* (100 spec.) consisted of 52 females and 48 males (a ratio close to 1:1). All specimens were captured at Stage IV gonadal development. The size of the individuals in the sample ranged in length from 10.1 cm to 15.2 cm and in weight from 22 g to 84 g. The body was elongated, with a mean body length-to-depth ratio of 3.1. The lateral line is perforated. The number of perforated scales in the lateral line varied from 10 to 33 in females and from 17 to 31 in males. The total number of scales along the lateral line ranged from 31 to 40 for females and from 33 to 38 for males. The lower jaw was upturned. The captured individuals had a dark spot at the base of the caudal fin (Fig. 1). These characteristics correspond to the description of the shallow-bodied dwarf form *C. carassius* morpha *humilis* Heckel, 1840, inhibiting small oxygen-depleted waterbodies [7; 12].

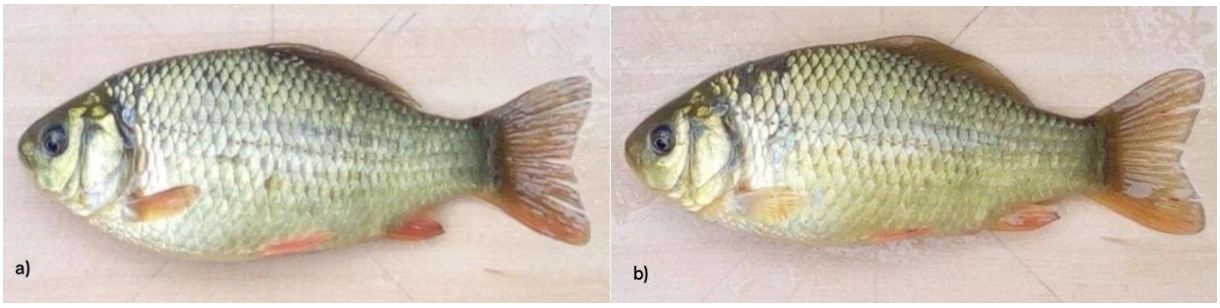


**Fig. 1. *Carassius carassius* specimens from Lake Srednee,**
**a) female (l = 10.5 cm, m = 38 g); b) male (l = 11.9 cm, m = 42 g)**

The age of the specimens in the sample was represented by four groups, from 5+ to 8+ years (Table 1). Younger age groups were not captured during the sampling period. The largest number of individuals (49 spec.) were 7+ years.

Table 1

**Size-age Characteristics of Female and Male Crucian Carp in the Sample from the Studied Waterbody**

| Sex, quantity | Age, year | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5+ | | 6+ | | 7+ | | 8+ | |
| | $L_{mean}$, cm | $m_{mean}$, g | $L_{mean}$, cm | $m_{mean}$, g | $L_{mean}$, cm | $m_{mean}$, g | $L_{mean}$, cm | $m_{mean}$, g |
| Females, 52 spec. | 10.9 | 28.0 | 12.3 | 43.9 | 13.6 | 63.1 | 14.1 | 68.1 |
| Males, 48 spec. | 11.0 | 29.3 | 11.3 | 32.1 | 11.6 | 33.6 | 11.6 | 34.2 |
| All individuals | 11.0 | 28.8 | 11.8 | 37.4 | 12.6 | 49.2 | 13.1 | 54.3 |

*Note.* $L_{mean}$ – mean length; $m_{mean}$ – mean mass.

Females in the entire sample were significantly larger in body length, depth and mass (Table 1 and Table 2). The differences in body depth and mass of the individuals are likely associated with the high (fourth) stage of gonad maturity, the size of which in females is usually 1–2 times larger during egg maturation [1; 4; 7].

Table 2

**Comparison of Morphological Traits between Female and Male Crucian Carp in the Studied Sample**

| Traits | Entire Sample (100 spec.) | | | | | Same-Age Individuals (49 spec.) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Females (52 spec.) | | Males (48 spec.) | | $pT_U$ | Females 7+ (27 spec.) | | Males 7+ (22 spec.) | | $pT_U$ |
| | $\bar{X}\pm m_X$ | CV | $\bar{X}\pm m_X$ | CV | | $\bar{X}\pm m_X$ | CV | $\bar{X}\pm m_X$ | CV | |
| L, cm | 13.5±0.2 | 4.2 | 11.4±0.1 | 9.1 | **0.000*** ** | 13.6±0.2 | 8.1 | 11.3±0.1 | 4.2 | **0.000*** ** |
| qh, cm | 4.4±0.1 | 10.9 | 3.6±0.0 | 5.7 | **0.000*** ** | 4.5±0.1 | 10.5 | 3.6±0.0 | 5.0 | **0.000*** ** |
| m, g | 60.7±2.2 | 26.0 | 32.8±0.7 | 13.8 | **0.000*** ** | 63.1±2.8 | 22.8 | 32.1±0.9 | 13.1 | **0.000*** ** |
| Sp.br. | 27.3±0.2 | 6.0 | 27.0±0.3 | 6.9 | 0.101 | 27.3±0.2 | 3.9 | 26.7±0.4 | 6.4 | **0.001*** ** |
| LLS | 35.8±0.2 | 3.7 | 35.9±0.1 | 2.6 | 0.595 | 36.0±0.3 | 3.6 | 35.3±0.3 | 3.4 | **0.000*** ** |
| LL | 24.9±0.7 | 20.2 | 24.8±0.5 | 13.7 | **0.000*** ** | 25.2±0.9 | 17.7 | 24.6±0.5 | 10.3 | **0.000*** ** |
| TRa | 5.9±0.1 | 6.1 | 5.8±0.1 | 7.4 | 0.179 | 5.9±0.1 | 6.2 | 5.7±0.1 | 7.4 | **0.029*** |
| TRb | 5.9±0.0 | 4.5 | 6.0±0.1 | 5.4 | 0.301 | 6.0±0.0 | 3.2 | 5.9±0.1 | 5.0 | **0.004**** |
| DSR | 4.0±0.0 | 0.0 | 4.0±0.0 | 0.0 | 1.223 | 4.0±0.0 | 0.0 | 4.0±0.0 | 0.0 | 0.771 |
| DBR | 17.7±0.1 | 4.1 | 17.3±0.1 | 3.8 | **0.016*** | 17.6±0.1 | 4.2 | 17.4±0.1 | 3.8 | **0.017*** |
| ASR | 3.0±0.0 | 0.0 | 3.0±0.0 | 0.0 | 1.223 | 3.0±0.0 | 0.0 | 3.0±0.0 | 0.0 | 0.771 |
| ABR | 6.8±0.1 | 6.3 | 6.7±0.1 | 7.3 | 0.096 | 6.9±0.1 | 6.7 | 6.9±0.1 | 7.1 | 0.089 |
| PR | 14.8±0.1 | 6.7 | 14.6±0.1 | 5.3 | **0.036*** | 14.8±0.1 | 4.7 | 14.6±0.2 | 5.4 | **0.028*** |
| VR | 9.8±0.1 | 4.8 | 9.7±0.1 | 5.5 | **0.045*** | 9.8±0.1 | 5.2 | 9.7±0.1 | 5.4 | **0.003**** |
| TV | 33.9±0.1 | 2.4 | 33.8±0.2 | 3.2 | **0.032*** | 34.0±0.2 | 2.4 | 33.7±0.3 | 3.5 | **0.000*** ** |
| PV | 16.6±0.1 | 4.8 | 16.7±0.12 | 5.0 | 0.646 | 16.4±0.1 | 4.6 | 16.6±0.2 | 5.2 | 0.077 |
| TRV | 4.2±0.1 | 19.3 | 4.1±0.1 | 18.5 | 1.030 | 4.4±0.1 | 15.7 | 4.0±0.2 | 20.4 | **0.005**** |
| CV+ ct | 13.3±0.1 | 6.2 | 13.1±0.1 | 5.2 | 0.141 | 13.0±0.2 | 6.0 | 13.1±0.2 | 5.4 | 0.115 |
| pop | 9.2±0.1 | 10.1 | 8.8±0.1 | 13.3 | **0.035*** | 9.2±0.2 | 9.6 | 8.8±0.3 | 14.0 | 0.117 |
| dn | 5.8±0.1 | 8.1 | 5.8±0.1 | 9.8 | 0.310 | 5.8±0.1 | 6.8 | 5.6±0.1 | 8.7 | 0.095 |
| f | 6.9±0.1 | 10.4 | 6.8±0.1 | 12.2 | 0.299 | 6.9±0.1 | 9.7 | 7.1±0.1 | 9.0 | 0.209 |

*Note.* $\bar{X}$ – mean value; $m_X$ – standard error of the mean; CV – Coefficient of Variation; $pT_U$ – calculated value of the Mann-Whitney U test; * – differences are significant at the 1st level ($p \leq 0.05$); ** – differences are significant at the 2nd level ($p \leq 0.01$); *** – differences are significant at the 3rd level ($p \leq 0.001$).

Sexual dimorphism among all individuals in the sample of the crucian carp from the eutrophic water body was identified for 6 meristic characters (Table 2).

In the sample, females showed a statistically significantly higher number of perforated scales in the lateral line, a greater number of branched rays in the dorsal, pectoral, and pelvic fins, as well as a higher number of vertebrae in the axial skeleton. When comparing individuals of the same age, differences were also found in the above-mentioned meristic features, as well as in the total number of scales in the lateral line, the number of scales above and below the lateral line, and the number of transitional vertebrae. The mean values of all these traits were higher in females at various levels of statistical significance.

There is evidence of both positive and negative correlations between certain countable traits and body length in C. carassius [12]. For example, the number of transitional vertebrae (Vi) shows a significant positive correlation with body length (l) in crucian carp inhabiting the Udmurt Republic [12], which is consistent with acquired data indicating significantly greater body length and a higher number of transitional vertebrae (Vi) in females compared to males of the same age from Lake Srednee. In the cited study [12], a negative correlation was found between body length and the total number of rays in the dorsal fin (D), along with the absence of sexual differences for this trait. In this study, the number of rays in the dorsal fin was divided into two traits (Dk and Dv), and significant differences at the first level of significance ($p \leq 0.05$) were found between females and males in the number of branched rays in the dorsal fin, both in the overall sample and among same-age individuals (Table 2).

Regarding the number of seismosensory canals (SSC) on the preopercular bone, females also showed a statistically higher count in the overall sample. However, when comparing same-age eight-year-old individuals, no differences were recorded for this trait. It is known that the number of SSC is related to the number of primary canal neuromasts – mechanoreceptive organs located in the canals of dermal bones, which play an important role in detecting moving food objects and other elements in the aquatic environment, and depends on the concentration of various chemical elements [13]. Further research is recommended to investigate the relationship between SSC on paired skull bones and the hydrochemical characteristics of the water body, as well as the potential for assessing environmental comfort based on fluctuating asymmetry of these traits.

Thus, sexual dimorphism in meristic features among same-age crucian carp from the eutrophic waterbody of the Tura river basin was found to be statistically significant in 55.6% of the studied meristic features (10 out of 18), confirming the necessity of accounting for differences between males and females of the species in morphological examination.

The average erythrocyte nuclear area (ENA) in females across the entire sample was $45.9 \pm 0.4$ μm², while in males it was $46.3 \pm 0.5$ μm². The differences between females and males in this trait, both in the overall sample and among eight-year-old individuals, were not statistically significant (Table 3). However, the greatest variability in ENA parameters was recorded for males, covering the full range from minimum to maximum values (37.0–54.0 μm²).

Table 3

**Comparison of Mean Dimensional Characteristics of ENA for Female and Male Crucian Carp in the Sample**

| Traits | All Females (52 spec.) | | | | | All Males (48 spec.) | | | | | Tst |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | max | $\bar{X}$ | $m_{\bar{X}}$ | CV | min | max | $\bar{X}$ | $m_{\bar{X}}$ | CV | |
| ENA,μm² | 39.0 | 51.0 | 45.9 | 0.4 | 6.2 | 37.0 | 54.0 | 46.3 | 0.5 | 7.8 | 0.58 |
| | Females 7+ (27 spec.) | | | | | Males 7+ (22 spec.) | | | | | |
| ENA,μm² | 39.0 | 50.0 | 45.5 | 0.7 | 7.4 | 43.0 | 54.0 | 46,4 | 0.7 | 6.9 | 0.90 |

*Note.* min – minimum value; max – maximum value; $\bar{X}$ – mean value; $m_{\bar{X}}$ – standard error of the mean; CV – Coefficient of Variation; Tst – calculated Student's t-test value.

The distribution of the erythrocyte nuclear area (ENA) by the quantity of individuals in the sample between females and males is presented in Figure 2.

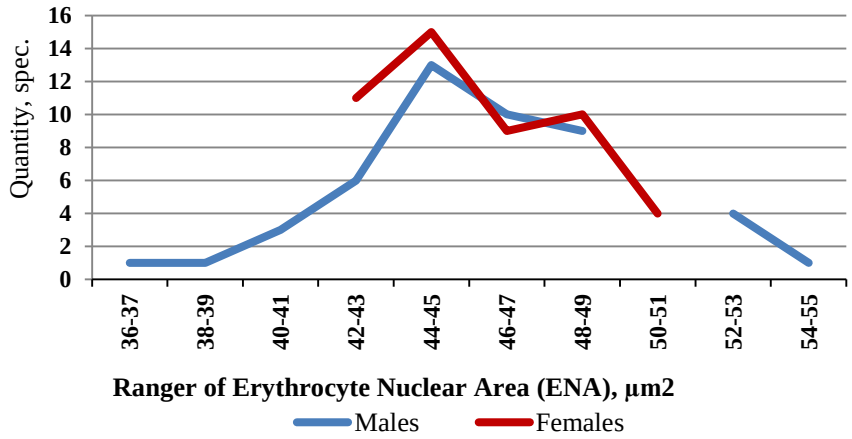


**Fig. 2. Erythrocyte Nuclear Area Distribution in Female and Male Crucian Carp**

The size range of the erythrocyte nuclear area (ENA) in female crucian carp (*Carassius carassius*) from Lake Srednee varied from 39.0 to 51.0 μm², which was five intervals smaller than in males.

It is known that erythrocytes with an unusual size and nuclear structure can serve as a marker trait for hybrids (or allopolyploids) in cyprinid fish [2]. It has also been demonstrated that in cyprinid species, males can undergo hybridization more readily than females when co-habiting with other related species, such as the silver crucian carp (*Carassius gibelio*) [2; 3; 8; 16]. To gain a more detailed understanding of potential hybridization between the two crucian carp species in the studied waterbody, additional cytometric studies of nuclear anomalies (size, number of nucleoli, segmentation) are recommended [2].

The analysis of the genetic diversity in the crucian carp from Lake Srednee, based on a representative fragment of the mtDNA control region (460 bp), revealed that all 30 specimens shared an identical haplotype, CCA2. This indicates a low genetic diversity within the studied population [27].

To further examine the phylogenetic relationships with other species of the genus Carassius, we obtained all available sequences of the mtDNA control region for the crucian carp from the GenBank database and reconstructed a phylogenetic tree. All identified *C. carassius* sequences

formed a distinct cluster separate from *C. gibelio* and *C. cuvieri*. This cluster splits into two clades corresponding to two different haplotypes, CCA1 and CCA2, as was previously shown by Sakai et al. [27]. The phylogenetic tree of the genus *Carassius* is presented in Figure 3, where the numbers at the branches (1; 0.814; 0.802) represent the bootstrap support values.

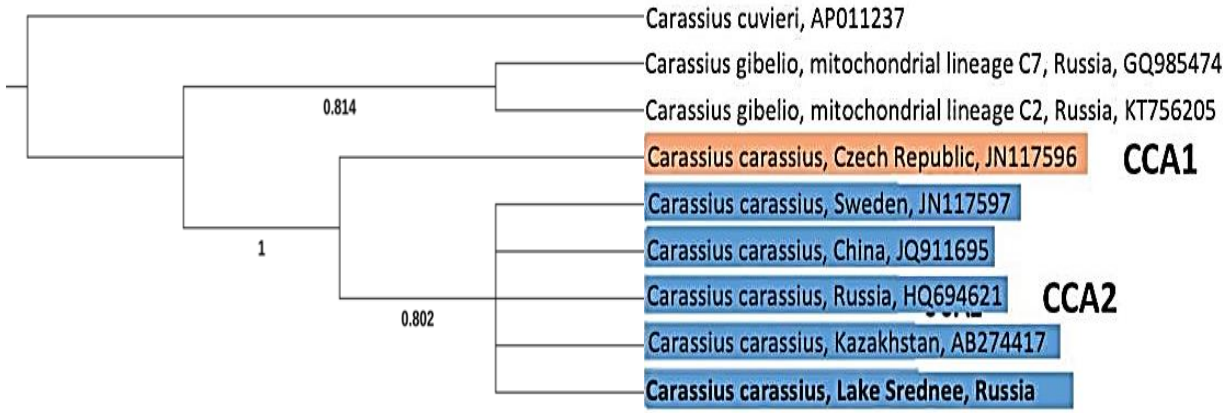


**Fig. 3. Phylogenetic relationship of the crucian carp with other species of the genus Carassius, where two haplotypes are highlighted in color: red – CCA1, blue – CCA2**

The sequences of the crucian carp from Lake Srednee obtained in this study show 100% sequence identity with AB274417 (Kazakhstan), JN117597 (Sweden), HQ694621 (Russia), and JQ911695 (China). These results indicate that the individuals from our sample belong genetically to the *Carassius carassius* species and correspond to the widely distributed Eurasian haplotype CCA2.

The detection of only two haplotypes of the crucian carp across the entire Eurasian range indicates a low genetic diversity, likely resulting from an ongoing population decline [15, 16, 22, 23, 26, 27, 30-32]. These findings underscore the need for continued monitoring of the population structure and provide a solid foundation for developing some targeted conservation measures.

**Conclusion**

The study of the crucian carp population in the eutrophic waterbody of the Tura river basin revealed distinctive features in both morphological and genetic structure. The population consisted of a small-bodied dwarf form (*C. carassius* morpha *humilis* Heckel, 1840) with a sex ratio approaching 1:1, consistent with previously published data and indicating stability in reproductive processes.

Pronounced sexual dimorphism in morphometric and meristic traits was observed, emphasizing the importance of further investigation into these characteristics to enhance understanding of the species' ecology.

A low genetic diversity, evidenced by the lack of mtDNA haplotype variation and the uniformity of erythrocyte nuclear area in both sexes, raises concerns regarding the population vulnerability. This highlights the need for ongoing monitoring and additional studies. Taking these findings into account, developing and implementing some conservation and population restoration measures are important steps toward ensuring the long-term survival of the species in its natural habitats.

Regular monitoring of the population and the habitat hydrochemical parameters is recommended to allow prompt adjustments to conservation strategies and to maintain the species' genetic integrity over time.

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