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STUDY OF THE GENETIC STRUCTURE OF TWO ECOMORPHOLOGICAL GROUPS OF ROACH (*RUTILUS RUTILUS*) OF THE KANIV RESERVOIR

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Materials & Methods: aim, object, sampling

The aim of the study was to investigate the genetic structure of two forms of roach (large fast-growing forms – group I and small slow-growing – group II) of the Kaniv reservoir using microsatellite loci (Rru: 3, 4 and 7; Ca: 3, 5 and 12).





Materials & Methods: Microsatellite Analysis

	Genbank accession	Marker sequence (5'→3')	PCR conditions					Notes:			
Locus					F	C	Reference	* - Temperature was optimized			
	number		D	AI	L	C		F - Iol ward primer's sequence,			
Rru 3	AB112739	F: GGGGAGTCTGGCTTCAGG R: CGGCACACAGGGAGGTTA		55°C 30 s				D - denaturation;			
Rru 4	AB112740	F: TAAGCAGTGACCAGAATCCA R: CAAAGCCTCAAAAGCACAA	90° C	55°C*	72 °C	33	[Barinova,	AT - annealing temperature; E - extension.			
Rru 7	AB112741	F: GTCTCCACAAAAATAGCGAACC R: CGATTGATGCCGTCAGAA	50 \$	50 s 55°C 30 s	50.8		2004]	C - Cycles			
Ca 3	AF277575	F:GGACAGTGAGGGACGCAGAC R:TCTAGCCCCCAAATTTTACGG		57°C* 1 min	72 °C 1.5 27 min			- Separation in 8% PAGE Gel analysis in Totallab v.2.01			
Ca 5	AF277577	F:TTGAGTGGATGGTGCTTGTA R:GCATTGCCAAAAGTTACCTAA	92 °C	57°C* 1 min		27	[Dimsoski, 2000]	Statistical Analysis in Genalex 6.5 (Peakall & Smouse, 2006, 2012).			
Ca 7	AF277579	F:ACACGGGCTCAGAGCTAGTC R:CAAATGTCAGGAGTTCTCCGA	1 min	59°C 1 min				PIC (The polymorphic information content) was calculated using the formulas generally accepted for codominant markers (Nagy et al., 2012).			
Ca 12	AF277584	F:GTGAAGCATGGCATAGCACA R:CAGGAAAGTGCCAGCATACAC		57°C* 1 min							
MFW 6	-	F*: ACCTGATCAATCCCTGGCTC R**:TTGGGACTTTTAAATCACGTTG						1. Barinova,A., Yadrenkina,E., Nakajima,M. and Taniguchi,N. (2004). Identification and characterization of microsatellite			
MFW 15	-	F: CTCCTGTTTTGTTTGTGAAA R: GTTCACAAGGTCATTTCCAGC	95°C	55 °C 45 s	72 °C 1 min	35	[Crooijmans, 1997]	 DNA markers developed in ide Leuciscus idus and Siberian roach <i>Rutilus rutilus. Mol. Ecol. Notes</i> 4, 86-88 2. Dimsoski P., Toth G. P., Bagley M. J. (2000) Microsatellite characterization in central stoneroller <i>Campostoma anomalum</i> (Pisces: Cyprinidae). Blackwell Science Ltd, <i>Molecular</i> 			
MFW 23	-	F:GTATAATTGGGAGTTTTAGGG R:CAGGTTTATCTCCCTTCTAG	30 s								
MFW 31	-	F:CCTTCCTCTGGCCATTCTCAC R:TACATCGCAGAGAATTCGTAAG						 <i>Ecology</i>, 9, 2187–2189 3. Crooijmans R., Bierbooms V., Komen J. et al. Microsatellite markers in common carp (<i>Cyprinus carpio L.</i>). Animal Genetics. 1997. V. 28. P. 129 – 134. 			
Hmo 02	AM086449	F: CATCTGTTCTGAGGGGGCTGAG R: CCCCACTTTACCACCAATTATTAT		60°C 50 s		28	[Gheyas, 2006]				
Hmo 27	AM086456	F: CTGTAATTCCGTTTTATCTGTGT R: ATTGCTGTAAACCATAAAATGTAA	94° C	60°C 50 s	72 °C			 4.Gheyas A. A., Cairney M., Gilmour A. E., Sattar M. A., Das T. K., Mcandrew B. J., Penman D. J. and Taggart J. B. (2006) Characterization of microsatellite loci in silver carp (<i>Hypophthalmichthys molitrix</i>), and cross-amplification in other cyprinid species. Molecular Ecology Notes 6, 656–659. doi: 10.1111/j.1471-8286.2006.01288.x © 2006 			
Hmo 33	AM086458	F: GTGCAGCAGTATGTGAATCAGGACAC R: GTGCTTCGGGATACCACACTCTTG	50 S	59°C 50 s	5U S						

Results: evaluation of the effectiveness of markers







Notes:

Na = No. of Different Alleles Ne = No. of Effective Alleles I = Shannon's Information Index uHe = Unbiased Expected Heterozygosity

Results: differences between groups

		Ν		Na		Ν	Ne	Ι		uHe	
0	Locus	Group 1	Group 2								
	Ca 3	8	6	3.000	5.000	2.667	4.800	1.040	1.589	0.667	0.864
)3	Ca 5	10	9	12.000	11.000	10.000	8.526	2.389	2.274	0.947	0.935
	Ca 12	9	8	9.000	7.000	7.364	4.414	2.091	1.689	0.915	0.825
	Rru 3	9	10	8.000	7.000	6.480	5.128	1.966	1.765	0.895	0.847
	Rru 4	10	10	6.000	7.000	4.545	3.704	1.614	1.591	0.821	0.768
	Rru 7	10	10	5.000	5.000	3.846	3.509	1.471	1.392	0.779	0.753
	Mean	9.333	8.833	7.167	7.000	5.817	5.013	1.762	1.717	0.837	0.832
	SE	0.333	0.654	1.302	0.894	1.092	0.747	0.198	0.123	0.043	0.027

Results: differences in Allele Frequency between groups



Notes: group I group II

Conclusion

- The studied loci were found to be polymorphic (100%) (average Ne = 7.9 ± 1.9).
- The average polymorphic information content increased in the following sequence Ca 3 (0.657) < Rru 7 (0.685) < Rru 4 (0.723) < Ca 12 (0.797) < Rru 3 (0.803) < Ca 5 (0.883).
- Since the PIC value was above 0.5, it was concluded that all SSR primers used were characterized by high polymorphism.

Key Features of studied groups

General:

- There are no significant differences in the value of Shannon index I in the groups (fast-growing 1.762 ± 0.198 and slow-growing 1.717 ± 0.123)
- For Ca5 locus allelic variant of 287 bp occurred in both groups (0.050 in group I and 0.222 in group II).
- For Rru7 locus 82 bp, which occurred in both groups with a high frequency: 40% and 30%, respectively.
- For Rru3 locus, the presence of a specific allele of 187 bp was found, which was typical for both groups and occurred with a frequency of 22% in group I and 30% in group II.
- For Rru4 variant of 140 bp occurring with a high frequency in both groups (30% and 45%, respectively)

Differences:

- For Ca3 locus:
 - in group I allelic variants of 224 bp. (50%) and 237 bp (25%) were private alleles
 - in group II 275 bp and 297 bp variants were the most frequent (25% each), which were specific for this group.

The obtained results allowed making assumptions about the different genetic origin of the studied roach groups. Confirmation of this hypothesis requires additional comparison of the genetic structure of fast-growing roach and *R. heckelii* inhabiting the lower Dnieper.

THANK YOU FOR YOUR ATTENTION!