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## REGULAR INTERGENERIC HYBRIDIZATION OF LEUCISCINE CYPRINIDS (CYPRINIDAE, LEUCISCINAE) IN THE DNIPRO RIVER AFFLUENTS

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**Regular Intergeneric Hybridization of Leuciscine Cyprinids (Cyprinidae, Leuciscinae) in the Dnipro Affluents.** Tsyba, A. A., Ghazali, M., Kokodiy, S. V., Mezhzherin, S. V. — The large-scale hybridization of fishes of the subfamily Leuciscinae in the subordinate systems of the Dnipro River basin is presented by the data on two pairs of species, roach *Rutilus rutilus* × bream *Abramis brama*, and bleak *Alburnus alburnus* × rudd *Scardinius erythrophthalmus*. The hybridization and occurrence of F1 hybrids are confirmed with allozyme spectra and morphological characters on series of samples. A complete morphometric analysis of the characteristics of bleak and rudd hybrids was performed. The paper discusses the intergeneric hybridization in nature, which is a unique phenomenon characteristic only of some groups of cyprinids. The most likely reason is the overestimation of the taxonomic status of European cyprinids, which is confirmed by the insignificant level of intergeneric genetic divergence.

Key words: Cyprinidae, bream, roach, bleak, rudd, hybridization, allozymes, morphometry.

The issues of natural hybridization are attracting a lot of attention. Interest is caused not only by the wide presence of this natural phenomenon that was previously thought to be exceptional, but also by its consequences, often in the form of irreversible evolutionary changes (Arnold, 1992; Barton, 2001; Abbott et al., 2013). The intensity of hybridization phenomena has increased in recent decades (Brennan et al., 2015) because of climate changes, destabilization of ecosystems, destruction of historically formed landscapes, and invasions.

Cases of interspecific hybridization of freshwater and anadromous fishes are especially numerous. This phenomenon began to be studied in detail in the middle of the 20th century (Hubbs, 1955; Schwartz, 1972) using morphological characters to identify hybrids, and gene markers are used since the 1970s. Hence, the fact of hybridization was unambiguously proved and the parental species were reliably established. At present, there are hundreds of hybridizing pairs of species, and hybridization mainly occurs in freshwater and anadromous fish of the Holarctic region (Scribner et al., 2000).

Depending on parent species and the degree of their genetic differentiation, hybridization can lead to different results. Crosses between genetically close vicarious species are accompanied by genetic recombination and introgression of genetic fragments of one species into the genome of another, and with the formation of hybrid zones of various widths (Arnold, 1992). The random hybridization is characteristic of sympatric genetically distant species (Mayr, 1963). In this case, allopolyploid offspring are formed. Typically, the proportion of such hybrids is negligible, produced in random recurring interspecific crosses. The allopolyploid hybrids can even numerically dominate over individuals of parental species in small water bodies for a while, as in the case with the hybridization of *Carassius carassius* and *Carassius auratus* (Mezhzherin et al., 2012).

Allopolyploid hybrids can be divided into two groups depending on the modes of gametogenesis. In some cases, meiosis is replaced by ameiosis (mitosis), which ultimately leads to the formation of diploid-polyploid hybrid complexes that self-reproduce by sperm-dependent parthenogenesis (Vasiliev, 1985). Alternatively, various kinds of meiotic anomalies are observed, leading to a significant loss of fertility.

The large cyprinid family comprising about 367 genera and more than 3,000 living species (Nelson et al., 2016) is of particular interest. Intergeneric hybridization is often observed for its extratropical representatives (Schwartz, 1972; Scribner et al., 2000). Hybrids identified by external characteristics as crosses of the roach *Rutilus rutilus* (Linnaeus, 1758) and the bream *Abramis brama* (Linnaeus, 1758) (Berg, 1912; Beling, 1928), bleak *Alburnus alburnus* (Linnaeus, 1758) and rudd (*Scardinius erythrophthalmus* (Linnaeus, 1758) (Berg, 1912; Velykokhatko, 1929), were noted in the Dnipro River system in the first quarter of the 20th century. Since then, the hybridization of these species in this river system was not reported. In order to resolve the problem, it is necessary to prove the fact of natural hybridization at the level of gene markers. In addition, it remains unclear how widespread this phenomenon is in the modern, anthropogenically transformed Dnipro River system.

## Material and methods

The materials of the present work are samples and single specimens of cyprinids with morphological characters either within the standard range or clearly deviating. A series of 16 supposed hybrids of *A. brama* and roach *R. rutilus* was collected in June 1991 on the mouth of the Sozh River (left tributary of the Dnipro) in a floodplain lake (51.975 N, 30.868 E).

A total of 51 putative hybrids of the bleak and rudd were caught in a small lake-type reservoir on the Snitka River (50.106 N, 29.976 E), located in the watershed of the upper reaches of Unava (basin of Irpin River, right tributary of the Dnipro River) and Stugna Rivers (right tributary of the Dnipro) in May–June 2016–2019. Fishes were caught with a sport fishing gear.

Alozyme analysis according to the standard method (Peacock, Buntig, 1965) was carried out on supposed hybrids and the following leuciscine species: bream, *Abramis brama*, blue bream, *Abramis ballerus* (Linnaeus, 1758), silver bream, *Blicca bjoerkna* (Linnaeus, 1758), common roach, *R. rutilus*, common rudd, *S. erythrophthalmus*, common bleak, *A. alburnus*, ide, *Leuciscus idus* (Linnaeus, 1758), chub, *Squalius cephalus* (Linnaeus, 1758), asp, *Aspius aspius* (Linnaeus, 1758), as well as a representative of the subfamily Acheilognathinae Bleeker, 1863, the Amur bitterling, *Rhodeus sericeus* (Pallas, 1776).

Multilocus analysis was performed using polyacrylamide gel electrophoresis and running tris-borate-EDTA buffer, pH 8.5, for a number of water-soluble and structural muscle proteins. The variability of the following enzymes and proteins encoded by the corresponding loci was analysed: aspartate aminotransferase (*Aat-1*, -2), glycerol-3-phosphate dehydrogenase (*G3pdh-1*), malate dehydrogenase (*Mdh-1A*, B, *Mdh-2A*), superoxide dismutase (*Sod-1*), lactate dehydrogenase (*Ldh-A*, -B), umbelliferyl esterase (*Es-D*), structural muscle proteins stained for total protein (*Pt-1*, -2, -3, -4) and albumin (*Alb*). A total of eight enzymes and proteins encoded by 15 loci were analysed.

Morphometric analysis was performed on a series of 30 hybrids of rudd and bleak, according to the standard scheme of body measurements for cyprinids (Pravdin, 1966). For this purpose, 22 body measurements (*H* — body depth, *iH* — body thickness, *aD* — antidorsal length, *pD* — postdorsal length, *aV* — anteventral length, *aA* — antianal length, *lD* — dorsal fin length, *hD* — dorsal fin height, *lA* — anal fin length, *hA* — anal fin height, *lP* — pectoral fin length, *lV* — pelvic fin length, *PV* — distance between pectoral and pelvic fin, *pl* — caudal peduncle length, *h* — caudal peduncle depth, *C1* — upper lobe of the caudal fin length, *C2* — lower lobe of the caudal fin length, *c* — head length, *hc* — head height, *r* — snout length, *o* — eye diameter, *io* — interorbital distance (standard length) were used. All plastic characters are given relative to *l* - standard body length, except for the plastic characters of head, they are given relative to head length. And also five meristic characters (*A* — the number of rays in the anal, *D* — dorsal, *P* — pectoral, and *V* — pelvic fins, and *ll* — scales in the lateral line) were used.

Group characterizing was performed with the principal component analysis (PCA) on correlation matrix. In total, we used 12 specimens of *A. alburnus*, 4 specimens of *S. erythrophthalmus* and 26 specimens of their hybrid for which all traits were available. We used function PCA of package FactoMineR, v. 1.42 (Le et al., 2008); visualization was done with package factoextra, v. 1.0.5 (Kassambara, Mundt, 2017) in statistical system R, v. 3.6.3 (R Core Team, 2020).

## Results

Selection of gene markers and assessment of genetic divergence. All studied species of cyprinids had a unique set of allelic variants of enzymes (allozymes) and structural proteins (table 1). Moreover, the degree of interspecies differences varied significantly. Minor fixed differences in alleles affecting two, less often three loci, took place in most pairwise interspecific comparisons. Minimal differences were found when

**Table 1. Allele pools of enzyme and protein loci in cyprinid species**

Loci	Allele	<i>Abr. bra.</i>	<i>Abr. bal.</i>	<i>Bl. bjor.</i>	<i>Alb. alb.</i>	<i>Rut. rut.</i>	<i>Sc. eryth.</i>	<i>L. idus</i>	<i>Sq. ceph.</i>	<i>As. asp.</i>	<i>Rh. ser.</i>
<i>Aat-1</i>	99										1
	100		1		1	1	1	1	1	1	
	110	1		1							
<i>Aat-2</i>	-90										1
	-95							1		1	
	-98						1		1		
	-100	1	1	1	1	0,8					
	-110					0,2					
<i>Ldh-B</i>	90					1					
	92										1
	100	1	1	1	1		1	1	1	1	
<i>Mdh-1A</i>	90	0,05									
	100	0,95	1	1	1	1	1	1	1	1	1
<i>Mdh-1B</i>	90										1
	100	1	1	1	1	1	1	1	1	1	
<i>Mdh-2A</i>	99	1		1							
	100		1		1	1	1	1	1	1	1
<i>Sod-1</i>	80										1
	90					0,1					
	100			1	1	0,9	1	1	1	1	
	110	1	1								
<i>G3pdh</i>	85										1
	90		1								
	100	1		1	1	1	1	1	1	1	
<i>Pt-2</i>	80				0,4						
	98							0,7			
	99										1
	100	1		1	0,6	1	1	0,3	1	1	
	120		1								
<i>Pt-3</i>	96							1			
	97								1		
	98										
	99				1						1
	100			1		1	1			1	
	102	1	1								
<i>Pt-4</i>	-100				1	1	1	1	1	1	1
	100	1	1	1							

Loci: *Alb*, *Es-D*, *Ldh-A*, *Pt-1* under used conditions of electrophoresis were monomorphic.

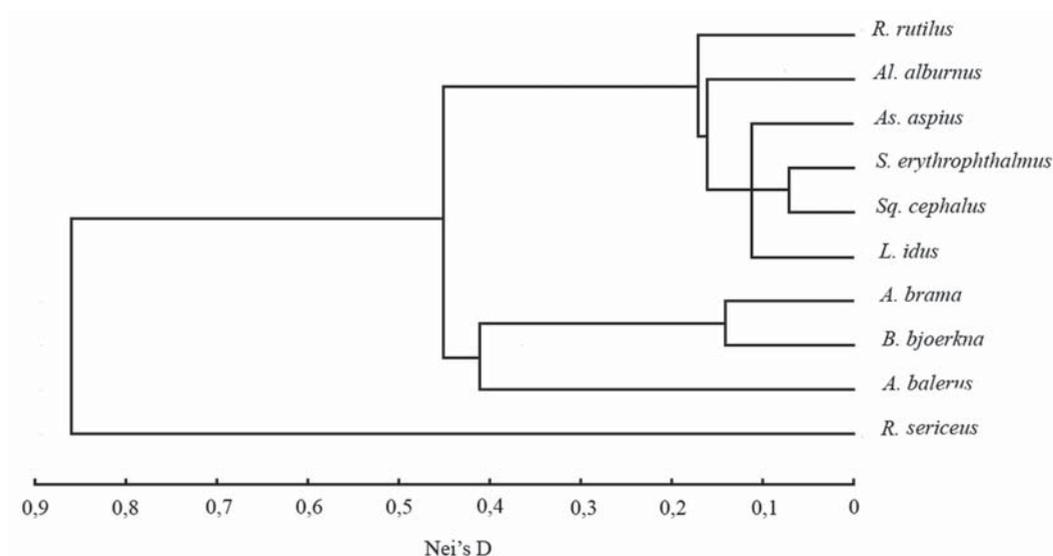


Fig. 1. Phenogram of genetic distances (Nei's D) between the studied species of cyprinids, UPGMA algorithm on the basis of 15 biochemical loci.

comparing *R. rutilus* and *A. alburnus*. In this case, fixation of alternative alleles affected only one *Ldh*-B locus, which in roach has an allele that distinguishes it from the rest of the studied cyprinids. In this case, fixation of alternative alleles affected only one locus. In 4–5 loci, differences were observed between representatives of the *Abramis* group and other European cyprinid species. Significant differentiation affecting 7–8 loci, largely expected, was observed by comparing the gene pool of *R. sericeus* with other species.

All these features of genetic relationships between the studied species are clear on a phenogram based on genetic distances (fig. 1). Three or four clusters (groups of species) can be distinguished on different hierarchical levels of genetic differences.

Hybridization of *A. alburnus* × *S. erythrophthalmus*. Analysis of enzymatic and protein spectra in 30 putative hybrids collected in an artificial reservoir on the Snitka River confirmed their status as hybrids of *A. alburnus* × *S. erythrophthalmus* for all of

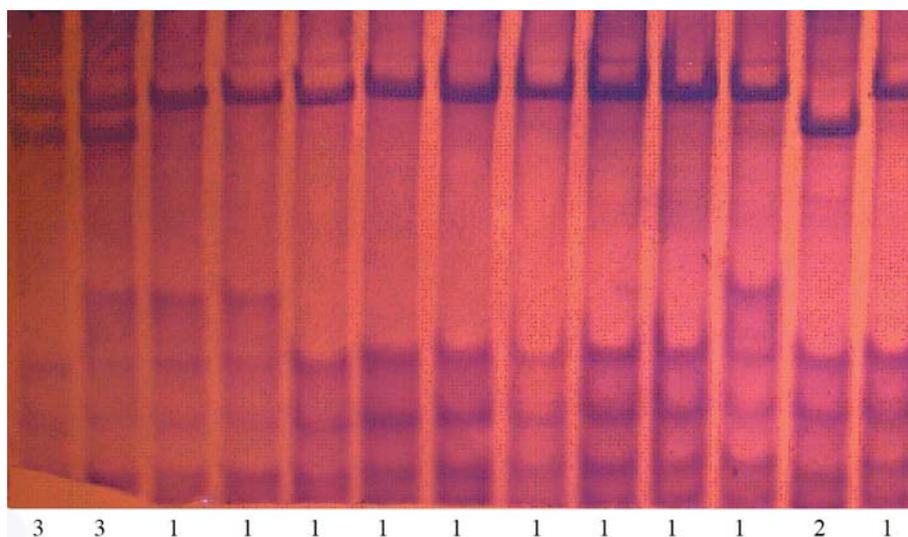


Fig. 2. Electrophoregram of muscle structure proteins spectra of *A. alburnus* (1), *S. erythrophthalmus* (2), and their hybrids *A. alburnus* × *S. erythrophthalmus* (3).



Fig. 3. Standard hybrid *A. alburnus* × *S. erythrophthalmus* from Snitka River.

them. The electrophoretic spectrum of the products of the *Pt-3* locus, presented in hybrids by two fractions (fig. 2), was found to be diagnostic and the most convenient in practice, which indicates its monomeric nature. Theoretically, hybridization of the above fish with *R. rutilus*, which is a common species in the Snitka River here, is quite possible in this reservoir. However, the absence of heterozygotes in hybrids at the *Ldh-B* locus does not confirm such an assumption.

A four-year study conducted on this reservoir showed a stable presence of hybrids in bleak schools, where their proportion in different samples varied from 5 to 25 % of caught fishes. This means that the hybridization between bleak and rudd is a common phenomenon, which repeats annually. The analysis of muscle spectra of samples from seven populations of the bleak from the rivers of the Kaniv reservoir, the Desna and Stugna Rivers showed the absence of hybrids. This indicates that hybridization between these species is possible primarily in “confined” conditions of reservoirs with a limited number of suitable spawning grounds.

Hybrids of bleak and rudd are more similar in size and externally to bleak, although they reliably differ from the latter in the reddish colour of the caudal and anal fins (fig. 3). For these hybrids, the almost red colour of the caudal fin is especially characteristic, which is never dark grey at the edge as in bleak. The body is elongated, laterally compressed. The upper mouth is small. The back is dark, while the sides are silvery.

Ovaries filled with eggs were found in 16 of 51 individuals. The number of eggs ranged from 31 to 435, with an average of 130. Such a low individual fecundity means a limited fertility of the hybrids.

**Table 2.** Statistical parameters of meristic characters and body indices in two parent species and hybrids

Characters	<i>A. alburnus</i> (n = 17)				<i>A. alburnus</i> × <i>S. erythrophthalmus</i> (n = 30)				<i>S. erythrophthalmus</i> (n = 4)			
	M	SE	Min	Max	M	SE	Min	Max	M	SE	Min	Max
<i>ll</i>	48	0.75	43	55	43.6	0.28	40	47	39.8	0.48	39	41
<i>A</i>	18.3	0.29	15	20	14.3	0.12	13	16	11.5	0.29	11	12
<i>H/l</i> , %	22.4	0.38	19.4	24.9	24.8	0.29	22.0	28.4	29.1	0.61	28	30.9
<i>aA/l</i> , %	64.0	0.33	62.0	66.8	66.7	0.47	56.0	70.1	70.1	1.32	66.9	73.2
<i>lA/l</i> , %	20.0	0.18	18.3	21.3	17.6	0.20	15.2	19.6	15.4	0.12	15.3	15.8
<i>Io/c</i> , %	26.3	0.46	22.2	29.4	30.9	0.30	28.3	34.9	37.1	0.4	36.1	38

Legend: *ll* — number of scales in the lateral line; *A* — number of branched rays in the anal fin; *l* — body length to the end of the scale cover; *H* — body depth; *aA* — anteanal distance; *lA* — anal fin length; *Io* — infraorbital distance; *c* — head length; SE — standard error.

**Table 3. The formula of meristic traits in hybrids, bleak, rudd**

Characters	Hybrids	Bleak*	Rudd*
D	III (8)9(10)	III (6-7)8-9	III (7) 8 (9-10)
A	III (13)14-15(16)	III 17-20	III 10-12
P	I (12)13-14(15)	I (11-12) 13-16	I 13-15 (16)
V	I 8	I (7) 8 (9)	II (7) 8
l.l.	40-47	45-48	(37) 38-42 (43)
d.f.	2.5-5.2, 2.4-5.2, 3.5-5.3, 2.5-5.1, 1.5-5.1, 2.5-6.2, 1.4-5.2	2.5-5.2	3.5-5.3, 3.5-5.2

\*Movchan, 2011

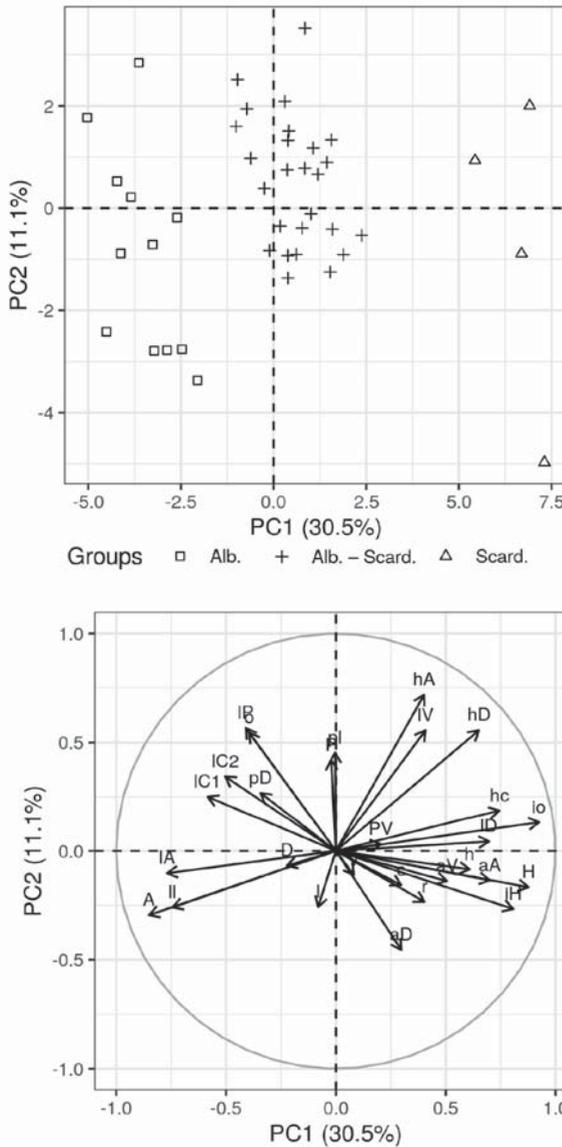


Fig. 4. Results of the principal component analysis of the *A. alburnus* (squares), *S. erythrophthalmus* (triangles) and their hybrid (crosses): Top — position of the specimens in the space of first two principal components; Bottom — loadings of the traits.

In a number of body proportions and some meristic characters, the hybrids significantly differ from the two parental species (table 2). The parameters in question are the relative height of the body, measurements associated with the anal fin, infraorbital distance, as well as the number of scales in the lateral line and branched rays in the anal fin.

By all these features, hybrids have intermediate values compared to the parental species. An important diagnostic feature of cyprinids is the formula of the pharyngeal teeth, which is 2.5-5.2 in bleak and 3.5-5.3 in rudd. Hybrids showed a high variability in that parameter: there were 1-2 teeth on the outer row, and at least 4-6 teeth on the inner row. The formula of the meristic traits of hybrids and parental species, for comparison, is presented in table 3.

First two PCs explain 42 % of total variance (table 4). Multivariate statistical analysis based on body indices and meristic parameters shows a clear separation of parental species and hybrids in the morphological space of the first two components (fig. 4). The most variation is associated with io, H, iH, hc, aA (larger in *A. alburnus*) and A, IA, II (larger in *S. erythrophthalmus*) (table 5).

Distribution of hybrids, although it is of an intermediate values, is somewhat biased towards bleak (fig. 5). Greater similarity

with bleak than rudd was also noted in offspring obtained under artificial conditions (Nikolyukin, 1952).

A high degree of morphometric differences between hybrids is also confirmed by the results of discriminant analysis. The level of identification of hybrids and parental individuals by 22 indices and five meristic characters is 100 %.

Hybridization between *Abramis brama* × *R. rutilus*. Hybridization between the bream and the roach was observed in floodplain of the Sozh River. They were established by the presence of fixed heterozygous states in the putative hybrids at the loci *Aat-1*, *Ldh-B*, *Pt-3*, and *Pt-4*. In the heterozygote, the product of the *Pt-4* locus, which had a spectrum of five fractions, was a tetramer (fig. 6). A similar set of diagnostic loci in relation to roach is also characteristic of the silver bream, which also looks very similar to the bream. The single fixed allele at the *Pt-3* locus is the same in roach and silver bream. Therefore, finding the bream and roach hybrids is proved by the presence of a constant heterozygote  $Pt-3^{100/102}$ .

Some of the found hybrids of roach and bream from the Sozh River sharply differed in size. In addition, they were collected in different years. That, together with the finds of similar hybrids in the floodplain water bodies of the Desna River, proves that the hybridization of roach and bream in the Dnipro basin from the Desna to Sozh Rivers has been a fairly common and recurring phenomenon in recent decades.

## Discussion

Factors and biological consequences of hybridization. Natural hybridization of the roach and bream has been repeatedly reported (Witkowski & Blachuta 1980; Pervozvanskiy & Zelinskiy, 1981; Blachuta, Witkowski, 1984; Economidis et al., 1989; Fahy et al., 1988; Pitts et al., 1997; Slynko, 2000; Kodukhova & Karabanov, 2013; Hayden et al., 2014; Kuparinen et al., 2014; Konopiński & Amirowicz, 2018; Wood & Jordan, 1987) and confirmed by the analyses of gene markers (Golubtsov et al., 1990; Slynko, 2000; Pitts et al., 1997; Konopiński & Amirowicz, 2018). In the middle of the 19th century, *Abramis leuckarii* Heckel et Kner, 1858 was even described from hybrid

**Table 4. Description of principal components**

Components	Eigenvalue	% of variance	Cumulative % of variance
PC1	8.536	30.5	30.5
PC2	3.121	11.1	41.6

**Table 5. Correlation of principal components and variables**

Characters	PC1	PC2
ll	<b>-0.744</b>	-0.260
D	-0.223	-0.068
A	<b>-0.851</b>	-0.295
P	-0.021	0.419
V	0.081	-0.110
L	-0.081	-0.258
H	<b>0.877</b>	-0.167
H	0.609	-0.085
iH	<b>0.808</b>	-0.267
aD	0.298	-0.454
pD	-0.343	0.268
aV	0.505	-0.139
aA	<b>0.700</b>	-0.135
PV	0.204	0.031
pl	-0.003	0.453
ID	0.697	0.044
hD	0.652	0.556
lA	<b>-0.768</b>	-0.103
hA	0.401	<b>0.719</b>
IP	-0.411	0.566
IV	0.408	0.556
IC1	-0.584	0.250
IC2	-0.504	0.344
c	0.297	-0.157
hc	<b>0.744</b>	0.187
r	0.404	-0.234
o	-0.395	0.546
io	<b>0.925</b>	0.132



Fig. 5. A putative recurrent hybrid *A. alburnus* × *S. erythrophthalmus* from Snitka River.

individuals of the bream and roach. Numerous cases of hybridization gave reason to believe that hybridization between these species, although it occurs randomly, is an obligatory event during cohabitation, and individuals of *A. brama* × *R. rutilus* are perhaps the most common interspecific freshwater fish hybrids in Europe. Studies at the Rybinsk Reservoir have shown that at unstable spring flood, the ratio of hybrids can be 1.5 % (Kodukhova & Karabanov, 2013). Hybrids reach sexual maturity, are fertile, and their gametogenesis occurs by semi-cloning, abnormal meiosis and ameiosis, judging by the size of gametes (Slynko, 2000). Under artificial conditions, backcrosses can be obtained from hybrids (Wood & Jordan, 1987; Slynko, 2000; Peittse et al., 2005). However, effective reproduction of hybrids in nature is questionable. Triploid hybrid associates of the bream and roach, which should form in backcrosses and copulations of diploid hybrid gametes with haploid gametes of parental species, have not been found in natural conditions.

In the Dnipro basin, hybridization between the bream and roach was noted by L. S. Berg (1912) and, somewhat later, by D. E. Beling (1928). Information about hybrids of these two species for the Dnipro basin has not been presented later. A series of 67 hybrids caught in the Hirskiy Tikych River (basin of the Southern Bug River) in 1990 is kept in the Department of Zoology of the National Museum of Natural History, National Academy of Sciences of Ukraine (Movchan et al., 2003).

Interestingly, the hybrids in the Sozh River were mostly caught under flooded willow shrubs. In years with low floods, coastal shrubs were not inundated, and hybrids were not

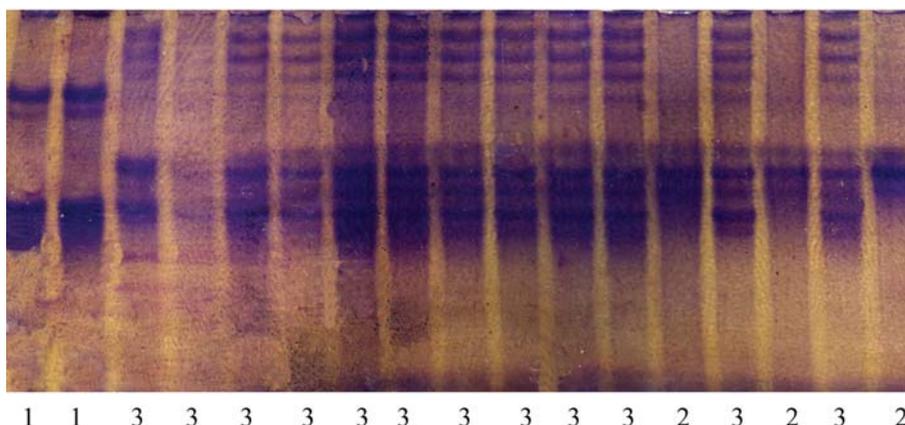


Fig. 6. Electrophoregram of muscle structure proteins spectra of *A. brama* (1); *R. rutilus* (2), and their hybrids *A. brama* × *R. rutilus* (3).

found. It seems obvious that the physically defective hybrids of bream and roach keep to poorly accessible microhabitats, occupying small riverside reservoirs, by which they are clearly inferior to the standards of parental species.

Hybrids of bleak and rudd were obviously known back in the 19th century. It is thought that their presence in the aquatic systems of Germany has served as the basis for erroneous descriptions of the species *Alburnus rosenhaueri* Jäskel, 1866 and *Scardiniopsis alburniformis* Beneske, 1884 (Berinkey, 1960). In the first decades of the 20th century, Berg (1912) pointed out the hybridization between the bleak and rudd, and F. D. Velykokhatko (1929) mentioned such hybrids in the middle reaches of the Ros River. In the 1970–1980s, hybrids of *A. alburnus* with species of the genera *Abramis*, *Rutilus*, *Squalius*, but never with *S. erythrophthalmus*, were often observed in European waters (Wheeler, 1978; Witkowski & Blachuta, 1980; Kammerad & Wuestemann, 1989). On the Iberian Peninsula, bleak is an invasive species that easily hybridizes with endemic representatives of the genus *Squalius* (Vinyoles et al., 2007), displacing them. Nevertheless, until now the fact of hybridization between bleak and rudd in nature has not been confirmed at the level of gene makers, which, due to the intermediate nature of the morphology of hybrids, raises the question of the correctness of their determination purely by external features. Therefore, our results, proving the high frequency of hybridization of rudd and bleak at the level of gene markers, are of interest.

The studied cases of hybridization of the roach and bream, bleak and rudd are obviously the most common cases of interspecific hybridization within the European cyprinid subfamily Leuciscinae. This tendency is mainly determined by the following two ecological factors. Firstly, they are the most numerous and widespread species of these subfamilies in Europe. Secondly, the spawning grounds and time of these fish overlap strongly. At the same time, intergeneric hybridization is in no way associated with the genetic proximity or remoteness of the crossing species and in this respect is completely random.

Intergeneric hybridization as a phenomenon of the cyprinid family. Intergeneric hybridization is extremely rare in nature, but it is common in the cyprinid family. The ratio of intergeneric hybrids within Cyprinidae is 84 % of the total number of recorded cases of natural hybridization in this family (Scribner et al., 2000). Most of them belong to the European-West Siberian subfamily Leuciscinae and, to a lesser extent, to small cyprinids of North America, genetically close to this subfamily. In the other 10 families of freshwater fishes, for which the natural hybridization was established, only one case of intergeneric hybridization was revealed within Catostomidae (Scribner et al., 2000). The ease and efficiency of intergeneric hybridization in cyprinids is also confirmed by artificial crosses (Nikolyukin, 1952), and the resulting hybrids often reach sexual maturity.

What is the reason for this strange phenomenon? Obviously, the more distant are the species are, the more reliable is the reproductive isolation and less viable and fertile are their hybrids. Usually, intergeneric genetic differences are so great that hybrids can only be obtained in vitro, and they are usually sterile. The ease and prevalence of intergeneric hybridization in European cyprinids, first of all, means that the generic status in this group is overestimated, which is confirmed by both the taxonomic structure of the Leuciscinae subfamily and the insignificant level of genetic differentiation of species in these taxonomic units. The latter circumstance has long attracted the attention of researchers and even became the reason for raising the question of a sharp ambiguity in the rates of molecular evolution in different taxonomic groups (Avisé, 1977). In the system of European cyprinids, monotypization at the genus level is expressed like in no other group of Holarctic fishes. In fact, all morphologically reliably isolated species of these European subfamilies are considered as a separate genus, and most of the recently recognized species are vicarious forms confined to different basins (Kottelat & Freyhof, 2007; Perea et al., 2010). The level

of genetic distances between genera of the subfamily Leuciscinae is drastically low for vertebrates and other freshwater fishes and is in sharp contrast to the differentiation of the East Asian and Trans-Palaearctic genera of this family (Hänfling, 2000; Briolaya et al., 1998; Perea et al., 2010).

According to genetic distances (Hänfling, 2000) and the molecular clock recalculated on this basis, the age of generic radiation in modern Leuciscinae should be estimated at a level from 1.0 to 2.4 million years and dated to the Pleistocene. The divergence of the East Asian genus *Rhodeus* from Europe occurred, apparently, about 4 million years ago. The first case corresponds to the period of formation of species, and the second one — to the genera of modern mammals (Mezhzherin, 1997). However, according to palaeontological data (Kovalchuk, 2017), the radiation of European cyprinid genera occurred in the late Miocene about 10–11 Ma. This contradiction between molecular and paleontological data can be explained by presenting the evolution of cyprinids not as a result of one-time radiation, but as a series of successive speciations that occurred every 2–4 million years. As a result, a number of species were formed with a similar set of morphological characters each time.

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