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SPINED LOACHE SETTLEMENTS STRUCTURE (COBITIDAE) OF THE EASTERN UKRAINE RIVER SYSTEMS AND ALTERNATIVE CHARACTER OF DIPLOID AND POLYPLOID POPULATIONS

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Spined Loache Settlements Structure (Cobitidae) of the Eastern Ukraine River Systems and Alternative Character of Diploid and Polyploids Populations. Mezhzherin, S. V., Kryvokhyzha, D., Tsyba, A. A., Rostovskaya, O. V. — The structure of spined loaches diploid-poliploid complex of *Cobitis* genera representatives as well as *Sabanjeewia aurata* of the Eastern Ukraine river systems were investigated by allozyme analysis and cytometry. In total 39 samples and 1412 specimens from the upper left tributaries of the Middle Dnipro River, the Donets Basin, the coastal rivers of the Sea of Azov and the Lower Dnipro were studied. We identified the representatives of *C.* (superspecies *taenia*) (51.6 % of all researched specimens). *C. melanoleuca* (4.1 %), *S. aurata* (1.1 %), allodiploids *C.* (superspecies *taenia*) × *C. melanoleuca* (0.3 %) and two groups of polyploid hybrids (46.4 %). EET-group includes mainly *C. elongatoides*–2 *taenia* and *C. elongatoides–taenia –tanaitica*. EET-group (2.8 %) is represented only by *C. elongatoides*–2 *tanaitica* biotype. In the Lower Donets and the Lower Dnipro, there is a zone of introgressive hybridization between *C. taenia* and *C. tanaitica*. The structure of settlements differs accordingly to the river basins and has a tendency to decreasing of polyploids frequency respetivly to meridional direction. The analysis of loaches samples from the coastal rivers of the Sea of Azov collected in 1938 and samples from the Middle part of the Donets River in 1971 leads to the conclusion that polyploids in mentioned above regions appeared in 1960–1970s. The structure of diploid-polyploid settlements has an alternative character. More often, there is a predominance of diploids or polyploids. The reason of such bipolarity of settlements could be as reproductive displacement so a relatively strong tolerance of polyploids to anthropogenic climate change.
Key words: spined loaches, *Cobitis*, *Sabanejewia*, hybridization, polyploidy, parthenogenesis, biological invasion, population structure.

Spined loaches are a particular interest for evolutionary and systematics studies as clonally reproducing groups of organisms. These are small fish that are traditionaly represented by two genera *Cobitis* Linnaeus, 1758 and *Sabanejevia* Vladykov, 1929. Using of genetic methods and detailed morphological analysis showed the appearance of great number of earlier unknown and often cryptic species (FishBase, 2017) in both European and East Asian parts of Palearctic. The number of such species can be up to 30–40, showing the contrast with data from XX century, when it was the only one genus and two species (Berg, 1949). Relevance of studies is given by the fact of extensive consistent hybridization of the forms with different ploidy within *Cobitis* (Boroṅ, 2003; Janko et al., 2007 b; Mezhzherin, Pavlenko, 2009) and rare hybridization between *C. taenia* Linnaeus, 1758 and *S. aurata* De Filippi, 1863 (Mezhzherin et al., 2014). In the first case it leads to the appearance of not less than 10 allodiplod biotypes (Janko et al., 2007 b; Mezhzherin, Pavlenko, 2009), reproducing by gynogenesis involving sperm of parental species (Janko et al., 2007 a). As a rule, there are mixed diploid-polyploid settlements the structure of which has a regional specificity caused by the composition of parental species and by the remote location from the centers of polypoid biotypes formation. Such centers currently are the Lower Danube, where *C. elongatoides* Bacescus & F. M. Mayer, 1969 and *C. tanaitica* Bacescus & F. M. Mayer, 1969, inhabit and hybridize with one another as well as the Upper Danube, Oder, and Rein where *C. elongatoides* hybridize with *C. taenia*.

The river systems of the Eastern part of Ukraine are of the particular interest, because they are the southeastern boarder of polyploid loaches distribution. Current knowledge relating to the structure of species communities and genomic instability of populations of the downstream of the Donets River (Mezhzherin, Lisetskaya, 2004) showed that *C. taenia*, *C. melanoleuca* and *S. aurata* are present here. Meanwhile *C. taenia* and *C. melanoleuca* hybridize producing allodiploids however the last ones can take part in producing tetraploid biotype, which is admitted for the Upper Volga (Janko et al., 2007 b). In the Donets Basin, there are triploid biotypes, which are formed through consistent hybridization of *C. elongatoides* and *C. taenia.* An interesting fact about loaches populations of the Donets River is that *C. taenia* and *C. tanaitica* coexist here (Freyhoff, 2011, a, b). All this indicates potentially high level of biotypic diversity of loaches population not only of the Donets River but other rivers of the East of Ukraine. This circumstance as well a fact of significant distancing from the center of polyploid formation stimulates a comprehensive study, the objectives of which is not only to find and to describe diploid-polyploid complexes of loaches in the rivers of Eastern Ukraine, but to discover features of their structure and patterns of their variability in space and time.

Material and methods

Samples of loaches, collected during 2002–2021, formed the basis of the study. Besides, we used earlier published materials (Mezhzherin, Lisetskaya, 2004; Mezhzherin, Verlatiy, 2019). As result, investigation is based on 41 samples and 1412 specimens. The researched populations can be divided into four groups respectively to the main river basins. The Donets River system is presented by 20 samples, which included 653 specimens. The Middle Dnipro basin is presented by seven populations (281 specimens) from the Desna River basin (the rivers Ivotka, Svyga and Seym), the basins of the Vorskla River and Sula River represented by samples from the Kolomak River and Uday River respectively. The small coastal rivers of the Sea of Azov are studied on the example of population of the Berda River (two samples from the Berda River and Karatysh River), the Gruzskiy Yelanchak River, the Kalmius River (directly the Kalmius River and Kalchik River), the Lozovatka River, the Molochna River, the Obitochna River system (sample from the Kiltychyia River). In general, it has been investigated 10 samples and 166 specimens. The Lower Dnipro is presented by two series that included 293 specimens.

The work analyzes the collections of the ichthyological fund of the National Museum of Natural History at the National Academy of Sciences of Ukraine (NNMP NASU). It is about a spined loach series (N 2740) collecting by D. E. Beling in 1938 in the Berda River and Obitochna River and two samples of 1971 (N 1627, 1665) from the Middle Donets.

As a rule, samples of loaches were taken from nature and delivered to the laboratory alive, where we took blood samples for cytometry and muscles for gel electrophoresis. In some cases, fishes were frozen and in this way were delivered to the laboratory.

The identification of species and polypoid biotypes was carried out using allozyme analysis and cytometric method. As diagnostic markers we used three loci: *Aat*-1, *Ldh*-B, *Mdh*-1 that proved their reliability in some similar researches (Šlechtova et al., 2003; Janko et al., 2007 b; Mezhzherin, Pavlenko, 2010). The pool of alleles of these genes allowed to discriminate reliably *C. elongatoides*, *C. taenia*, *C. melanoleuca* specimens and to identify hybrids (table 1). As for polyploid biotypes, we can differ them with 100 % reliability from diploid species due to the fixation of heterozygous genotypes at *Aat*-1, *Mdh*-1A loci that are diagnostic to parental species. By the feature of gene dozage in spectrum, polyploid hybrids divide in to two groups: with one (ETT), two or three (EET) genomes of *C. elongatoides*. For a identification specimens of *S. aurata* and *C. melenoleuca*, as well as possible hybrids with their participation, additionally, other enzyme systems were used. In particular, applied loci *Es*-1, *Es*-2, *Pgm*-1, *Gpi*-1 (Mezhzherin, Lisetskaya, 2004). In addition, some morphological features were also analyzed, primarily the presence, shape and location of melanistic spots on the base of the caudal fin.

Table 1. Allozyme pools of species and hybrid forms of spined loaches of the genus *Cobitis* for three diagnostic loci

Species and bitypes	*Aat*-1	*Ldh*-B	*Mdh*-1A
C. taenia s. l.	cc	ab, bb	bb
C. melanoleuca	aa	cc	aa
C. taenia × C. *melanoleuca*	ac	bc	ab
S. aurata	ac	bc	ab
ENT-group	acc	abb, bbb	abb
ETT-group	aac	bbb	aab

One of the structural muscle proteins encoded by the gene *Pt*-3 allows to identify specimens of two genetically very close species *C. taenia* and *C. tanaitica* which cannot be divided by the standard combination of enzymes. (Šlechtova et al., 2003; Janko et al., 2007 a). In populations of *C. tanaitica* the allele *Pt*-3^{b} is fixed. It is identical to allele of *C. elongatoides* and has lower electrophoretic mobility than allele intrinsic to *C. taenia* and characterized by the dimeric structure. On the electropherogram of polypoid hybrids the products of this locus manifests as a triple spectrum with visible effect of gene dosage (fig. 1). This allows not only to identify species of *C. tanaitica* but also to detect a genome of this species in polypoid set of chromosomes. However, using of this locus has some limitations explained by the fact that for getting an electrophoretic picture of a high quality we need fresh samples of muscles from adult animals and better if it is during the spawning. Due to this locus in the combination of three other alozyme loci five samples were studied: from the river Seym (the Desna River system) within the town of Baturyn (51.33/32.88), the Uday River (the Sula River system) within Pyriatyn towm (50.24/32.50), the river Donets near Kharkiv City (49.99/36.29) and within the Stanytsia Luhanska town (48.67/39.47), and from the Lower Dnipro near Kherson City (46.65/32.61).

Cytomenric analyses was carried out by the standard technique of measuring of erythrocytes size on the coloured blood slides (Shandikov, Krivohizha, 2008).

Sex determination was done by the presence of Canestrini scale — a small bone structure on the pectoral fin.

Results

As a result of biochemical gene marking of loaches population of Eastern Ukraine river systems by the loci *Aat*-1, *Ldh*-B, *Mdh* -1A such species and hybrid forms were identified (table 2). First, this is *C. taenia* s. 1. specimens which can be identified depending on the river system as *C. taenia* s. l., or as *C. tanaitica,* or as introgressive hybrids of these species. Two species *S. aurata, C. melanoleuca* and its allodiploid hybrid with *C. taenia* s. l. are significantly less often. Besides, there are two groups of polypoid biotypes. EET-group is represented by the biotypes with one genome of *C. elongatoides* and two genomes of *C. taenia* or *C. tanaitica* or by their combination. The alternative to previous group is EET-group, whose biotypes have two genomes of *C. elongatoides* and one genome of *C. taenia* or much less often *C. tanaitica*.

The analysis of genetic structure of some samples including locus *Pt*-3 allowed to identify specimens of diploid species and polypoid biotypes with genome of *C. tanaitica* (table 3). As a result, it becomes obvious that in populations of *C. taenia* of the Middle Dnipro and the Upper Donets basins is genetically homogeneous whereas in the Lower Donets and the

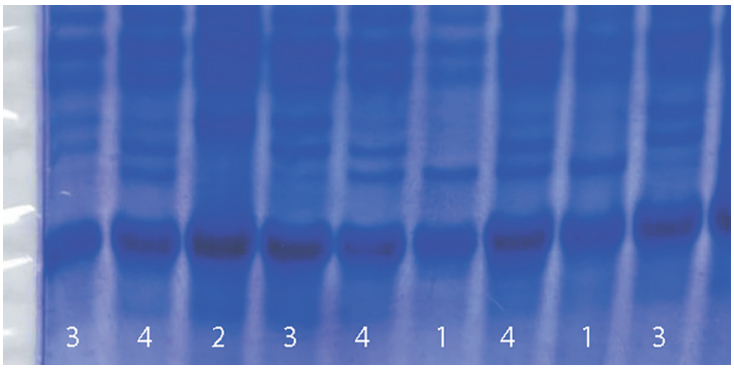

Fig. 1. Electrophoretic variation of the muscles structural proteins of spined loaches. Electromorphs corresponding to genotypes and biotypes: 1 — *Pt*-3^{aa} (*C. taenia*); 2 — *Pt*-3^{cc} (*C. elongatoides*, *C. tanaitica*, *C.* 2 (3) *elongatoides–tanaitica*, *C. elongatoides–C.* 2 (3) *tanaitica*); 3 — *Pt*-3^{acc} (*C. elongatoides–taenia*–1 (2) *tanaitica*); 4 — *Pt*-3^{aac} (*C. elongatoides*–2 (3) *taenia*).

Table 2. Sampling sites, species and biotypes structure of the spined loach settlements of the rivers of Eastern Ukraine on the basis three diagnostic loci

River basins	Rivers/stations	Coordinates	Species and biotypes					
			T	M	A	TM	ETT	EET
Berda	Berda	46.93/36.83	–	–	–	–	19	–
	Karatysh-1	47.31/37.09	18	–	–	–	–	–
	Karatysh-2	?					21	–
Gruzskiy Elanchik	Elanchik -1	38.07/47,12	47	–	–	–	–	–
	Elanchik -2	47.57/38.25	20	–	–	–	–	–
Kal'mius	Kal'mius	47.47/37.89	5	–	–	–	–	–
	Kal'chik	47.40/37.60	27	–	–	–	–	–
Lozovatka	Lozovatka	46.77/36.16	9	–	–	–	–	–
Molochna	Molochnaya	46.97/35.44	4	–	–	–	1	–
Obitochna	Kil'tichia	46.84/36.57	2	–	–	–	11	1
Donets	Aydar	49.00/38.96	–	1	–	–	2	–
	Kamyshevaha	49.11/37.03	–	–	4	–	16	–
	Donets-1	48.58/39.54	11	–	–	–	–	–
	Donets-2	48.65/39.45	56	9	6	3	7	–
	Donets-3	48.77/38.89	7	–	–	–	4	–
	Donets-4	48,88/37.90	–	–		–	1	–
	Donets-5	49.62/36.33	5	30		1	21	–
	Donetz-6	49.94/36.95	–	–	–	–	46	–
	Donets-7	50.17/36.84	–	–	–	–	1	–
	Evsiug-1	48.96/39.16	1	–	–	–	29	–
	Evsiug -2	47.47/37.89	17	–	–	–	1	–
	Zherebetz	?	2	4	–	–		–
	Merla	51.09/36.22	2	–	–	–	1	–
	Merchik	50.08/35.28	26	–	–	–	47	7
	Mzha	49.79/35.86	5	–	–	–	14	–
	Oskol-1	49.10/37.41	3	1		–	15	–
	Oskol-2	49.11/37.40	1	–	–	–	11	1
	Udy	49.96/36.17	1	–	–	–	1	–
	Khar'kiv	50.23/36.40	130			4	84	10
	Chepel	?	–	–	–	–	4	–
Middle Dnipro	Sviga	52.21/33.40	10	–	1	–	34	1
	Ivotka	51.98/33.78	1	–	–	–	6	1
	Kolomak-1	49.82/35.29	15	–	–	–	1	–
	Kolomak-2	49.80/35.25	30	–	–	–	3	–
	Kolomak-3	49.74/35.18	7	–	–	–	6	–
	Uday	?	4	–	–	–	100	1
	Seym	51.34/32,89	21	–	6	–	33	–
Lower Dnipro	Dnipro-1	?	116	–	–	–	–	–
	Dnipro-2	46.63/32.64	155	–	–	–	5	17

Note. T — *C. taenia* s. l.; M — *C. melanoleuca*; A — *S. aurata*; TM — *C. taenia* s. l. × *C. melanoleuca*; ETT — presumably biotypes *C. elongatoides*–2 *taenia*, *C. elongatoides*–3 *taenia*, *C. elongatoides*–*taenia*–*tanaitica* and *C. elongatoides*– *taenia*–2 *tanaitica*; EEN — presumably biotypes *C.* 2 *elongatoides*–*tanaitica* and *C. elongatoides*–3 *tanaitica*.

Lower Dnipro there are introgressive hybrids with approximately equal *C. taenia*/*C. tanaitica* genes ratio. In the Dnipro basin within EET-group predominated trihybrid biotype *C. elongatoides*–*taenia*–*tanaitica* whereas dihybrid biotype *C. elongatoides*–2 *taenia* was a quite rear. Meanwhile in the basin of Donets there is only dihybrid biotype *C. elongatoides*–2 *taenia*. Hybrid polyploids of EET-group are presented by the *C.* 2 *elongatoides*–*tanaitica*.

Table 3. Distribution of biotypes of the genus *Cobitis* spined loache settlements in the rivers of Eastern Ukraine on the basis of four diagnostic loci

Samples	T	N-T			Triploid biotypes			
		Pt-3 genotypes			ENT	ETT	ENN	EEN
		aa	ab	bc				
Seym	16	–	–	–	6	1	–	–
Uday	4	–	–	–	86	8	–	1
Kharkiv	2	–	–	–	–	5	–	–
Donets-2	–	7	28	13	–	4	–	–
Dnipro-2	–	25	54	30	1		1	8

Note. T — *C. taenia*; N-T — introgressive hybrids between *C. taenia* and *C. tanaitica*; ENT — *C. elongatoides–taenia– tanaitica*; ETT — *C. elongatoides–*2 *taenia*; ENN — *C. elongatoides–*2 *tanaitica*; EEN — *C.* 2 *elongatoides–tanaitica*.

Empirical distribution of genotypes of *Pt*-3 locus in the populations of diploid species *C. taenia* s. 1. (table 3) unequivocally corresponds to theoretical one, calculated on the basis of Hardi-Weinberg formula. The situation is true both for the Donets River ($\chi^2 = 1.65$; d. f. = 2; $p > 0.05$) and the Lower Dnipro ($\chi^2 = 0.01$; d. f. = 2; $p > 0.05$) that means unrestricted recombination of the genetic material of parental species in hybrids and their introgressive nature.

The most numerous in the region are representatives of *C. taenia* s. l. (fig. 2). They account for 53.9 % of all individuals. They were found in the most parts of samples of all four basins. *C. melanoleuca* is found in series of samples within the riverbed of the Donets River, where its frequency is 8.3 %. Hybrids *C. melanoleuca* × *C. taenia* s. l. were rare. In general, there were found only four allodiploid hybrid specimens. In the studied samples *S. aurata* was represented by a single or in small series in the rivers Ivotka, Seym and Donets. Polyploid biotypes of ETT-group are the second in frequency and represented within the whole studied region. They account for 38.3 %, meanwhile biotypes EET-group accounts for 2.8 % from the summarised number of studied animals. The last group is found in single specimens or in small series in populations of the Middle Dnipro (the Uday River), the Donets River and the small coastal rivers of the Sea of Azov. In the Lower Dnipro, their frequency is not high as well, but they exceeded in frequency EET-group.

Within the riverbeds, the situation is as follows (table 4). In the tributaries of the Middle Dnipro, about two-thirds are polypoid biotypes ETT-group, when *C. taenia* is one third. In the basin of the Donets polyploids form a relative majority and almost equal to diploids. In the rivers of the Sea of Azov polyploids make up one third and in the basins of Lower Dnipro — only 0.3 %, significantly inferior to alternative biotypes EET-group.

The distribution of diploid and polyploid specimens by the average erythrocyte size shows two overlapping unimodal ranges of values, which correspond to diploid and polyploid (fig. 3). At the same time, about 15 % specimen of the total number get into the transgression zone. According to data getting on the karyotyping material (Vasil'ev, 1985), a size of erythrocytes has to increase by one

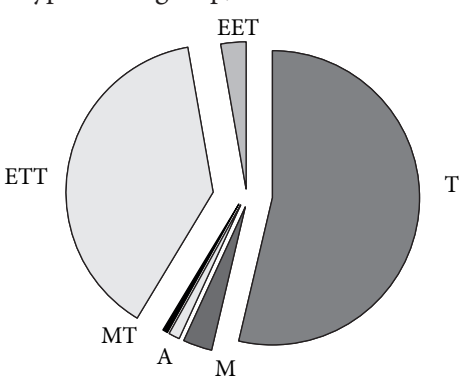

Fig. 2. Ratio of species and hybrid biotypes in summarized sample of loaches from the Eastern Ukraine. Marking: T — *C. taenia* s. l.; M — *C. melenoleuca*; A — *S. aurata*; MT — *C. melanoleuca* × *C. taenia* s. l.; ETT — *C. elongatoides–*2 *taenia*, *C. eleongatiides–taenia–tanaitica*; EET — *C.* 2 *elongatoides–tanaitica*.

Table 4. Frequencies of species and biotypes of the spined loaches in the river basins

Basins	T, T-N	M	A	MT	ETT	EET
Middle Dnipro	0.313	–	0.025	–	0.651	0.004
Donets River	0.413	0.081	0.009	0.006	0.462	0.002
Azov sea rivers	0.714	–	–	–	0.281	0.005
Lower Dnipro	0.980	–	–	–	0.003	0.017

third with increasing the number of each genome. It means that with the modal range of erythrocyte size of diploids within 80–90 units, triploids will have the range within 106–120 and tetraploids within 133–150. In case of, calculation is according to empirical series of polyploids, meanwhile tetraploids include only one specimen. That means that tetraploids are very rare in the settlements of the Sea of Azov and the Donets River.

The average proportion of male within *C. taenia* s. l. populations is 0.32 (n = 502), among *C. melanoleuca* 0.36 (n = 32). All hybrids *C. melanoleuca* × *C. taenia* s. l. were female. The proportion of polyploid specimens with Canestrini scale was 0.01 (n = 300).

The proportion of polyploids and diploids in populations of loaches has distinct bipolar nature and shows an extreme bimodal allocation of frequencies (fig. 4). Most of all there

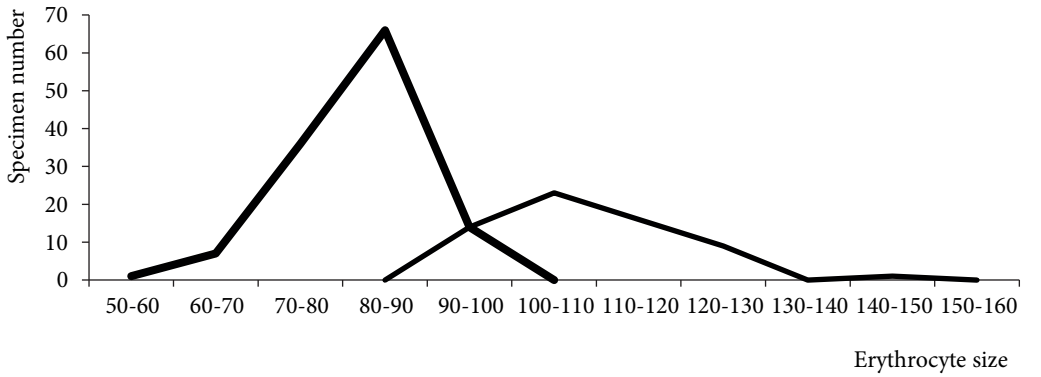

Fig. 3. Distribution of diploid and polyploid loaches due to erythrocyte size in the populations of the Donets and small rivers of the Sea of Azov.

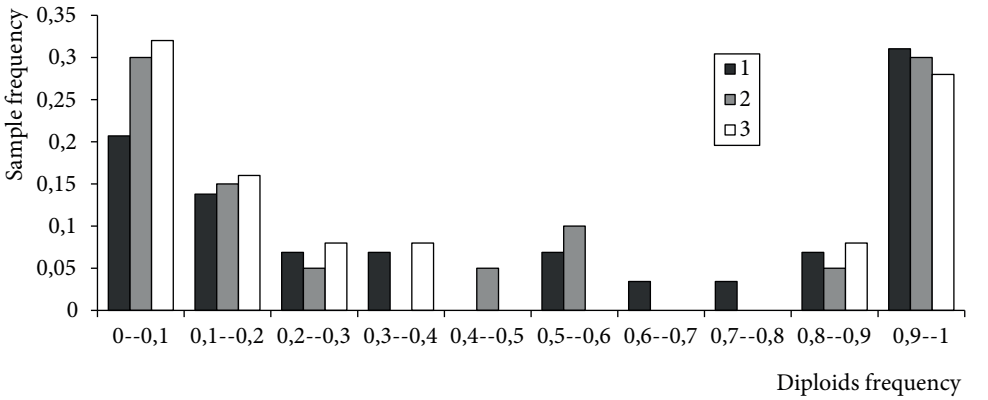

Fig. 4. Distribution of diploid frequency specimens (*C. elongatoides*, *C. taenia*, *C. tanaitica*) within samples: 1 — river systems of the Eastern Ukraine; 2 — the basins of Vistula and Oder rivers (by after Boroń, 2003, Janko et al., 2007 with the addition data from Ukraine); 3 — the Danube basins (after Janko et al., 2007; Lusková et al., 2004 with the addition data from Ukraine).

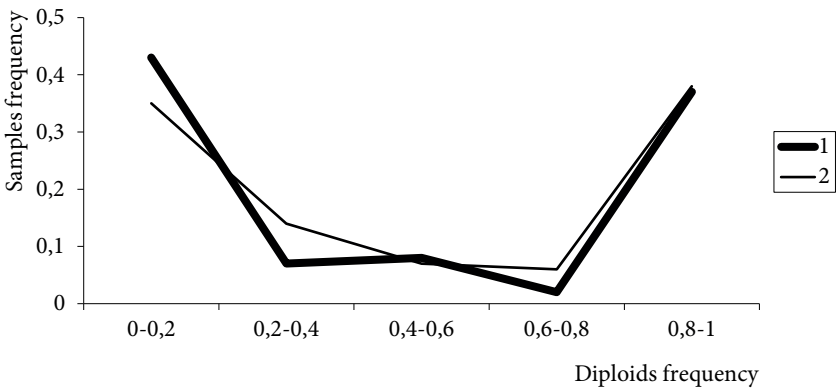

Fig. 5. Distribution of loaches samples of group of species *C. elongatoides, C. taenia, C. tanaitica* by the average ratio of diploids in the river systems of Eastern Ukraine, the Oder, Vistula, and Danube (1) against a similar distribution in the river systems of the Eastern Ukraine.

are populations with diploid predominance or polyploids. Populations with the frequency of diploids from 0 to 0.2 account for 35 % from the total number, and populations from 0.8 to 1 — 38 %. Besides, the cases of total absence of diploids as well as homogeneous diploid populations are not rare. First cases are less common than second ones. The groups with relatively equal ratio of diploids and polyploids (0.4-0.6) have 7 %, groups with small predominance of diploids (0.2–0.4) amounted to 6%, and group with deficit of diploid (0.6–0.8) is equal to 14 %.

The bipolar feature of distribution of diploids and polyploids occurs in populations of loaches of other water basins with a diploid-polyploid settlements. These were in the river systems of the Baltic Sea Basin (the Oder River and the Vistula River) and the Danube River basin (fig. 4). The averaged data for three rivers shows not only an extreme bimodality (fig. 5), but some asymmetric features in distribution of diploids and polyploids in populations. The least frequent are populations with the ratio between 0.6 and 0.8.

In general, in the East of Ukraine there is a tendency of decreasing of polyploids in the south region and predominance of diploid populations (fig. 2). It formally confirms a correlation analysis between latitude of location of samples and the number of diploids there ($r = 0.38$, $n = 41$, $p = 0.01$). Obviously, this is due to the fact that the lower Dnipro and the rivers adjacent to the Sea of Azov are as far as possible from the northern route of invasion (Mezhzherin et al., 2022), by which the biotypes ETT–group and invasion here had limited nature. The invasion nature of polyploid spined aches in the coastal rivers of the Sea of Azov is proved by the collections, stored in the ichtyological fund of NNMP NASU. In the series of loaches ($n = 36$), which were collected in 1938 in the rivers of Berda and Obitochna the proportion of males was 28 %, while in the samples of these basins collected in 2010, males were not found. It means that in the coastal rivers of the Sea of Azov as well as in the basins of the Middle Dnipro (Mezhzherin et al., 2022) in the second part of XX there was the outbreak of populations of all-female polyploids, which was caused by the invasion from the basin of the Middle Dnipro (fig. 6).

Two samples of loaches of 1971 from the basin of Donets, from the suburbs of town Izium (49.21/37.29) are stored in the ichtyological fund of NNPM NANU. The average proportion of males is 13 % ($n = 44$), that corresponds to the proportion of diploids 35–45 % (Mezhzherin et al., 2022). The nearest location of collections of material in 2010 was the mouth of the river Velyka Kamyshevaha (49.11/37.03). The proportion of diploids in this sample of the Donets River is at the level of 11 % ($n = 51$), this is approximately in three times less than it was in 1971.

Fig. 6. Ratio of diploid (grey fill) and popyploid (black fik) specimens in settlements and invasion routs of polyploid spined loaches.

Discussion

The populations of loaches of Eastern Ukraine are characterized by the insignificant predominance of specimen of the only one parental species of *C. taenia* s. l. and the obvious dominance among the polyploids of ETT-biotype. One more species of this genus *C. melanoleuca* is only in the Donets River. In the whole region it is not numerous, but in the Middle Donets it could be predominant among the population of loaches. Hybrids *C. melanoleuca* × *C. taenia* are the results of rare random crosses. They have allodiploid all-female nature and are occurred significantly rare than parental species. It means that intraspecific hybridization is not a factor of depression of rare species by the numerous species, as it is occurred among crucians (Mezhzherin et al., 2014). The absence of allodiploids with genome *C. melanoleuca* is a significant feature, which proves the limited evolution capacity. One more species *S. aurata* occurs sporadically and its hybrids were not found (Mezhzherin et al., 2014) in contrast to other rivers. Inappropriate catching tools for mass catching of such a small fish could explain the latter.

A very important feature of loaches populations of the region is the fact of introgression hybridization between *C. taenia* and *C. tanaitica*. The absence of restrictions for hybridization confirms the correspondence of the observed and expected distribution by the diagnostic loci of *Pt*-3 for these species. The basins of the Lower Donets and Lower Dnipro could be considered as wide hybrid zones or as zones of intergradation of these species. The introgression feature of hybridization is resulted from the close genetic relations of these species and inability to form allodiploid hybrids without genetically distant species *C. elongatoides*.

Minimal differences at the level of nuclear genes (Perdices et al., 2016), easily occuring introgressive hybridization, the absence of diagnostic morphological features give a reason to consider *C. taenia* and *C. tanaitica* as semispecies within the supraspecific complex *Cobitis* (superspecies *taenia*), which was suggested earlier (Mezhzherin, Pavlenko, 2009).

A bipolar character of spatial distribution of diploids and polyploids is of particular interest. This tendency is a characteristic of not only Eastern part of Ukraine but of other zones of cohabitation of diploids and polyploids, which is universal regularity. The reason of such difference between diploid and polyploid populations can be spatial and environmental circumstances, ethological mechanisms, for example competition between diploid and polyploid females for males and different reproductive potential. However, competitive relations between polyploid and diploid females should be considered the most probable factors where polyploids are more successful or a stability of polyploids is higher compared to diploids in the anthropogenically modified ecosystems. Those circumstances could complement each other. A similar situation is between diploid crucian and goldfish *Carassius auratus* with gynogenetic triploid Prussian carp *C. gibelio* (Bloch, 1782) (Mezhzherin et al., 2012), which also have a tendency to make bipolar populations. The difference is that polyploids of loaches have a more powerful potential and occupy an area of diploids (Mezhzherin et al., 2022), while in crucian carp it is the opposite: triploids give way to diploids (Mezhzherin et al., 2015).

The absence of *C. elongatoides* in the study region, which is mandatory in a chromosome set of polyploid loaches and impossibility of hybridization of this species with representatives of *Cobitis* (superspecies *taenia*) with the subsequent formation of aborigine polyploids is the evidence of allochthonous status of polyploid loaches. Basing on the fact that in the first part of XX cent. in the coastal rivers of the Sea of Azov, as well as in the Middle Dnipro (Mezhzherin et al., 2022) polyploid loaches were absent or extremely rare, we can suppose that the high number of them now is a result of expansion which was in 1960s. It was then that hydrological regime of the Donets River was fundamentally violated and *Carrasius auratus* (Mezhzherin et al., 2021) appeared in this region, becoming the most plentiful among the species here. There could be two historical ways. The first: polyploids in a very limited quantity preserved in local populations because of recolonization of rivers after the last glaciation (Janko et al., 2005; Culling et al., 2006). The second way (Mezhzherin et al., 2022) explains the appearance of polyploids in the Eastern part of Ukraine relatively modern invasion into the basins of the Dnipro River through the middle-European invasion corridor, which connects the Rein, Oder, Vistula, Bug, Prypiat, and Dnipro (Bij de Vaate et al., 2002) in 1960–1970s. Later from the left bank tributaries of the Dnipro polyploids of EET-group got into the Upper Donets and started distributing along the river from north to south to the rivers of the cost of the Sea of Azov. Meanwhile the southern corridor where EET-group moved to the East did not take a significant part. It leads from the Lower Danube through Lower Dniester, Southern Bug and Lower Dnipro. The proportion of these biotypes is insignificant here.

In the context of this study, the appearance of polyploids could be considered as a result of cryptic invasion which has happened in the middle of XX cent. This interpretation is more real than the outburst of numbers of local polyploids that got to the basins of the rivers of Eastern Ukraine during postglacial period about 10 thousand years ago. There are some arguments. Firstly, why during recolonization of the rivers of Eastern Ukraine only hybrid forms got there but the main parental species *C. elongatoides* distributed only in the basin of the Danube reaching only the Oder. Secondly, in the East of Ukraine, resettlement was from North to South and while for the postglacial refugiums characterized by distribution in opposite direction. Moreover southern way along the Black Sea coast was not involved. Thirdly, along the main rivers and first-order tributaries in case of cohabitation of diploid and polyploids the last one always dominate (Boroń, 2003; Janko et al., 2007). It means that in the case of recolonization by the middle of the 20th century, the number of polyploids should not be less than the number of diploids. This could lead to a deficit of males that we cannot see in the samples of 1938. Fourthly, there is a mosaic of populations in the coastal rivers of the Sea of Azov, where in one river the population consists of polyploids, and in the other — of diploids. Such type of populations of loaches could be

considered as a consequence of a founder effect. It arose because of recent invasions, and not as a population structure that has developed over the years.

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