

Triploid forms' karyotypes of spined loaches from the genus *Cobitis* (Actinopterygii: Cypriniformes: Cobitidae) of the upper Dnieper and Western Dvina rivers: Analysis of the triploids' origin

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Abstract

Unisexual forms of lower vertebrates (fish, amphibians, and reptiles) reproduced by parthenogenesis, gynogenesis, or hybridogenesis are represented by diploids, triploids, or tetraploids, whose origin is associated with interspecific hybridization. Among fish species, the highest variability of unisexual polyploids was found in the genus *Cobitis*. The structure of their genomes and putative parental species holds great interest for the investigation in association with questions about possible evolutionary success. In particular, it serves to elucidate the possible high colonization properties of a few polyploid forms, in contrast to the local history of rather numerous hybrid forms with a limited distribution. Therefore, the aim of this study was to describe the karyotype structure of two newly discovered triploid forms of the genus *Cobitis*, to analyze their origin and putative parental species. The karyotype structure of 182 spined loach individuals from the Western Dvina River and 91 individuals from the upper Dnieper River of the Smolensk District of Russia was studied. A total of 121 studied individuals from the Western Dvina comprised triploid females with a chromosome number 74 and karyotype consisting of 13 meta-, 39 submeta-, and 22 subtelo-acrocentric chromosomes. Among loaches collected in the upper Dnieper River, 42 triploid females were found with 74 chromosome number including 23 meta-, 26 submeta-, and 25 subtelo-acrocentric chromosomes. Other individuals from both localities were karyologically identified as *Cobitis taenia* Linnaeus, 1758 with $2n = 48$. The triploid form of spined loaches of the Western Dvina River most likely arose as a result of the hybridization of *Cobitis tanaitica* Băcescu et Mayer, 1969 and *C. taenia*. The range of *C. tanaitica*, whose karyotype is characterized by an evolutionarily fixed Y-autosomal translocation, is limited to the rivers of the northern coast of the Black Sea. Therefore, hybridization probably happened in late Pleistocene in the Dnieper River system, where both parental species occur. The triploid form that arose here is unique for the Baltic Sea basin. Probably, it colonized the Western Dvina through the artificial Berezinskaya water system (Berezina Canal = Daugava–Dnieper Canal), but at the same time it was forced out of its area of origin by other triploid forms which are now widespread there. According to the karyotype structure, the triploid form, common for both the upper and lower reaches of the Dnieper, has a trihybrid origin, with probable hybridization of *Cobitis elongatoides* Băcescu et Mayer, 1969, *C. tanaitica*, and yet unidentified species *Cobitis* sp. Both studied triploid forms are parts of unisexual-bisexual complexes, in which their putative diploid maternal species (*C. tanaitica* and *C. elongatoides*) are absent, and the role of the host species involved in reproduction belongs to *C. taenia*.

Keywords

hybrid origin, parental diploid species, polyploidy, unisexual-bisexual complexes

Introduction

About 90 unisexual forms reproducing by parthenogenesis, gynogenesis, or hybridogenesis are known among fish, amphibians, and reptiles at present. These forms can be diploid, triploid, and tetraploid; however triploid unisexual forms are much more widespread, while tetraploids are extremely rare. The genesis of unisexual forms is associated with interspecific hybridization, while triploid and tetraploid forms can have not only dihybrid, but even trihybrid origin (see Vasil'ev et al. 1989, 2011; Vrijenhoek et al. 1989).

In contrast to parthenogenesis in several reptiles (see Vrijenhoek et al. 1989), natural gynogenetic reproduction of unisexual forms requires males whose sperm stimulates egg development without fertilization. Therefore, gynogenetic fishes and amphibians are found together with one or two related bisexual species, the hybridization of which led to their origin. Rarely, clonal gynogenes reproduce with less related bisexual species. Thus, gynogenetic reproduction of unisexual forms results in the formation of unisexual-bisexual (clonal-bisexual, diploid-polyploid) complexes. Such complexes have been found in fish from the genera *Poecilia*, *Poeciliopsis* (Poeciliidae), *Fundulus* (Fundulidae), *Menidia* (Atherinopsidae), *Chrosomus*, *Carassius*, *Squalius* (Cyprinidae), *Misgurnus* and *Cobitis* (Cobitidae) (see Vasil'ev 1985; Vrijenhoek et al. 1989; Dawley et al. 2000; Lamatsch and Stöck 2009; Arai and Fujimoto 2013).

The first clonal-bisexual (diploid-polyploid) complex in the genus *Cobitis* was noted in 1981 in the Volga River basin (Vasil'ev and Vasil'eva 1982; Vasil'ev et al. 1989). Further studies revealed the wide distribution of diploid-polyploid loach complexes in the Volga River basin, as well as in the rivers of the Black, Baltic, and North seas (Vasil'ev 1990; Boroń 1992, 1995, 1999, 2003; Ráb and Slavik 1996; Vasil'eva and Vasil'ev 1998; Boroń and Danilkiewicz 1998; Bohlen and Ráb 2001; Bohlen et al. 2002; Vasil'ev et al. 2005, 2007, 2011; Janko et al. 2007, 2012; Choleva et al. 2008; Buj et al. 2008; Mezherin et al. 2022). The structure of these complexes varies, but their obligatory elements are at least one all-female form and one diploid bisexual species (host), which is a sperm donor for gynogenetic reproduction. The most common complexes include a diploid and/or triploid unisexual form and one diploid bisexual species (Vasil'ev et al. 2011). The high variability of unisexual forms in *Cobitis* observed in ploidy levels and in karyotype structure is the result of different combinations of haploid sets from several diploid bisexual species distributed in European waters. According to various genetic studies, the presumed parental species for the unisexual forms are as follows: *Cobitis taenia* Linnaeus, 1758, distributed in the North Atlantic basin from France and Great Britain to Russia; *Cobitis melanoleuca* Nichols, 1925 widespread from the Volga River system to the waters of the Western Pacific in the Far East and China; the Black Sea species *Cobitis tanaitica* Băcescu et Mayer, 1969, *C. taurica* Vasil'eva, Vasil'ev, Janko, Ráb et

Rábová, 2005, and *C. pontica* Vasil'eva et Vasil'ev, 2006; *Cobitis elongatoides* Băcescu et Mayer, 1969, common in the Danube basin; *C. strumicae* Karaman, 1955, recorded in the basins of the Aegean and southwestern Black Seas (Vasil'ev et al. 1989, 2011; Ráb et al. 2000; Bohlen et al. 2002; Boroń 2003; Janko et al. 2005a, 2007, 2018; Choleva et al. 2008). It is assumed that their ranges fluctuated during the Quaternary (Janko et al. 2005a; Culling et al. 2006) and overlapped in Central Europe, the Lower Danube basin, and southern Ukraine (Janko et al. 2007). Hybrid unisexual forms that arose in the mentioned hybrid zones achieved outstanding evolutionary successes and colonized most of the European continent (Janko et al. 2018). However, the observed diversity of unisexual genotypes suggests a local history of bisexual-unisexual complexes (Bohlen and Ráb 2001; Vasil'ev et al. 2011). That is why further work on the discovery of new complexes, the study of their origin and distribution in our opinion holds great interest for the investigation.

In this study, we describe the karyotypes of two triploid forms found in the Western Dvina and Upper Dnieper rivers, respectively, and analyze their origin and putative parental species.

Materials and methods

Materials for karyological research were collected in June 2003 and June 2005 in the Western Dvina River (vel Zapadnaya Dvina vel Daugava) at Velizh City, Smolensk District of Russia, 55°36'N, 031°12'E, and in June 2003 and June 2005 in the Upper Dnieper at Bilino village, Smolensk District of Russia, 55°13.4'N, 033°28.7'E (Fig. 1). In total, 182 spined loach individuals were studied karyologically from the first locality, and 91 individuals from the second. The voucher specimens were deposited in the collection of the Zoological Museum of Moscow State University under the numbers P-21434, P-21800, P-21801, P-21805, P-23738, P-24010. All individuals were injected with about 0.05 mL colchicine solution (0.3%–0.4%). After 4 h cells of anterior part of kidney were used for chromosomal slides prepared according to Vasil'ev (1978). The study was performed using anesthesia of fishes with a solution of MS-222. Metaphase chromosomes stained in 4% Giemsa solution (pH 6.8) were counted with PC software Quick Photo Micro. From 3 to 5 metaphase plates were analyzed for each studied fish. The chromosomes were classified according to Levan et al. (1964). Photos of metaphase chromosomes were taken with a Leica DFC 295 digital camera. For a comparative analysis, we used previously published data on loaches collected in the Lower Dnieper and Dnieper estuary (Vasil'ev 1990).

Results

Among the karyotyped spined loaches of the Western Dvina River, 44 (63.8%) were identified as triploid females



Figure 1. Localities of sampling of triploid loaches of the genus *Cobitis*: 1 = Western Dvina (=Daugava), 2 = upper Dnieper.

in the sample collected in 2003 and 77 (68.1%)—in the sample collected in 2005. These triploids had 74 chromosomes, including 13 meta-, 39 submeta- and 22 subtelo- and acrocentrics (Table 1, Fig. 2). Other fishes were karyologically identified as *C. taenia*. The karyotype of this species was described earlier (Vasil'ev and Vasil'eva 1982; Vasil'ev et al. 1989) and is shown in Table 2. Karyotyped loaches of the Upper Dnieper included 22 triploid females (46.8%) in the sample collected in 2003 and 20 (45.9%)—in the sample collected in 2005. Their karyotype included 74 chromosomes: 23 meta-, 26 submeta- and 25 subtelo- and acrocentric (Table 1, Fig. 3). Other spined loaches found in this location belonged to *C. taenia*.

Discussion

Triploid form in the Western Dvina basin. According to the number of chromosomes (Fig. 2A), the karyotype of this triploid form includes two haploid sets of 50-chromosome species and one haploid set of 48-chromosome species. *Cobitis taenia* Linnaeus, 1758 is the only 48-chromosome species among the karyotyped European spined loaches (Table 2). After removing the haploid set of *C. taenia* with 5 meta- (m), 9 submeta- (sm) and 10 subtelo- and acrocentric (sta) chromosomes (Fig. 2B) from the triploid karyotypes, the remaining chromosomes of the triploid form of the Western Dvina ($8\text{ m} + 30\text{ sm} + 12\text{ sta}$) correspond fairly well to the sum of

Table 1. The karyotype structure of the studied triploid forms, their putative parental diploid species of *Cobitis* and diploid species coexisting with triploids.

River system	Chromosome number	m	sm	sta	Putative parent species	Coexisting diploid species
Western Dvina	74	13	39	22	2 <i>C. tanaitica</i> – <i>C. taenia</i>	<i>C. taenia</i>
Upper Dnieper	74	23	26	25	<i>C. elongatoides</i> – <i>C. tanaitica</i> – <i>Cobitis</i> sp.	<i>C. taenia</i>

m = meta-, sm = submeta-, sta = subtelo- and acrocentric chromosomes.

Table 2. The karyotype structure of diploid spined loach species of the genus *Cobitis* apparently participated in the origin of polyploid forms.

Species	River system	Chromosome number	m	sm	sta	Reference
<i>C. taenia</i>	Volga, Dnieper, Dniester, south Bug, Elbe, Weser, Vistula, Odra	48	10	18	20	Vasil'ev and Vasil'eva 1982; Vasil'ev et al. 1989; Vasil'eva and Vasil'ev 1998; Ráb and Slavík 1996; Ráb et al. 2000; Boroń 2003
<i>C. taenia</i>	Odra	48	10	20	18	Bohlen et al. 2002; Janko et al. 2007
<i>C. taenia</i>	Vistula	48	12	18	18	Boroń 1995, Boroń 1999, Boroń 2003
<i>C. elongatoides</i>	Elbe, Danube, Odra, Tisza	50	30	16	4	Ráb et al. 2000; Boroń and Kotusz 2000 (as <i>C. danubialis</i>); Lusk et al. 2003
<i>C. elongatoides</i>	Odra	50	28	18	4	Boroń 2003
<i>C. elongatoides</i>	Danube, Kamchya, Elbe, Odra	50	22	26	2	Janko et al. 2007
<i>C. tanaitica</i> , females	Dnieper estuary	50	8	24	18	Vasil'ev 1990
<i>C. tanaitica</i> , males		49	9	24	16	
<i>C. tanaitica</i> , females	Don, Dnieper, Dniester, Kuban	50	8	28	14	Vasil'ev 1995; Vasil'eva and Vasil'ev 1998; Vasil'ev et al. 2007
<i>C. tanaitica</i> , males		49	9	28	12	
<i>C. tanaitica</i> ^A p. 395	Danube, Odra, Sinoe, Don	50	10	24	16	Janko et al. 2007
<i>C. tanaitica</i> ^A Fig. 1c		50	10	26	14	
<i>C. tanaitica</i> ^A	Danube, Sinoe	50	10	26	14	Majtánová et al. 2016
<i>C. tanaitica</i> ^A	Danube	50	10	22	18	Bohlen et al. 2002
<i>C. taurica</i>	Crimea	50	10	30	10	Janko et al. 2005b
<i>C. pontica</i>	Veleka	50	10	30	10	Janko et al. 2005b; Vasil'eva and Vasil'ev 2006
<i>C. taurica</i> ^A	Crimea, S. Bug, Veleka	50	10	30	10	Janko et al. 2007
<i>C. melanoleuca</i>	Volga	50	8	18	24	Vasil'ev and Vasil'eva 1982; Vasil'ev et al. 1989
<i>C. strumicae</i>	Kamchya, Jantra	50	10	20	20	Janko et al. 2007

^A Species identification is provided by Bohlen et al. 2002 and Janko et al. 2007 (see comments in the text); m = meta-, sm = submetacentric, sta = subtelocentric and acrocentric chromosomes.

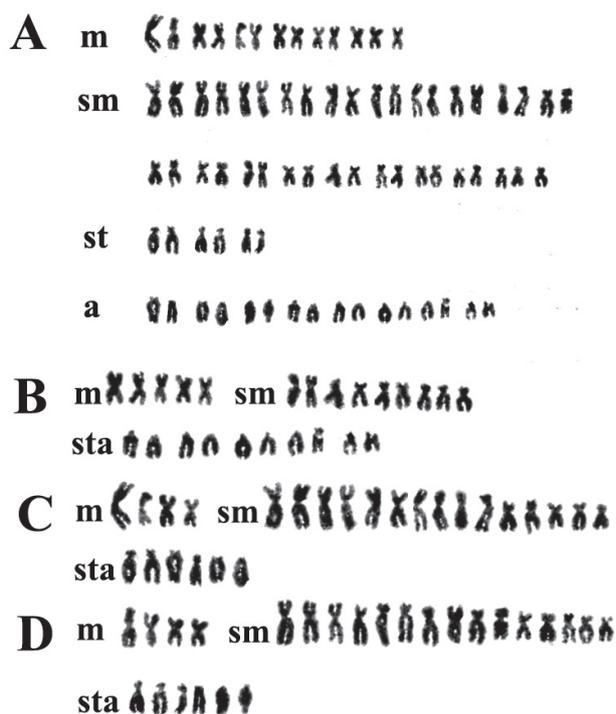


Figure 2. Karyotype of triploid form of the genus *Cobitis* from the Western Dvina River (A) and haploid sets of the putative parental species involved: B, *C. taenia*; C, *C. tanaitica*, female; D, *C. tanaitica*, male; m = metacentric, sm = submetacentric, sta = subtelocentric, a = acrocentric chromosomes.

the haploid set of *C. tanaitica* females (4 m + 15 sm + 6 sta) and the male of the same species (4 m + 15 sm + 6 sta) (Fig. 2C, D). Differences in the ratios of submeta- /

subtelocentrics may be associated with a difference in the classification associated with various degrees of spiralization of chromosomes in the analyzed triploids or diploid species. Other karyotyped 50-chromosome species are unsuitable as putative parental species: they have a higher number of metacentrics and differ in sm/sta ratio (Table 2). Accordingly, the proposed scheme of the origin of this triploid form should include the following stages: 1) hybridization of a female *C. tanaitica* and a male *C. taenia*; 2) hybridization between a hybrid *C. tanaitica* × *C. taenia* and a male *C. tanaitica*. This scheme is due to the unique karyotype of *C. tanaitica*, characterized by an evolutionarily fixed Y-autosomal translocation (Vasil'eva and Vasil'ev 1998), leading to differences in the number of chromosomes in males and females (Table 2). It is also confirmed by our preliminary results that demonstrated a close relation between the triploids of the Western Dvina and *C. tanaitica* from the Don River based on the variability of the cytochrome *b* gene (Vasil'ev et al. 2005).

In this line, it is necessary to discuss the differences observed in the structure of the karyotype of *C. tanaitica* in our studies and in publications of other authors (Bohlen et al. 2002; Janko et al. 2007). Previously, we studied the karyotype of *C. tanaitica* (the species was originally described from the Don River basin) based on materials, collected from 7 populations of the Black Sea and Sea of Azov basins: Don River at Rogozhkino (47°10.367'N, 039°20.8'E), Obitochnaya River (about 46°40.217'N, 036°11.8'E), Beisug (46°2.533'N, 038°34.717'E), Kalka River, tributary of the Kal'chik River (47°7'N, 037°36.75'E), Kazachii Erik, Kuban River system (45°18.467'N, 037°14.567'E), Dniester River at Mayaki (46°24.733'N, 030°16.383'E),

Dnieper estuary (46°32.333'N, 032°3.967'E) (Vasil'eva and Vasil'ev 1998). A total of 50 individuals were studied, all of them had a characteristic karyotype with $2n = 50$ with 8 metacentrics in females and $2n = 49$ with 9 metacentrics (an unpaired large chromosome is noticeably prominent) in males (Vasil'ev 1990, fig. 1a–b; Vasil'ev 1995, fig. 1). All studied specimens, as well as non-karyotyped fishes from the same samples and fishes from the south Bug system (Savranka River) were morphologically identified and described as a new species *Cobitis rossomeridionalis* Vasil'eva et Vasil'ev, 1998, a junior synonym of *C. tanaitica*. This species differed from *C. taenia* sympatrically found in the Kalka and Obitochnaya rivers, Dnieper estuary, and Dniester River at Mayaki both in the karyotype structure (36 individuals were karyotyped) and morphological features (Vasil'eva and Vasil'ev 1998).

Bohlen et al. (2002) presented the karyotype of *C. tanaitica*, which they isolated from the chromosome set of the tetraploid specimen ($4n = 100$) from the Danube River in Germany. They proposed that the genome of this tetraploid includes 3 haploid sets of *C. elongatoides* and 1 set of *C. tanaitica*. Janko et al. (2007) indicated that they karyotyped or examined by flow cytometry (?) 8 specimens of *C. tanaitica* from the Odra River, 4 specimens from the Danube River, 6 specimens from the Lake Sinoe, and 9 specimens from the Don River (Janko et al. 2007, Table 1). They stated that a biotype corresponding to *C. tanaitica* ($2n = 50$, 10 m + 24 sm + 8 st + 8 a) was found in both sexes in the lower Danube River, upper Odra River, and in the Don River (p. 395); this karyotype was presented by the authors in Fig. 1C. However, the presence of diploid bisexual *C. tanaitica* specimens has never been confirmed in the Odra basin despite the fine-scale intensive researches (Janko et al. 2012; Kotusz et al. 2014). The karyotypes proposed by Bohlen et

al. (2002) and Janko et al. (2007) are similar in structure, but differ significantly from the karyotype described by us from the confirmed range of *C. tanaitica* not only in the absence of sex differences, but also in the higher number of metacentric (Table 2) and acrocentric chromosomes: 8 acrocentrics versus 4–6 in males and females from the Sea of Azov basin. Janko et al. (2003, 2007) have never confirmed that the studied fish belong to *C. tanaitica* according to the accepted morphological diagnostic characters (Vasil'eva and Vasil'ev 1998; Vasil'eva 2004; Kottelat and Freyhof 2007). Most likely, Janko et al. (2007) studied the karyotype of some other spined loach species, found only in the lower reaches of the Danube and in Lake Sinoe, a lagoon in northern Dobrudja, Romania, in the Danube Delta. Indeed, Bohlen and Ráb (2001) noted that only specimens from Sinoe Lake were karyotyped. It should also be noted that during two years of our field work in the Danube Delta and in the adjacent territories of Ukraine (2010–2011) we did not find a single specimen of *C. tanaitica*. The spined loaches in our samples from the Danube system were mainly represented by triploids ($3n = 75$), a small number of tetraploids ($4n = 100$), and diploid males and females with $2n = 50$, which were identified as *C. elongatoides* according to our preliminary DNA studies (unpublished). Therefore, in association with the foregoing, in this paper we designate the diploid loach species found in the lake Sinoe, in the Odra and the lower reaches of the Danube with $2n = 50$, 10 m, 22–26 sm, 14–18 sta as *Cobitis* sp.

Among the described triploid karyotypes, the fishes of the Western Dvina are most similar to the fishes of the Oława (Odra River system) and Bug (Vistula River system) rivers. They also have $3n = 74$ with a low number of metacentric and a high number of submetacentric chromosomes (Table 3). It has been suggested that the

Table 3. The karyotype structure of previously studied triploid forms, their putative parental diploid species of *Cobitis* (as identified in the cited publication) and diploid species coexisting with triploids.

River system	Chromosome number	m	sm	sta	Putative haploid sets of parental species	Coexisting diploid species	Reference
Odra (Oława)	74	16	36	22	2 <i>Cobitis</i> sp. ($2n = 49$)– <i>Cobitis</i> sp. 1 ($2n = 50$)	$2n = 49$ (19 m + 18 sm + 12 sta)	Boroń and Kotusz 2000
Vistula, Bug	74	18	33	23	<i>C. taenia</i> –2 <i>Cobitis</i> sp. ($2n = 50$, 12 m + 24 sm + 14 sta)	<i>C. taenia</i>	Boroń 1999, 2003
Lower Dnieper	74	23	25	26	<i>C. taenia</i> –2 <i>Cobitis</i> sp.	<i>C. tanaitica</i> ^A	Vasil'ev 1990
Volga	74	23	27	24	<i>C. taenia</i> –2 <i>Cobitis</i> sp. or <i>C. taenia</i> – <i>C. tanaitica</i> – <i>Cobitis</i> sp. 1 or <i>C. taenia</i> – <i>C. elongatoides</i> – <i>Cobitis</i> sp. 2	<i>C. taenia</i> , <i>C. melanoleuca</i>	Vasil'ev and Vasil'eva 1982; Vasil'ev 1990; Vasil'ev et al. 2011
Vistula, Odra	73	24	27	22	2 <i>C. taenia</i> – <i>C. elongatoides</i>	<i>C. taenia</i>	Boroń 2003
Odra (Polska Woda)	74	24	36	14	2 <i>C. elongatoides</i> – <i>C. taenia</i>	<i>C. elongatoides</i> ^{AA}	Boroń and Kotusz 2000
Vistula, Odra	74	21	31	22	<i>C. taenia</i> –2 <i>Cobitis</i> sp.	<i>C. taenia</i> , <i>C. taenia</i> × <i>C. elongatoides</i>	Boroń 2003
Elbe (Pšovka creek)	74	35	25	14	2 <i>C. elongatoides</i> – <i>C. taenia</i>	<i>C. elongatoides</i> , <i>C. taenia</i>	Ráb et al. 2000
Odra	74	33	27	14	<i>C. taenia</i> –2 <i>C. elongatoides</i>	<i>C. elongatoides</i>	Boroń 2003
Danube (Dyje River)	75	38	29	8	2 <i>C. elongatoides</i> – <i>Cobitis</i> sp.1	<i>C. elongatoides</i>	Ráb et al. 2000
Tisza basin	75	35	27	13	2 <i>C. elongatoides</i> – <i>C. tanaitica</i>	<i>C. elongatoides</i>	Lusk et al. 2003
Odra (Polska Woda)	75	24	36	15	2 <i>C. elongatoides</i> – <i>C. taenia</i> or <i>Cobitis</i> sp.	<i>C. elongatoides</i>	Boroń and Kotusz 2000; Boroń 2003
Odra (Polska Woda)	75	24	35	16	2 <i>C. elongatoides</i> – <i>C. taenia</i> or <i>Cobitis</i> sp.	<i>C. elongatoides</i>	Boroń 2003

^AIdentified as *Cobitis* sp., but not the same as suggested in the triploid genome; ^{AA} identified as *C. danubialis* Nalbant, 1993. m = meta-, sm = submeta-, sta = subtelo- and acrocentric chromosomes.

genome of triploids of the Vistula basin includes a haploid set of *C. taenia* and two haploid sets of an unknown species with $2n = 50$, 12 m + 24 sm + 14 sta (Boroń 1999). Taking into account the different degree of chromosome spiralization, we can assume the already mentioned *Cobitis* sp. as the second parental species for this triploid. The same dihybrid origin can be assumed for the triploids of the Odra River. Boroń and Kotusz (2000) revealed in the Odra River a diploid spined loach with $2n = 49$ (19 m + 18 sm + 12 sta) and primarily identified it as a separate undescribed species, parental to sympatric triploids. However, according to the described karyotype structure, this diploid form should be considered as a hybrid of *C. taenia* and *C. elongatoides* (see Table 2), as Boroń (2003) later concluded; both species are known from the Odra system (Boroń 2003).

Thus, the triploid form of the Western Dvina differs from other triploids found in the Baltic Sea basin (Odra, Vistula) by the karyotype structure and the supposed origin; this form also differs from other triploids revealed in various European river systems (Table 3). Judging by the present-day distribution of its parental species, *C. taenia* and *C. tanaitica*, the triploid form did not originate from the Baltic Sea basin; its origin, of course, should be associated with the Black Sea basin and dated to the Late Pleistocene according to Culling et al. (2006).

It is well known that the recent freshwater fish fauna of the Baltic Sea is one of the youngest. The Baltic Sea depression was covered with ice during the last glacial advance in the Pleistocene and was filled with fresh water at the end of the Quaternary after the retreat of the ice sheet, starting about 13 thousand years ago (Reid and Orlova 2002). Berg (1949) associated the genesis of southern fish forms in the Baltic zoogeographic province with their northward dispersal after the retreat of the ice. However, Reid and Orlova (2002) concluded that much of the current biological diversity in the Baltic Sea reflects relatively recent colonization of non-native species, intentionally or unintentionally introduced by human activities. In our case, an important transport artery connecting the Western Dvina with the Dnieper basin was the Berezinskaya water system (Berezina Canal)—a former artificial waterway that connected the Berezina River (tributary of the Dnieper) with the Western Dvina on the territory of the Vitebsk region of (the present day) Belarus, built within 1797–1805. The total length of this system was 169 km, it had 14 locks and connecting and bypass canals with a length of about 24 km. The system was opened to traffic in 1805; timber rafting continued until the early 1950s; but at present, the canals are impassable due to blockages (Snytko et al. 2016). As follows from the current distribution of polyploid forms of spined loaches in the river systems of Europe, after penetrating the Baltic Sea basin (about 13 000–200 years ago), the analyzed triploid form disappeared from its natural range. Probably, it was replaced by another triploid form, which is now widespread in the upper Dnieper basin.

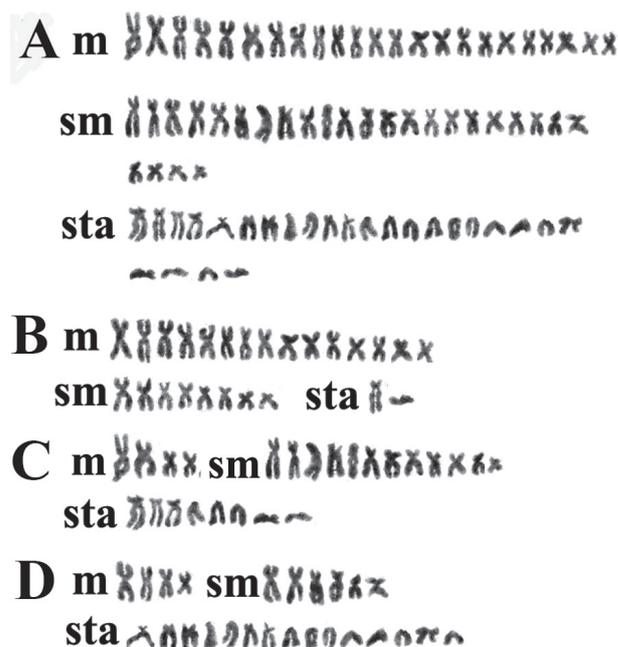


Figure 3. Karyotype of triploid form of the genus *Cobitis* from the upper Dnieper River (A) and haploid sets of the putative parental species involved: B, *C. elongatoides*; C, *C. tanaitica*, male; D, *Cobitis* sp.; m, metacentric, sm, submetacentric, sta, subtelo- and acrocentric chromosomes.

Triploid form in the upper Dnieper. The karyotype structure of triploid females in the upper Dnieper basin indicates its trihybrid origin (Fig. 3A). A similar karyotype structure was found in triploid forms from the Lower Dnieper and Volga systems (Table 3). Since their genomes consist of 74 chromosomes, one of the parental species must have 24 chromosomes in the haploid set. According to the first hypothesis of the origin of studied triploids, *C. taenia* can be such a parental species. After the removal of its haploid set, the remaining chromosomes of triploids (18 m + 16–17 sm + 15–16 sta) suggest that the other parental species was a diploid spined loach with a high number of metacentrics and a low number of uni-armed chromosomes; the only corresponding karyotyped species is *C. elongatoides* (Fig. 3B, Table 2). The rest of the chromosomes (4–7 m + 4–8 sm + 13–15 sta) are similar to haploid sets of spined loach species common in the Caucasus (Eastern Black Sea region and Caspian basin) (Vasil'ev 1995; Vasil'eva et al. 2021): *Cobitis saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh et Esmaili, 2017, *Cobitis satunini* Gladkov, 1935, *Cobitis derzhavini* Vasil'eva, Solovyeva, Levin et Vasil'ev, 2020, and populations requiring identification. It should be noted that our long-term studies in the Caucasus showed the absence of polyploid loaches in this area (Vasil'eva et al. 2021). *C. taenia*, *C. elongatoides* and unknown species *Cobitis* sp. were previously assumed as parental species for the triploid form, widespread in the Volga River basin (Vasil'ev et al. 2011). *C. taenia* was confirmed as its maternal species by our preliminary study of the variability of the cytochrome b

gene (Vasil'ev et al. 2005). At the same time, triploids from the Lower Dnieper basin showed pronounced divergence from both *C. taenia* and triploids of the Volga system and were closer to *C. tanaitica* and triploids from the Western Dvina basin (Vasil'ev et al. 2005).

According to the second hypothesis of the origin of triploids from the Dnieper, the parental diploid with 24 chromosomes in the haploid set may be a male of *C. tanaitica*. After removing its haploid set (Fig. 3C), the remaining genome of triploids should include 19 m, 14 sm, 17 sta. And after further removal of the haploid set of *C. elongatoides* (Fig. 3B), the resulting set with 4 m + 6 sm + 15 sta (Fig. 3D) will be similar to haploid sets of spined loaches from the Caucasus, as in the previous hypothesis. Based on our preliminary mtDNA data (Vasil'ev et al. 2005), we suggest that the most probable origin of the Dnieper triploids is via hybridization of *C. elongatoides*, *C. tanaitica*, and *Cobitis* sp., with the first species as the maternal form. Anyway, this triploid form undoubtedly arose in the water bodies of the northwestern part of the Black Sea basin, where *C. elongatoides*, currently found in the Danube basin, could meet the Caucasian *Cobitis* sp., as well as *C. tanaitica* (or *C. taenia*), living in northeastern Europe. The strong competition and deep northward dispersion observed for this triploid form may explain the absence of the putative maternal species in diploid-triploid complex, where the role of the host species belongs to *C. taenia*.

Conclusions

The triploid form of spined loaches of the Western Dvina River most likely arose as a result of the

hybridization of *Cobitis tanaitica* and *C. taenia*. Since the range of *C. tanaitica*, whose karyotype is characterized by an evolutionarily fixed Y-autosomal translocation, locally distributed in the rivers of the northern coast of the Black Sea, hybridization probably happened in the Dnieper River system, where both parental species occur. The triploid form that arose here, unique for the Baltic Sea basin, probably colonized the Western Dvina through the artificial Berezinskaya water system (Berezina Canal), but at the same time it was forced out of its area of origin. According to the karyotype structure, the triploid form, common both in the upper and lower reaches of the Dnieper, has a tri-hybrid origin, with probable hybridization of *C. elongatoides*, *C. tanaitica* and an unknown species *Cobitis* sp. Both studied triploid forms are parts of unisexual-bisexual complexes, in which their putative diploid maternal species (*C. tanaitica* and *C. elongatoides*) are absent, and the role of the host species involved in reproduction belongs to *C. taenia*. This phenomenon can be explained by the strong ecological competition of the unisexual form and its maternal species, as well as the deep northward dispersion observed for the triploid forms of the Western Dvina and upper Dnieper basins, where maternal species cannot exist due to their more southerly origin.

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