Zoodiversity, **58**(2):129–136, 2024
DOI 10.15407/zoo2024.02.129

UDC 597.551.2(477.8:477.4)

SPATIAL SEGREGATIN OF DIPLOID AND POLYPLOID SPINED LOACHES, *COBITIS ELONGATOIDES–TAENIA–TANAITICA* (CYPRINIFORMES, COBITIDAE), IN RIVER SYSTEMS OF WESTERN AND CENTRAL UKRAINE

S. V. Mezhzherin*, A. O. Tsyba & O. V. Rostovska

Schmalhausen Institute of Zoology NAS of Ukraine,
vul. B. Khmelnytskogo, 15, Kyiv, 01054 Ukraine
**Corresponding author*
E-mail: smezhzherin@gmail.com

S. V. Mezhzherin (https://orcid.org/0000-0003-2905-5235)
A. A. Tsyba (https://orcid.org//0000-0001-5838-0948)
O. V. Rostovska (https://orcid.org/0000-0002-0712-6365)

urn:lsid:zoobank.org:pub:3BCEFA3B-F262-4D4F-9E85-68C112838D76

Spatial Segregation of Diploid and Polyploids Spined Loaches, *Cobitis elongatoides–taenia–tanaitica* (Cypriniformes, Cobitidae), in the River Systems of Western and Central Ukraine. Mezhzherin, S. V., Tsyba, A. A., Rostovska, O. V. — The investigation examines the structure of diploid and polyploid populations within the spined loach group (*Cobitis elongatoides–taenia–tanaitica*) in the water systems of Western and Central Ukraine, including the Danube River, Dniester River, Southern Bug River, Vistula River, Middle and Upper Dnipro River basins. The study reveals a bipolarity in settlement structure, with a sharp prevalence of either diploids or polyploids, and no discernible geographical trends in spatial distribution. Clonal hybrid polyploids, which make up 70 % of the individuals studied in the region, are most common in settlements along the main river channels, as well as in the middle and lower reaches of first and second-order tributaries. Populations where diploid specimens of the parent species are predominant are located in accessory systems, tributaries of the third and higher orders, upper reaches of first and second-order tributaries, and lakes. This spatial segregation arises from historical circumstances rather than ecological preferences, primarily due to the distance from water corridors through which polyploids invaded in the 1960s–1970s. The displacement of diploid individuals by clonal hybrid polyploids is a universal phenomenon. This is due to the advantages of parthenogenetic reproduction and higher survival rates of polyploids in modern environmental situations.
Key words: diploid-polyploid complex, spatial segregation, invasion, parthenogenesis, Ukraine, C*obitis*.

SIntroduction

In the early 21st century, the small loach species of the genus C*obitis* became one of the most popular subjects for evolutionary and taxonomic research in fish. The application of various genetic methods such as karyotyping (Ueno, Ojima, 1076; Vasil'ev, 1985; Vasil'ev et al., 1989; Rab et al., 2000; Boroń, 2003), biochemical gene marking (Vasil'ev et al., 1989; Sezaki, 1994; Šlechtova et al., 2000, 2003; Mezhzherin, Pavlenko, 2009), and sequencing (Janko et al., 2007 b; Perdices et al., 2008, 2016; Bartoš et al., 2019) led to a radical shift in the understanding of the taxonomic structure of this complex. This resulted in a taxonomic revision, which described dozens of new species instead of one transpalearctic species C*obitis taenia* Linneaus, 1758 (FishBase, 2017). Most of these new species are likely to have valid status.

Of particular interest is the multiple inter-species hybridization, resulting in the formation of unisexual (all-female) triploid and tetraploid biotypes (Vasil'ev, 1985; Vasil'ev et al., 1989; Kim & Lee, 1990; Bohlen & Rab, 2001; Boroń, 2003; Janko et al., 2007 b). Specifically, in the European part of the C*obitis* genus, a diploid-polyploid complex C. *elongatoides*–C. *taenia*–C. *tanaitica* is formed through hybridization of the Danube spined loach C. *elongatoides* on one side, and the common spined loach C. *taenia* and/or the Don spined loach *C. tanaitica* on the other (Bohlen & Rab, 2001; Janko et al., 2007). Additionally, rare biotypes are found that have probably formed without the involvement of C. *elongatoides* (Mezhzherin & Pavlenko, 2009; Mezhzherin et al., 2014; Vasil'ev & Vasil'eva, 2022).

The reproduction of polyploids occurs through gynogenesis with the involvement of males from parent species. Considering that the vast majority of polyploid biotypes formed with the participation of *C. elongatoides*, which inhabits the Danube basin, the upper reaches of the Oder and Rhine Rivers, it can be concluded that they are elements of the adventive ichthyofauna of the Vistula, Dniester, Southern Bug, Dnipro, Daugava, and Volga Rivers. Furthermore, it is highly likely that in the Middle Dnipro basin, polyploids entered in large numbers during the mid-20th century, coinciding with a period of global hydrotechnical construction and intensive reclamation (Mezhzherin & Chudakorova, 2001; Mezhzherin et al., 2022 b). The structure of diploid-polyploid communities exhibits a pronounced bipolar structure. Polyploids tend to predominate more frequently, followed by diploids, while an equal ratio of polyploids to diploids is a rare situation (Mezhzherin et al., 2022 a). The reasons for the exclusion of diploids and triploids can be attributed to three circumstances: geographic separation of diploids and polyploids with limited contact zone (hybrid zone model (Barton, Hewitt, 1985)), habitat segregation, and competitive interactions between diploids and polyploids.

In contrast to the water systems of Eastern Ukraine, where diploid specimens *C. taenia* s. l. predominate in the south and polyploids in the north (Mezhzherin et al., 2022 a), populations with a dominance of diploids and polyploids in the river basins of the western and central regions (Danube River, Dniester River, Southern Bug River, Western Bug River (a tributary of the Vistula River), and Dnipro River) are distributed without apparent geographical preferences (Mezhzherin et al., 2009, 2010). This allows for the exclusion of the geographical component and facilitates a study aimed at demonstrating peculiarities of spatial segregation of diploid and polyploid spined loaches. This involves a comparative analysis of the structure of spined loach settlements in different aquatic systems (rivers, lakes, streams, oxbows etc). This research is particularly relevant in light of understanding the reasons for the successful invasion of polyploids in the rivers of Eastern Europe.

Thus, the aim of the investigation was to establish the features of spatial segregation of diploid and polyploid spined loaches in a region where there are no geographical preferences for their distribution.

Material and Methods

Sampling. The factual basis of the study consisted of a series of samples, totaling 5078 specimens collected in 104 sampling points that fairly evenly covered the distribution zone of polyploid spined loaches in the water systems of the Danube, Dniester, Southern Bug, Western Bug Rivers (a tributary of the Vistula River), Middle and Upper Dnipro basins (see: Supplementary material).

River systems description. Special attention was paid to four tributary systems of the Upper Dnipro basins. The Desna River is one of the largest left tributaries of the Dnipro River, with a channel length of 1300 km. A total 9 samples were taken from the channel, accessory system, and tributaries within a distance of up to 350 km from the mouth. The Teteriv River is a right tributary of the Upper Dnipro basin (with a channel length of 365 km). Fourteen samples were collected from the channel over a stretch of 251 km. The Zdvyzh River is a right tributary of the Teteriv River (with a channel length of 145 km). Material was collected over a stretch of 120 km. The Irpin River is a right tributary of the Dnipro River (with a channel length of 162 km), and several samples were collected over a stretch of 125 km from the mouth. The rivers have varying degrees of anthropogenic transformation. The ecosystems of the Desna and Teteriv Rivers have been altered to the least extent. The first river is unregulated, and dams have only been built in the upper part of the second. The Irpin and Zdvyzh Rivers have numerous dams throughout their channels and partially channelized streams.

Catching. Spined loaches were caught using an ichthyological net with a diameter of up to 1 meter, while adhering to the daily catch limit. The fishing sites were small stretches of rivers with moderate flow and limited vegetation in which the maximum density of spined loach populations occurred.

Taxonomy. In accordance with previous studies (Mezhzherin, Pavlenko, 2009, 2010), the following taxonomic concept was used in the work. Parental diploid species: *C. taenia* s. str. is represented in the populations Dniester, Southern Bug, Western Bug Rivers and Middke and Upper Dnipro basins; *C. elongatoides* dwells in the Danube basin; *C. tanaitica* (= ? cf. *taurica*) was identifies in the Lower Danube. Hybrid biotypes are triploids and tetraploids formed by hybridization *C. elongatoides* with *C. taenia* and/or *C. tanaitica* s. l. mainly.

Biotype identification. Discrimination between diploid and polyploid dpecimens was carried out through allozyme analysis, with results complemented by cytometry. When using allozymes, individuals with fixed heterozygous combinations for alleles at the *Aat*-1 and *Mdh*-1 loci, diagnostic for parent species, were classified as polyploids (Mezhzherin & Chudakorova, 2002; Šlechtova et al., 2000, 2003). Electrophoresis was conducted in a continuous buffer system (Peacock et al., 1965). Cytometric analysis was implemented by measuring erythrocytes on blood smears preparations (Sezaki & Kobayashi, 1978), the areas of which show a sufficiently clear hiatus between diploids and polyploids (Vasil'ev, 1985; Mezhzherin & Chudakorova, 2002).

Results

In the spined loach setlements of the studied river systems, polyploids clearly dominate, accounting for approximately 70 % of the total frequency. However, communities with varying ratios of diploids and polyploids are formed, ranging from the complete absence of polyploids to their 100 % representation. The distribution of the proportion of polyploid individuals in spined loach settlements exhibits a bipolar nature, indicating the mutually exclusive occurrence of diploids and polyploids (fig. 1).

The settlement structure depends on the location within the river system (fig. 2). In samples from the main river channels (Danube, Dniester, Southern Bug, and Dnipro Rivers), polyploids completely dominate, with an overall frequency of $P = 0.882$ ($SE = 0.013$; $N = 593$). In tributaries, their frequency is high but significantly lower, and it consistently decreases depending on the subordination of the tributary to the main channel. In first-order tributaries, the frequency of polyploids is $P = 0.805$ ($SE = 0.009$; $N = 1916$), in second-order it decreases to $P = 0.657$; ($SE = 0.011$; $N = 1845$), and in third-order it drops to 0.298 ($SE = 0.03$; $N = 205$). The lowest frequency of polyploids occurs in lakes, with $P = 0.140$ ($SE = 0.02$; $N = 293$).

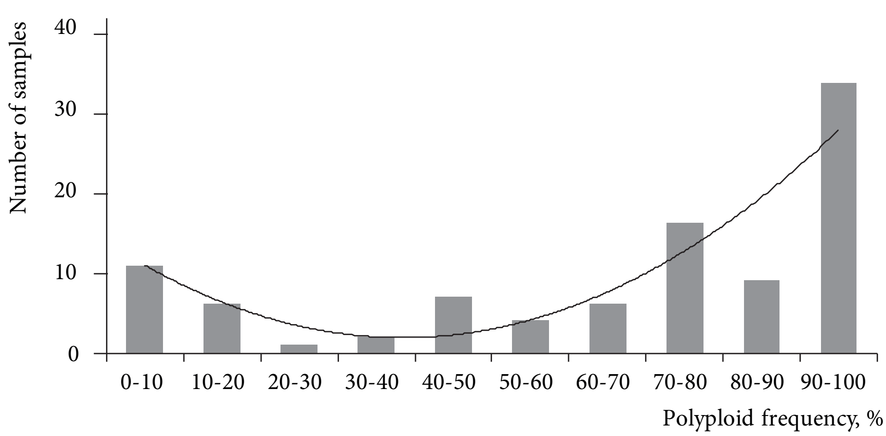

Fig. 1. Distribution of polyploid frequencies in spined loach settlements of the water systems of the Western and Central Ukraine. The approximation is performed with a polynomial function.

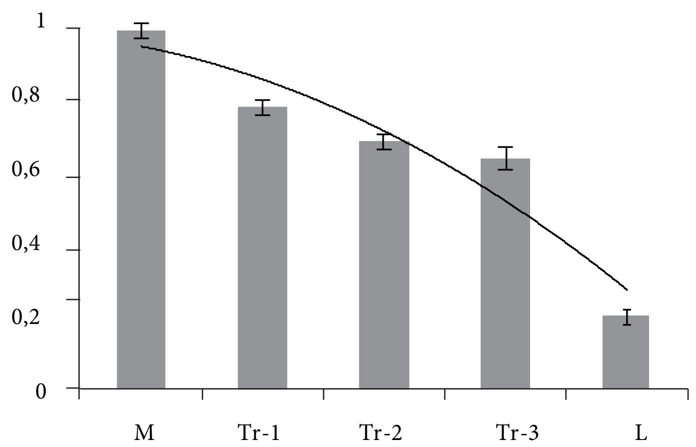

Fig. 2. Frequencies of polyploids in spined loach settlements and its standard errors depending on the type of water system of the Western and Central Ukraine. M — main channel, Tr-1, Tr-2, Tr-3 — first, second, and the third-order tributaries, L — lakes. The approximation is performed with a polynomial function.

The ratio of diploids to polyploids also depends on the size of the river (fig. 3). In samples from rivers with a channel length of more than 700 km, the overall frequency of polyploids is P = 0.886 (SE = 0.01; N = 871), while in rivers with lengths of 300–700 km, it decreases to P = 0.832 (SE = 0.014; N = 688). In rivers with lengths of 100–300 km, it reaches P = 0.759 (SE = 0.009; N = 2156), and in rivers less than 100 km, it drops to P = 0.395 (SE = 0.02; N = 715). The lowest frequency of polyploids is observed in populations of accessory systems (backwaters and floodplain lakes) (P = 0.258; SE = 0.02; N = 311).

The frequency of polyploids also undergoes significant changes within the river channels (fig. 4). For instance, in the lower third of the channel of first and second-order tributaries of the Dnipro River system, polyploids dominate with a frequency of P = 0.851 (SE = 0.009; N = 1626), while in the middle stretch, their frequency is even higher than that of diploids, at P = 0.696 (SE = 0.011; N = 1196). However, in the upper third of the channel, they are outnumbered by diploids with a frequency of P = 0.361 (SE = 0.011; N = 355).

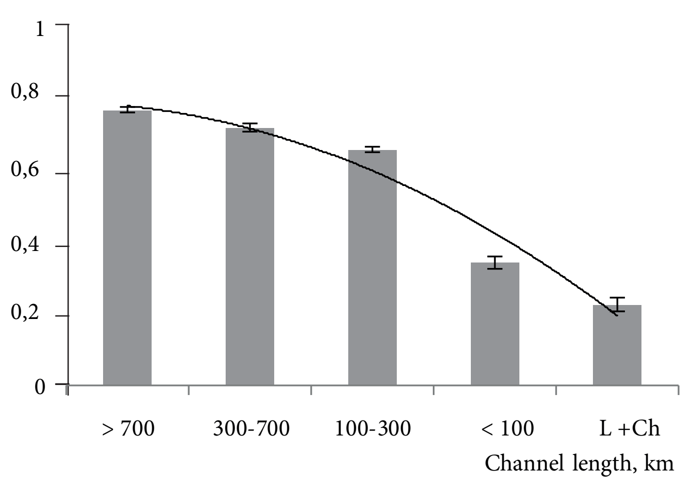

Fig. 3. Frequency of polyploids in spined loach settlements and its standard errors of rivers with different channel lengths, as well as in accessory systems of the rivers (ASR) of the Western and Central Ukraine. The approximation is performed with a polynomial function.

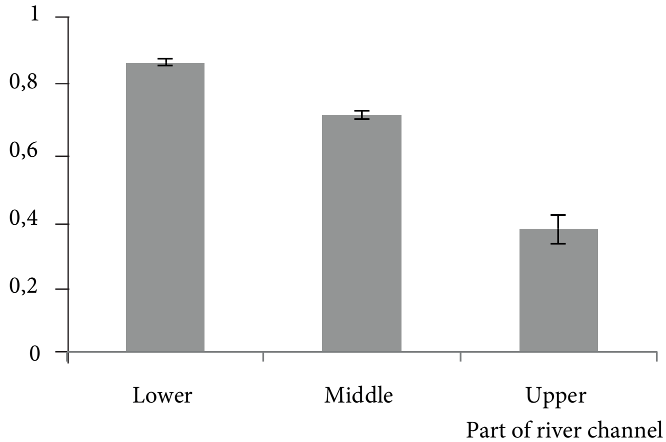

Fig. 4. Frequencies of polyploids and its standard errors in different parts of the first and second-order tributaries of the Dnipro River system: Lower — lower third of the channel, Middle — middle part of the channel, Upper — upper third of the channel. The approximation is performed with a polynomial function.

The decrease in the frequency of polyploids and the increase of diploids as one move upstream along the rivers is confirmed by the analysis of individual well-studied rivers. For example, the representation of polyploids in samples of spined loaches collected from the main channel of the Teteriv River decreases from 100 % in a sample from the lower part of the river (23 km from the river mouth) to complete absence in the highest sample taken 251 km from the mouth (fig. 5) ($r = -0.82$; $p < 0.001$; $n = 12$). The trend of increasing diploid frequencies is observed in the Zdvyzh River. The frequency of polyploids in the lower part of the river (samples from 9 to 64 km from the mouth) is P = 0.752 (SE = 0.037), while in the upper part it is P = 0.450 (SE = 0.023). In the Irpin River, the frequency of polyploids in the segment 3–27 km from the mouth was P = 0.899 (SE = 0.025), in the middle stretch (35–62 km) it remained at a similar level, P = 0.917 (SE = 0.011), and in the upper part (75–113 km) it decreased to P = 0.719 (SE = 0.031). The decrease in the frequency of polyploids also occurs within the Desna River system. The overall frequency of polyploids within 200 km from the mouth is P = 0.89 (SE = 0.011; N = 782; Min-max = 0.72–0.98). In samples collected above 200 km, their frequency is only P = 0.60 (SE = 0.044; N = 121; Min-max = 0.125–0.778).

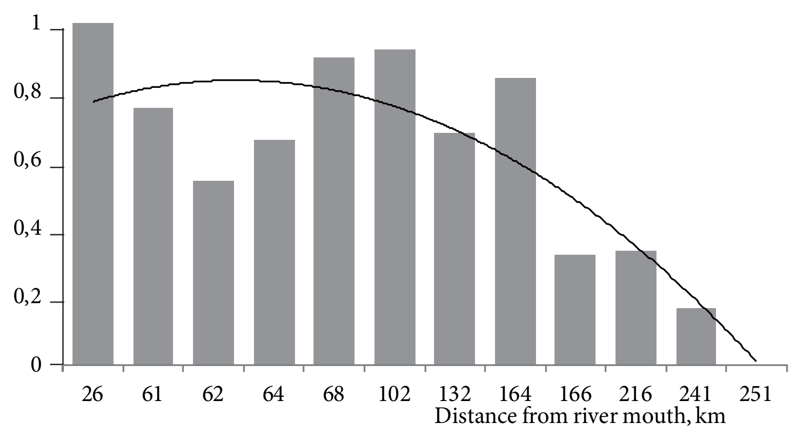

Fig. 5. Changes in the frequency of polyploids in samples of spined loaches from the Teteriv River, depending on the distance from the mouth. The approximation is performed with a polynomial function.

Discussion

Based on own results, as well as literature data (Boroń, 2003; Lusk et al., 2003; Luskova et al., 2004; Janko et al., 2007), spined loaches settlements can be divided into two main groups: in some, polyploids make up more than 80 % of individuals, while in others, diploids predominate to the same extent. Populations with an equal ratio of diploids and polyploids likely represent unstable communities and are relatively rare. Moreover, there are no clear geographical tendencies in the spatial distribution of populations of extreme types. Their spatial segregation and, accordingly, the maintenance of their structure is realized through the water system type preferences of diploids and polyploids. Spined loach settlements with an obvious predominance of polyploids are found in central watercourses: main channels, first-order tributaries, and major second-order tributaries. In contrast, the dominance of diploid individuals occurs on the periphery of river systems: in adnexal systems, small second-order tributaries, third-order tributaries, and upper reach of rivers, especially where the river turns into a stream. Additionally, populations consisting exclusively of diploid individuals are mostly localized in lakes.

Overall, the results of the analysis conducted in Central and Western Ukraine correspond to the results of similar studies in other regions where diploids and polyploids coexist within the *Cobitis elongatoides–taenia–tanaitica* group. For example, in the tributaries of the Tisza River (Lusk et al., 2003; Luskova et al., 2004), spined loach populations are clearly divided into two groups: diploid and those with a clear dominance of polyploids. The first type of populations tends to be found in streams, while the second predominates in rivers. In the Oder and Vistula Rivers basins, diploid populations are found only in lakes, while communities with a dominance of polyploids are widespread along the rivers (Boroń, 2003). The habitat preference of diploid populations is that they are associated with alternative hydrosystems: lakes and small rivers (streams). This means that for the preservation of diploid populations, it is not the ecological conditions per se that are crucial, such as flow rate, but rather historical circumstances. It is evident that the original diploid settlements have been preserved in relatively native ecosystems, distant from potential corridors of polyploid invasion. In the main channels, polyploids have displaced diploids, which is likely associated with the advantages of parthenogenetic reproduction and the polyploid genome structure.

The advantages of parthenogenesis over sexual reproduction are supported by data on the population structure and spatial distribution of communities of other freshwater fish, in which allopolyploids reproduce by gynogenesis using sperm from males of parent or closely related species. This applies to the Transpalearctic species complex *Carassius auratus–gibelio* (Shimuzu et al., 1993; Mezhzherin et al., 2020), American diploid-triploid complexes *Poecilia formosa* (*P. mexicana–latipinna*) (Rasch & Balsano, 1974; Warren et al., 2018), *Poeciliops monacha–lucida* (Moore et al., 1970; Rasch & Balsano, 1974), and the Pyrenean tri-tetraploid species complex *Squalius alburnoides* (Martins et al., 1998; Cunha et al., 2010). A similar situation is observed in the diploid-polyploid group of salamanders, formed as a result of hybridization of four species of the genus *Ambystoma*, where polyploid reproduction occurs through gynogenesis (Bogart et al., 1987).

The groups of species mentioned above, including the spined loaches diploid-polyploid group, share several seemingly non-obvious features: 1) the frequent dominance of hybrid polyploids over individuals of the parent species in mixed populations; 2) the bipolarity of the structure of mixed settlements based on the principle of sharp dominance of diploids or polyploids; 3) the occupation of central habitats by polyploids and the displacement of parent diploids to the periphery of their living space, especially in the headwaters of rivers. The established trends in spatial segregation can be considered universal rules that reflect the general patterns of formation of diploid-polyploid complexes, and not just limited to fish.

Supplementary material. Supplementary material is available online at: https://www.gbif.org/dataset/32e79b23-ced1-49e1-a579-90d97e675125.

References

Barton, N. H. & Hewitt, G. M. 1985. Analysis of hybrid zones. *Annual review of ecology and systematic*, **16**, 113–148. https://doi:10.1146/annurev.es.16.110185.000553.

Bartoš, O., Röslein, J., Kotusz, J., Paces, J., Pekárik, L., Petrtýl, M., Halačka, K., Štefková, K. E., Mendel, J., Boroń, A., Juchno, D., Leska, A., Jablonska, O., Benes, V., Šídová, M. & Janko, K. 2019. The legacy of sexual ancestors in phenotypic variability, gene expression, and homoeolog regulation of asexual hybrids and polyploids. *Molecular Biology and Evolution*, 36, 1902–1920. https://doi.org/10.1093/molbev/msz114

Bogart, J. P., Lowcock, L. A., Zeyl, C. W. & Mable, B. K. 1987. Genome constitution and reproductive biology of hybrid salamanders, genus *Ambystoma*, on Kelleys Island in Lake Erie. *Canadian Journal of Zoology*, **65** (9), 2188–2201. https://doi.org/10.1139/z87-333.

Bohlen, J. & Rab, P. 2001. Species and hybrid richness in spined loaches of the genus *Cobitis* (Teleostei: Cobitidae), with a cheklist of European forms and suggestions for conservation. *Journal of Fish Biology*, **59**, 75–89. https://doi.org/10.1111/j.1095-8649.2001.tb01380.x.

Boroń, A., 2003. Karyotypes and cytogenetic diversity of the genus *Cobitis* (Pisces: Cobitidae) in Poland: a review. Cytogenetic evidence for a hybrid origin of some *Cobitis* triploids. *Folia Biologica*, **51**, 49–54.

Cunha, C., Doadrio, I., Abrantes, J. & Coelho, M. M. 2010. The evolutionary history of the allopolyploid *Squalius alburnoides* (Cyprinidae) complex in the northern Iberian Peninsula. *Heredity*, **106**, 100–112. https://doi.org/10.1038/hdy.2010.70.

FishBase. 2017. Rainer, F. & Daniel, P., eds. Species of *Cobitis* in FishBase. June 2017 version. https://fishbase.mnhn.fr/identification/SpeciesList.php?genus=*Cobitis*.

Janko, K., Bohlen, J., Lamatsch, D., Flajahans, M., Epplen, J. T., Rab, P., Kotlik, P. & Alechtova, V. 2007 a. The gynogenetic reproduction of diploid and triploid hybrid spined loaches (*Cobitis*; Teleostei), and their ability to establish successful clonal lineages — on the evolution of polyploidy in asexual vertebrates. *Genetica*, **131**, 185–194. https://doi: 10.1007/s10709-006-9130-5.

Janko, K., Flajshnans, M., Choleva, L., Bohlen J., ŠLechtová V., Rábová M., Lajbne, Z., Šlechta V., Ivanova, P., Dobrovolov, I., Culling M., Persa H., Kotusz J. & Ráb, P. 2007 b. Diversity of European spined loaches (genus *Cobitis* L.) an update of the geographic distribution of the *Cobitis taenia* hybrid complex with a description of new molecular tools for species and hybrid determination. *Journal of Fish Biology*, **71**, 387–408. https://doi.*org*/10.1111/j.1095-8649.2007.01663.x.

Kim, I. S. & Lee, E. H. 1990. Diploid-triploid hybrid complex of the spined loach *Cobitis sinensis* and *C. longicorpus* (Pisces: Cobitidae). *Korean Journal of Zoology,* **1**, 203–210.

Lusk, S., Koščo, J., Halačka, K., Luskova, V. & Flajšmans, M. 2003. Identification of *Cobitis* from Slovakian part of Tizsa basin. *Folia Biologica*, 51, Suppl., 61–65.

Luskova, V., Koščo, J., Halačka, K., Straňai, I., Lusk, S. & Flajšhahs, M. 2004. Status of populations of the genus *Cobitis* in Slovakia. *Biologia*, **59** (5), 621–626.

Martins, M. J., Colares-Pereira, M. J., Cowx G. & Coelho, M. M. 1998. Diploids v. triploids of *Rutilus alburnoides*: spatial segregation and morphological differences. *Journal of Fish Biology*, 52, 817-828. https://doi.org/10.1111/j.1095-8649.1998.tb00822.x.

Mezhzherin, S. V. & Chudakorova, T. Yu. 2001. Expansion of triploid unisexual spined loaches *Cobitis taenia* L., 1758 (Cypriniformes, Cobitidae) in the watercourses of Ukraine. *Reports of the National Academy of Sciences of Ukraine*, **9**, 153–157 [In Russian].

Mezhzherin, S. V. & Chudakorova, T. Yu. 2002. Genetic structure of the diploid polyploid complex of spined loaches *Cobitis taenia* (Cypriniformes: Cobitidae) of the Middle Dnipro drainage. *Russian Journal of Genetics*, **38**, 86–92. https://doi:10.1023/A:1013719912744 [In Russian].

Mezhzherin, S. V. & Pavlenko, L. I. 2009. Species composition and diversity of hybrid biotypes of the genus *Cobitis* (Cypriniformes, Cobitidae) of the fauna of Ukraine. *Reports of the National Academy of Sciences of Ukraine*, 2009, 10, 172–178 [In Russian].

Mezhzherin, S. V. & Pavlenko, L. I. 2010. Genetic diversity, origin, and geographic distribution tendencies of biotypes of polyploid spined loaches (Cypriniformes, Cobitidae, *Cobitis*) in Ukraine. *Cytology and Genetics*, **44**, 317–327.

Mezhzherin, S. V., Pukhtaevych, P. P. & Kokodiy, S. V. 2020. The polyclonal structure of unisexual Prussian carp (*Carassius gibelio* (Bloch, 1782)) settlements in Northern Ukraine: comparison analysis of allozyme markers and chromosome numbers. *Cytology and Genetics*, **54**, 55–62. https://doi: 10.3103/S0095452720010119.

Mezhzherin, S. V., Kryvokhyzha, D., Tsyba, A. A. & Rostovskaya, O. V. 2022 a. Spined loache settlements structure (Cobitidae) of the Eastern Ukraine river systems and alternative character of dioloid and polyploidy populations // *Zoodiversity*, **56**, 331–340. https://doi.org/10.15407/zoo2022.04.331

Mezhzherin, S. V., Tsyba, A. A. & Kryvokhyzha, D. 2022 b. Cryptic expansion of hybrid polyploid spined loaches *Cobitis* in the rivers of Eastern Europe. *Hydrobiologia*, **849**, 1689–1700. https://doi.org/10.1007/s10750-022-04813-z.

Moore, W. S., Rush, M. R. & Schultz, R. J. 1970. Distribution, adaptation and probable origin of an all-female form of *Poeciliopsis* (Pisces: Poecilidae) in NorthWestern Mexico. *Evolution*, **24**, 789–795. http:// doi: 10.1111/j.1558-5646.1970.tb01813.x.

Peacock, F. C., Bunting, S. L. & Queen, K. G. 1965. Serum protein electrophoresis in acrylamide gel patterns from normal human subjects, *Science.* **147**, 1451–1455.

Perdices, A., Bohlen, J. & Doadrio, I. 2008. The molecular diversity of adriatic spined loaches (Teleostei, Cobitidae). *Molecular Phylogenetics and Evolution*, **46**, 382–390. https://doi:10.1016/j.ympev.2007.05.007.

Perdices, A., Bohlen, J., Slechtova, V. & Doadrio, I. 2016. Molecular evidence for multiple origins of the European spined loaches (Teleostei, Cobitidae). *PLoS ONE*, 11 (1): e0144628. https://doi.org/10.1371/journal.pone.0144628.

Rab, P., Rabova, M., Bonlen, J. & Lusk, S. 2000. Genetic differentiation of the two hybrid diploid-polyploid complexes of loaches, genus *Cobitis* (Cobitidae) involving *C. taenia*, *C. elongatoides* and *C.* sp. in the Czech Republic: кaryotype and cytogenetic diversity. *Folia Zoologica*, **49**, 55–66.

Rasch, E. M. & Balsano, J. S. 1974. Biochemical and cytogenetic studies of *Poecilia* from Eastern Mexico II. Frequency, perpetuation and probable origin of triploid genomes in females associated with *Poecilia formosa*. *International Journal of Tropical Biology and Conservation*, **21**, 351–381.

Shimuzu, Y., Oshiro, Ò. & Sakaizumi, M. 1993. Electrophoretic studies of diploid, triploid, and tetraploid forms of the Japanese silver crucian carp, *Carassius auratus langsdorfii*. *Japan Journal of Ichthyology*, **40**, 65–75. https://doi.org/10.11369/jji1950.40.65.

Šlechtova, V., Luskova, V., Slechta, V., Lusk, S., Halacka, K. & Bohlen, J. 2000. Genetic differentiation of two diploid–polyploid complexes of spined loach, genus *Cobitis* (Cobitidae), in the Czech Republic, involving *C. taenia*, *C. elongatoides*, and *C.* spp.: allozyme interpopulation and interspecific differences. *Folia Zoologica*, **49**, 67–78.

Šlechtova, V., Luskova, V., Slechta, N. V., Lusk, S. & Pivonkova, J. 2003. Potential species identification by allozyme/protein markers in European spined loaches, *Folia Biologica.* **57**, 43–47.

Sezaki, K, Watabe, S., Ochiai, Y. & Hashimoto, K. 1994. Biochemical genetic evidence for a hybrid origin of spined loach, *Cobitis taenia taenia* in Japan. *Fish Biology,* **44**, 683–691. https://doi.org/10.1111/j.1095-8649.1994.tb01244.x.

Sezaki, K. & Kobayashi, H. 1978. Comparison of erythrocytic size between diploid and tetraploid in spinous loach *C. biwae*. *Bulletin of the Japanese Society for the Science of Fish*, **44**, 851–864. http://doi:10.1016/j.cbpa.2007.03.006.

Ueno, K. & Ojima, Y. 1976. Diploid–tetraploid complexes in the genus *Cobitis* (Cobitidae, Cyprinida). *Proceedings of the Japan Academy*, **52**, 446–449. https://doi:10.2183/PJAB1945.52.446.

Vasil'ev, V. P. 1985. Evolutionary karyology of fish. Nauka, Moskow, 1–300 [In Russian].

Vasil'ev, V. P., Vasil'eva, K. D. & Osinov, A. G. 1989. Evolution of a diploid-triploid-tetraploid complex in fishes of the genus *Cobitis* (Pisces, Cobitidae). *Evolution and ecology of unisexual vertebrates*, eds Dawley, R. M., Bogart & Albany, J. P. New York State Museum, New York, **466**, 153–169.

Vasil′ev, V. P. & Vasil′eva, K. D. 2022. Triploid forms' karyotypes of spined loaches from the genus *Cobitis* (Actinopterygii: Cypriniformes: Cobitidae) of the upper Dnipro and Western Dvina rivers: Analysis of the triploids' origin. *Acta Ichthyologica et Piscatoria,* **52**, 67–75. https://doi 10.3897/aiep.52.81191 [In Russian].

Warren, W. C., García-Pérez, R., Xu, S. & Lampert, K. 2018. Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nature Ecology* & *Evolution*, **2**, 669–679. http:// doi:10.1038/s41559-018-0473-y.

Received 16 October 2023
Accepted 22 February 2024