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PRIMARY RESEARCH PAPER

Natural colonization and localized admixture across the Adriatic–Danube divide: insights from *Telestes souffia* and *Telestes muticellus*

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Abstract Along the Adriatic–Danube divide, Pleistocene climatic oscillations intermittently permitted faunal exchange across closely spaced headwaters. We investigated two non-commercial cyprinids, *Telestes souffia* and *T. muticellus*, across the Soča drainage (Slovenia–Italy) and adjacent Sava reaches using mitochondrial (cytochrome *b*) and nuclear (microsatellite) markers. We reveal clear genetic differentiation between *T. souffia* populations from the Sava and Soča drainages and show that its occurrence within the Soča drainage is restricted to the middle Soča. Genetic patterns indicate that its presence there reflects natural postglacial colonization from the upper Sava rather than recent anthropogenic translocations, highlighting the historical permeability of the Soča–Sava drainage boundary. We further confirm *T. muticellus* in previously unverified parts of Slovenia, including the Nadiža and Brda watercourses, providing evidence for its native status and supporting refinement of Natura 2000 site designations. A genetically distinct *T. muticellus* individual from the Vipava river system suggests a historically broader distribution in the lower Soča. Microsatellite data reveal localized introgressive hybridization between *T. souffia* and *T. muticellus* in the Volarja Stream, a middle Soča tributary, consistent with recent human-mediated translocations. No evidence for a broad or persistent hybrid zone was detected within the sampled sections of the Soča drainage.

Keywords Cytochrome b · Introgression · Microsatellites · Postglacial colonization · Soča River · Translocation

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Introduction

Freshwater systems of the Alpine–Dinaric region show a complex phylogeographic structure shaped by Pleistocene climatic oscillations, geomorphological instability, and repeated drainage rearrangements (Hewitt, 1999, 2000). Along the Adriatic–Danube divide, closely spaced headwaters have occasionally permitted faunal exchange via stream captures, meltwater corridors, or short-distance

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headwater connections (Costedoat & Gilles, 2009; Buj et al., 2017). Such conditions often create narrow contact zones where lineage boundaries are dynamic, necessitating multilocus data to distinguish recent secondary contact from older historical signals, such as Pliocene divergence associated with the Messinian salinity crisis versus Late Pleistocene drainage rearrangements.

The genus *Telestes* comprises 14 small-bodied leuciscids inhabiting clear, fast-flowing streams across southern and central Europe, typically showing strong population structuring and endemism (Buj et al., 2017). As non-managed, rarely translocated taxa, *Telestes* species preserve natural phylogeographic signatures and therefore provide valuable models for reconstructing historical drainage connectivity (Dubut et al., 2012).

Within the genus, *Telestes souffia* (Risso, 1827) and *Telestes muticellus* (Bonaparte, 1837) are of particular biogeographic interest because of their contrasting yet complementary distributions. *Telestes souffia* is widespread across the Danube and Rhône basins, with additional occurrences in the upper Rhine watershed (Gilles et al., 1998; Machordom et al., 1999). In the Adriatic basin its range is confined to the Soča/Isonzo drainage in Slovenia and north-eastern Italy (Crivelli & Bianco, 2006). In contrast, *T. muticellus* is largely restricted to Italy, occupying the Po–Venetian region, parts of the Soča drainage, and several Mediterranean watersheds draining to the Tyrrhenian and Ligurian Seas (Stefani et al., 2004; Marchetto et al., 2010). This configuration places *T. muticellus* within a relatively narrow Italian core area surrounded by the broader Alpine–Danubian distribution of *T. souffia*, giving rise to two major contact zones: (i) along the Mediterranean border of Italy and France and (ii) across the northern Adriatic basin in the border region between Italy and Slovenia.

Diagnostic differences between *T. souffia* and *T. muticellus* were originally established on morphological grounds and later corroborated by allozymes and mitochondrial lineages, with both species corresponding to well-supported and deeply divergent mtDNA haplogroups (Machordom et al., 1999; Ketmaier et al., 2004; Dubut et al., 2012; Buj et al., 2017). Natural contact between the species is genetically well documented in the France–Italy border region, where cross-drainage transitions occur but extensive hybridization has not been detected (Zaccara et al., 2007; Gilles et al., 2010; Dubut et al., 2012). A putative secondary-contact zone has also been proposed for the Soča drainage, though the evidence is based solely on mtDNA (Salzburger et al., 2003; Bertoli et al., 2024).

The Soča–Sava drainage boundary represents a broader biogeographical interface between the Adriatic and Danube basins. Several freshwater taxa—including *Squalius* spp., *Austropotamobius* crayfish, and *Salmo marmoratus* Cuvier, 1829—show signatures of historical cross-drainage connectivity in this region (Govedič, 2013; Sušnik Bajec et al., 2015; Mrzelj & Sušnik Bajec, 2022), indicating that the drainage boundary has not always been a strict barrier. However, in these taxa, historical patterns are obscured either by limited sampling (in *Squalius* and *Austropotamobius*) or by documented translocations and introgression (in *Salmo*), making it difficult to distinguish natural from anthropogenic processes. The two *Telestes* species, largely unaffected by stocking and characterized by clear species-level diagnostic differentiation, offers an excellent opportunity to examine historical versus anthropogenic processes without the confounding effects present in the other freshwater taxa.

Although *T. souffia* populations occurring in the Adriatic basin are generally associated with the Soča drainage of western Slovenia and north-eastern Italy (Kottelat & Freyhof, 2007; Ford, 2024), genetically confirmed records are so far limited to side pools of the Soča near Tolmin (Machordom et al., 1999; Gilles et al., 2010; Dubut et al., 2012) and from the Soča near Gorizia/Gorica (Bertoli et al., 2024). Additional populations reported from the Soča drainage (Podgornik et al., 2015) remain taxonomically unresolved because *T. souffia* and *T. muticellus* are morphologically very similar, making field identification difficult (Veenvliet & Kus Veenvliet, 2006).

Several localities delineate the distributional interface between *T. souffia* and *T. muticellus* in the Soča drainage and adjacent Adriatic tributaries (Fig. 1). The Volarja Stream represents a putative contact site based on the presence of mitochondrial haplotypes of both taxa, in the absence of nuclear data (Podgornik et al., 2015). Near Gradisca d'Isonzo/Gradišče ob Soči, only *T. muticellus* has been reported (Ketmaier et al., 1998, 2004). Records from the upper Vipava/Vipacco River indicate the presence of *Telestes*, but without species-level assignment (Semrajc, 2021; P. Valič, pers. comm.). Comparable uncertainty applies to populations

in the Adriatic tributaries of the Brda/Collio region draining toward the Torre/Ter River, located near the expected transition between the two species (Semrajc, 2021). In the Italian section of the Nadiža/Natisone River, *T. souffia* mitochondrial haplotypes have been detected within a population otherwise attributed to *T. muticellus* (Bianco, 1994; Salzburger et al., 2003; Bertoli et al., 2024).

Taken together, these observations reveal a geographically complex mosaic of *Telestes* occurrences in the Soča drainage and highlight unresolved questions regarding species delimitation, historical connectivity, and potential introgression.

In this study, we combine sequences of the mitochondrial cytochrome b gene and 11 microsatellite loci to (i) characterize the phylogeographic structure of *T. souffia* and *T. muticellus*, (ii) assess the permeability of the Adriatic–Danube divide, and (iii) evaluate evidence for potential interspecific introgression. Our multilocus analyses refine the evolutionary history of these *Telestes* taxa in a biogeographically complex region and provide a clearer basis for distinguishing species boundaries in areas where morphological identification alone remains unreliable—an issue of practical importance for biodiversity monitoring and Natura 2000 species designation.

Material and methods

Sampling design and study area

A total of 324 *Telestes* specimens were collected from Slovenia and Italy (Tables 1 and S1,). Sampling sites were selected to cover the known and suspected ranges of *T. souffia* and *T. muticellus* within the North Adriatic basin (with particular emphasis on the Soča drainage), and within the Sava drainage of the Danube basin (Fig. 1).

In the Sava drainage, sampling was conducted across a large portion of the known range of *Telestes* in Slovenia, based on inventory data from Fisheries Research Institute of Slovenia (FRIS). This included sites in the upper Sava drainage (Sora river system and Bohinjka River), the central section (the rivers Gameljščica, Borovniščica, and Gradaščica), and the lower drainage (the Mirna River and tributaries of the Savinja and Krka rivers). Particular attention was given to the Poljanska Sora river system, where three sites were sampled—Sovra, Gorenja vas, and Srednja vas (designated in figures as Poljanska Sora 1, 2, and 3, respectively). This area is of special interest because some of its tributaries lie near the headwaters of the Adriatic basin.

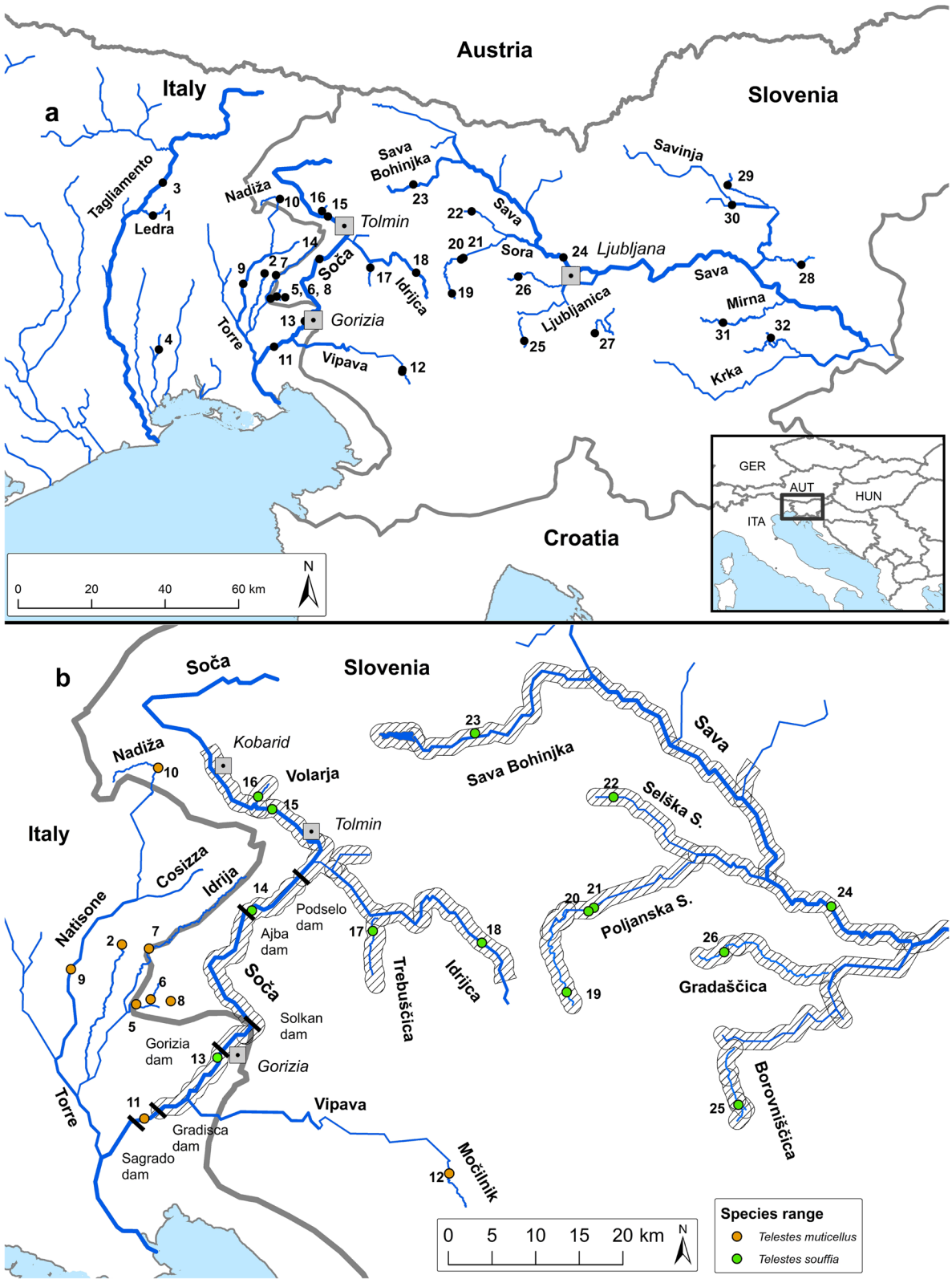
In the Soča drainage, sampling included the Soča River near Gorizia in Italy (site Soča_Gorizia), the Ajba hydropower reservoir (site Soča_Ajba), and the Volarja Stream upstream from Tolmin. Additional sites included the Idrijca River, its tributary, the Trebuščica River, and the Močilnik Stream, a tributary of the Vipava River—the only known site in that river system hosting *Telestes* sp. Further sampling sites included the Nadiža River—near Orsaria in Italy (site Nadiža 1) and near Logje and Kred in Slovenia (site Nadiža 2). Finally, the streams in the Brda region—the Idrija /Udrio River and its tributaries Reka, Kožbanjšček, and Vedrijanšček, collectively referred to as the Brda watercourses—were also sampled.

The Nadiža and Idrija rivers both flow into the Torre River in eastern Friuli–Venezia Giulia, which joins the Soča just a few kilometers before its outflow into the Adriatic Sea.

In addition, several sites in Italy were sampled to capture the broader geographical range of *T. muticellus* within the North Adriatic basin and to provide reference populations with confirmed species identity (Bertoli et al., 2024). These included rivers within the Soča drainage, such as the Chiarò Stream (a tributary of the Torre via the Corno and Idria rivers), as well as nearby Adriatic systems, including the Tagliamento River and its tributary the Ledra, and the Miliana Stream in the Grado–Marano lagoon basin.

Geographical coordinates are provided in Table S1.

Italian samples (extracted DNA) were the same as those used in Bertoli et al. (2024), with mitochondrial sequences adopted from that study. In addition, the Soča River sequence (GenBank acc. no. AY509852) from Ketmaier et al. (2004) was included in our sample set (Soča_Grad in Table 1). Reference sequences from Dubut et al. (2012), Buj et al. (2017) and Veličković et al. (2020), representing *T. souffia* from various parts of its range, served as orientation references.


a
Austria
Italy
Slovenia
Croatia
Tagliamento
Ledra
Nadiža
Tolmin
Soča
Idrijica
Gorizia
Torre
Vipava
Sava Bohinjka
Sava
Sora
Ljubljanica
Ljubljana
Savinja
Mirna
Krka
GER
AUT
HUN
ITA
0 20 40 60 km
N
b
Soča
Slovenia
Nadiža
Kobarid
Volarja
Tolmin
Italy
Cosizza
Idrija
Natisone
Podselo dam
Ajba dam
Trebuščica
Idrijica
Soča
Solkan dam
Gorizia dam
Gorizia
Torre
Gradisca dam
Sagrado dam
Vipava
Močilnik
Sava Bohinjka
Sava
Selška S.
Poljanska S.
Gradaščica
Borovniščica
0 5 10 15 20 km
N
Species range
Telestes muticellus
Telestes souffia

◂**Fig. 1** **a** Overview map showing all sampling locations across the Soča and Sava catchments, including the location of the study area within a broader European context (inset). **b** Detailed map of the Soča drainage and Sava catchments. Shaded areas represent the approximate extent of *Telestes souffia*. Sampling sites are numbered and correspond to those listed under the "Map code" column in Table 1; geographical coordinates are listed in Table S1. Locations for sites 11 (Ketmaier et al. 2004) and 15 (Dubut et al. 2012) are approximate, as precise coordinates were not available

DNA extraction

DNA was extracted from fin-clip tissue preserved in 96% ethanol using a salt-extraction protocol (Miller et al., 1988) for Slovenian samples, and the PCRBIO Rapid Extract PCR Kit (PCR Biosystems, London, UK) for Italian samples.

Mitochondrial cytochrome b gene analysis

PCR amplification of the mitochondrial (mt) cytochrome b (*cyt-b*) gene fragment was performed using primers Glu-F and Thr-R (Zardoya & Doadrio, 1998), with the following PCR conditions: initial DNA denaturation (94 °C for 60 s) and 35 successive cycles of strand denaturation (94 °C for 30 s), primer annealing (55 °C for 30 s), DNA extension (72 °C for 60 s), and final DNA extension (72 °C for 3 min). A total volume of 25 µL of reaction mix contained 100 ng of genomic DNA, 0.5 µM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1×PCR buffer, and 1 U of Taq polymerase (TaqOne, New England Biolabs).

Amplified DNA fragments were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermofisher Scientific) prior sequencing. Sequencing reactions were prepared using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations. The sequencing products were salt-precipitated and analyzed on an ABI Prism 3130xl Genetic Analyzer.

Details of PCR amplification and *cyt-b* sequencing of Italian samples are given in Bertoli et al. (2024).

Sequences were aligned using Clustal X (Thompson et al., 1994) implemented in MEGA version X (Kumar et al., 2018).

The genealogy of haplotypes was presented as haplotype network constructed using the software DNAsp (Librado & Rozas, 2009) and PopART 1.7 (Leigh & Bryant, 2015) following the median-joining network algorithm (Bandelt et al., 1999).

Mitochondrial DNA diversity metrics—including haplotype diversity (Hd), nucleotide diversity (π), haplotype count (h), the number of segregating sites (S), and the average number of nucleotide differences (k)—were calculated in DnaSP v6 (Rozas et al., 2017). Population groups were defined geographically as follows: (1) *T. souffia*—Sava drainage, (2) *T. souffia*—middle Soča excluding Volarja, (3) *T. muticellus*—Italian rivers and lower Soča, and (4) *Telestes* sp.—Volarja. All sequences were included in the analyses to ensure accurate representation of haplotype frequencies.

Microsatellite DNA

Eleven polymorphic microsatellite loci were amplified in 324 individuals using fluorescently labeled forward primers (Table S2) in 10 µl reaction mixtures with 50 ng of sample DNA, 0.5 µM of each primer, 0.2 mM dNTP, 1.0 mM MgCl2, 1×PCR buffer, and 1 U *Taq* polymerase (TaqOne, New England Biolabs) following the so-called "touchdown" protocol described in Hamilton and Tyler (2007) with the annealing temperature decreasing every five cycles (62 °C, 58 °C, 55 °C, 53 °C, 51 °C, 49 °C, 47 °C).

Aliquots of amplified fluorescently labeled DNA were mixed with formamide and GENESCAN-500 LIZ Size Standard (Applied Biosystems) and genotyped on the ABI Prism 3130xl with GeneMapper® Software v6.0 (Applied Biosystems). Possible null alleles were assessed using Micro-Checker v. 2.2.3 (Van Oosterhout et al., 2004).

Levels of genetic diversity were estimated from expected (Hexp) and observed (Hob*s*) heterozygosity (Nei & Chesser, 1983), total number of alleles across all loci (Aall), average number of alleles per locus (Aave), all calculated using Genetix v. 4.05 software (Belkhir et al., 1996–2004), and Factorial Correspondence Analysis (FCA) implemented in Genetix v. 4.05 were used to visualize genetic distance and relatedness between the tested individuals and to check for genetically homogenous groups within the entire sample set.

Table 1 Haplotypes observed in this study

Map code	Sampling sites	Nseq	Hap. No	*T. souffia* lineage (SOU) haplotypes											*T. muticellus* lineage (MUT) haplotypes							Rare haplotypes
				SOU1	SOU2	SOU5	SOU6	SOU7	SOU14	SLV07	SLO03	SOU16	SOU21	SOU22	MUT15	MUT20	MUT36	MUT37	MUT40	MUT48	MUT49	
1	Ledra	8	4												2	3	2					MUT41
2	Chiarò	10	5												1	6		1				MUT41, MUT42
3	Tagliamento	25	9												3	8	4	3	2			MUT43(2)-44, MUT46-47
4	Miliana	10	4												1	6	2	1				
5	Reka	/	/																			
6	Kožbanjšček	7	2													4	2	1				
7	Idrija	3	2													2						MUT38
8	Vedrijanšček	3	2													2	1					
9	Nadiža 1	6	6					**1**							1	1						MUT42, MUT44-45
10	Nadiža 2	26	6												1	12	7	1	3			MUT39, MUT50
11	Soča_Grad.*		1																	3		
12	Vipava	1	1																		1	
13	Soča_Gorizia	18	6			1		9						5								SOU23-24, SLO01
14	Soča_Ajba	11	4			2		6		1	2											
15	Soča_Tolmin**		6			8		26	1	1	1											SLO01
16	Volarja	15	3			1		9	2							**3**						
17	Trebuščica	11	4			4		6		1												
18	Idrijca	8	2			2		6														
19	Poljanska Sora 1	10	5	1	2	5			1													SOU17
20	Poljanska Sora 2	3	2			2																SOU18
21	Poljanska Sora 3	8	3	3		4																SOU19
22	Selška Sora	10	2			1							9									
23	Sava Bohinjka	9	3	4			2					3										
24	Gameljščica	10	3	6		3																SOU17
25	Borovniščica	12	2	11					1													
26	Gradaščica	4	2	1					3													
27	Bičje	8	3	1		6	1															
28	Gračnica	4	2					3														SOU15
29	Ložnica	4	1		4																	
30	Bolska	6	3		1	4			1													
31	Mirna	4	2	2					2													
32	Laknica	4	2			3																SOU20

Nseq, number of sequenced individuals; Hap. No., number of distinct haplotypes. Rare haplotypes (≤ 2 sites, $< 1\%$ total frequency) are listed in the "Rare haplotypes" column, with the exception of haplotypes from Vipava (site 12) and Soča_Grad (site 11), which are highlighted due to their biogeographic relevance Shading distinguishes three drainage-based regions: (1) western Adriatic tributaries (including Torre–Nadiža, Brda, and adjacent Italian streams), (2) middle Soča drainage, and (3) Sava drainage. Individuals carrying haplotypes unexpected for their drainage region are shown in bold. Asterisks indicate haplotypes reported by Ketmaier et al. (2004)* and Dubut et al. (2012)**

To quantify genetic variation within and among populations, hierarchical AMOVA was performed using Arlequin v3.5.1.3 (Excoffier & Lischer, 2010).

The genetic structure of the sample set was also estimated by using the Bayesian method implemented in STRUCTURE v. 2.3.4 (Pritchard et al., 2000; Falush et al., 2007; Vähä et al., 2007). The admixture model, which correlated allele frequencies between populations without sampling location information was used. MCMC was run for 1,000,000 replicates subsequent to 250,000 burn-in generations. The number of clusters (K) in the dataset was explored from $K = 1$ to 15, and for each value seven run replicates were performed. The most probable K was inferred using the Evanno (Evanno et al., 2005) and Puechmaille method (Puechmaille, 2016) implemented in Structure Selector (Li & Liu, 2018).

Results

Analysis of mitochondrial *cyt-b* gene

Sequencing of the *cyt-b* gene produced fragments of approximately 1150 bp, which were subsequently trimmed to a uniform length of 956 bp for consistency with previous published datasets. The analysis revealed 36 distinct haplotypes (Table 1).

All novel mitochondrial *cyt-b* sequences generated in this study have been deposited in GenBank under accession numbers PV792936-PV792959.

In the haplotype network, the sequences clustered into two well-supported mitochondrial lineages corresponding to the established *T. souffia* and *T. muticellus cyt-b* clades (SOU and MUT, respectively) described in earlier studies (Machordom et al., 1999;

Dubut et al., 2012; Buj et al., 2017) (Fig. 2). In total, 19 haplotypes fell within SOU and 17 within MUT.

In addition, the published sequence from the Soča River near Gradisca (AY509852; Ketmaier et al., 2004) clustered within the *T. muticellus* clade.

Mitochondrial DNA diversity

Mitochondrial DNA diversity metrics for *T. souffia* in the Sava drainage, *T. souffia* in the middle Soča (excluding Volarja), *T. muticellus* in the Italian sampled rivers and lower Soča, and the admixed Volarja population are summarized in Table 2.

In *T. souffia*, nucleotide diversity was uniformly low across both drainages ($\pi \approx 0.0014$), with similarly low average numbers of nucleotide differences, whereas haplotype diversity differed markedly between drainages, being higher in the Sava drainage (Hd $\approx$ 0.81) than in the middle Soča (Hd $\approx$ 0.58; Table 2). Despite this contrast, the number of segregating sites differed only slightly between drainages, indicating that the middle Soča haplotype pool represents a reduced subset of the broader Sava assemblage.

The Volarja Stream showed a strikingly different pattern, characterized by very high nucleotide diversity and average pairwise differences ($\pi \approx 0.03$; $k \approx 29$) despite low haplotype richness ($h = 4$; Table 2).

By comparison, *T. muticellus* from Italian rivers and the lower Soča exhibited intermediate levels of mitochondrial diversity relative to both *T. souffia* drainages and the Volarja population (Table 2).

Haplotype geographic distribution

In the Sava drainage, only haplotypes belonging to the *cyt-b* haplogroup—here denoted as SOU—associated with *T. souffia* in previous studies were detected. In the Adriatic basin, the same haplogroup occurred throughout the middle Soča. The only exception was the Volarja Stream, where haplotypes characteristic of *T. muticellus*—MUT haplogroup—were found in three individuals (Table 1).

The most common SOU haplotype, SOU5, was similarly represented in both the Sava drainage and middle Soča. Other SOU haplotypes showed clear geographic patterns, occurring either exclusively or predominantly within a single basin. For example, SOU7 was frequent across all middle Soča localities but appeared at only a single site in the eastern Slovenian Sava range, whereas SOU1 occurred exclusively in the Sava drainage (Table 1).

No phylogeographic structure was detected between SOU haplotypes from the Sava drainage and those from the middle Soča (Fig. 2).

Haplotypes from the MUT lineage were present in all sampled Italian rivers except the Soča at Gorizia (site 13). MUT haplotypes were also found in the Nadiža and in the Brda watercourses; however, only one individual carrying a SOU haplotype (SOU7) was detected in the Nadiža near Orsaria (site "Nadiža 1").

The haplotype recovered from the Vipava (MUT49), as well as the AY509852 sequence (MUT48) from the Soča at Gradisca (Ketmaier et al., 2004), both clustered within the MUT haplogroup, with the latter showing substantial divergence relative to other MUT haplotypes.

Microsatellite DNA

Only specimens with complete genotypes at a minimum of eight microsatellite loci—i.e., with no missing or null alleles at those loci—were retained for the final analysis. This filtering resulted in a dataset of 304 *Telestes* individuals (Table S1).

Total number of alleles across all loci (Aall), average number of alleles per locus (Aave), and expected (Hexp) and observed heterozygosity (Hobs) for each population are provided in Table S1. Aave ranged from 2.7 (Laknica) to 10.91 (Tagliamento), and Hobs from 0.39 (Laknica) to 0.82 (Chiarò). Observed heterozygosity (Hobs) was highest in *T. muticellus* and lowest in *T. souffia* from the middle Soča. Estimates of Hexp and Hobs were not calculated for populations with small sample sizes ($n < 8$).

Factorial correspondence analysis

FCA based on microsatellite genotypes revealed two clearly separated genetic clusters along the first axis (Fig. 3a). Cluster identity was defined using reference populations with known species status: samples from Italian rivers formed the *T. muticellus* reference cluster, whereas samples from the Sava drainage defined the *T. souffia* cluster. Most individuals from the Brda region and the Nadiža river system grouped with *T.*

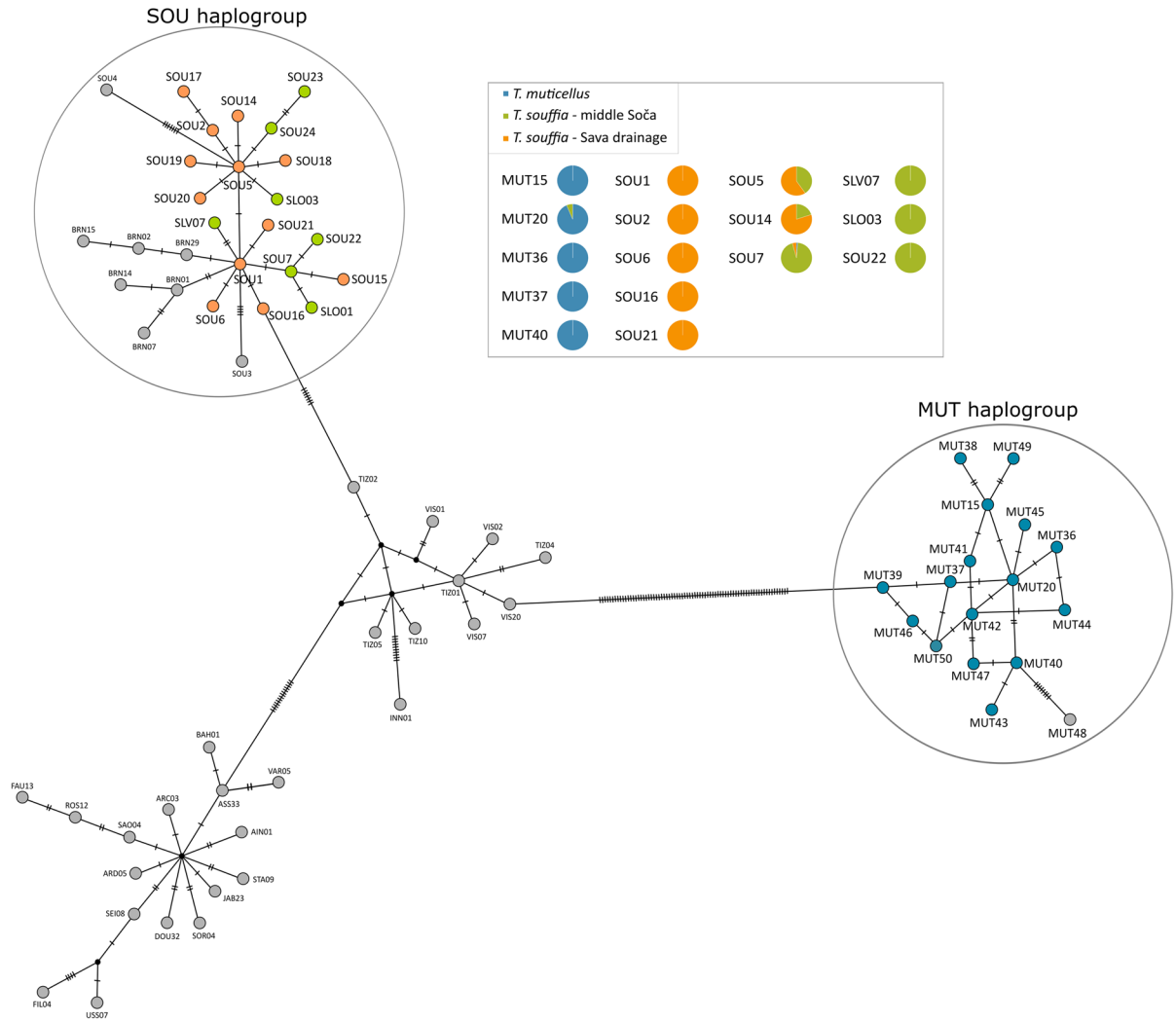


Fig. 2 Haplotype network based on *cyt-b* sequences. Two distinct clusters are apparent: a compact *T. muticellus* cluster on the right (blue), and a more heterogeneous *Telestes souffia* cluster on the left. The latter includes haplotypes from this study (top left, color-coded) as well as reference sequences from the Tisa River (central cluster) and the Rhône basin (bottom left). Colors indicate the predominant geographic distribution of haplotypes observed in this study: orange—primarily in the Sava drainage; green—primarily in the Soča drainage. Pie charts represent relative haplotype frequencies; hatch marks denote the number of mutational steps. Reference sequences (in gray) are from Ketmaier et al. (2004), Dubut et al. (2012), Buj et al. (2017), and Veličković et al. (2020)

muticellus, while samples from the middle Soča (excluding Volarja) clustered with *T. souffia*.

Most individuals from the Volarja were positioned within the *T. souffia* cluster, although several occupied intermediate positions and one fell at the margin of the *T. muticellus* cluster, consistent with localized admixture. A single Nadiža individual carrying a *T. souffia* mitochondrial haplotype was located between the two clusters, whereas a few other Nadiža individuals occurred near the boundary of the *T. muticellus* cluster. The Vipava (Močilnik) individual grouped with *T. muticellus* along the first axis but showed a pronounced divergence along the second axis, indicating a distinct genetic position relative to all other samples (Fig. 3a).

After excluding the Vipava individual (Fig. S1), the cohesion or separation of the two main species-level clusters were not affected, but resolution among

Table 2 Mitochondrial DNA diversity indices for four geographic groups of *Telestes* spp. based on *cyt-b* sequences

Geographic group	N	S	h	Hd	π	k
T. souffia — Sava drainage	96	12	13	0.807	0.00139	1.331
T. souffia — middle Soča excluding Volarja	86	10	9	0.581	0.00135	1.295
T. muticellus — Italian rivers-lower Soča	99	90	16	0.759	0.00319	3.048
Telestes sp. — Volarja	15	85	4	0.619	0.03056	29.219

Values are shown for *T. souffia* in the Sava drainage, *T. souffia* in the middle Soča (excluding Volarja), *T. muticellus* in Italian rivers and the lower Soča, and the admixed Volarja population. N = number of sequences; S = number of segregating sites; h = number of haplotypes; Hd = haplotype diversity; π = nucleotide diversity; k = average number of nucleotide differences

T. souffia samples increased along the second axis. In this reduced dataset, Selška Sora formed a small drift-driven outlier positioned below the main Sava cluster. After subsequently removing the Selška Sora samples, the *T. souffia* populations arranged themselves along a clear geographic gradient—from the middle Soča through the Poljanska Sora, Bohinjka and central Sava, to the eastern Sava tributaries (Krka, Savinja, Mirna) (Fig. 3b). Throughout all analytical steps, Volarja individuals retained intermediate positions between the two species clusters.

Genetic structure and geographic distribution

STRUCTURE analyses (Fig. 4) identified K = 2 and K = 8 as the most likely numbers of clusters according to the ΔK criterion (Evanno et al., 2005) and the MaxMean K method (Puechmaille, 2016), respectively.

At K = 2, individuals were assigned to two primary clusters corresponding to the species-level division between *T. muticellus* and *T. souffia*, with no evidence of widespread genetic mixing.

At K = 3, the *T. souffia*-associated cluster split into two components: one predominant in the middle Soča (excluding Volarja) and another more common in the Sava drainage. These components appeared in variable proportions in upper Sava tributaries (e.g., Bohinjka, Poljanska Sora) but did not follow a consistent spatial pattern.

At K = 4–6, additional clusters emerged that were increasingly localized to individual tributaries (e.g., Selška Sora, which formed the first such cluster at K = 4 and persisted across all higher K values). These fine-scale subdivisions did not correspond to major drainage boundaries, either between the middle Soča and Sava or within the Sava drainage.

This trend continued at K = 8, where clusters reflected fine-scale, tributary-level drift rather than major hydrological divisions (Fig. S2).

The single specimen from the Vipava (Močilnik) was assigned to the *T. muticellus* cluster at K = 2, and this dominant assignment persisted across higher K values. Minor fractions of *T. souffia*-associated components appeared only at K ≥ 4. Because this sample represents a single individual, no population-level inference can be drawn for this locality.

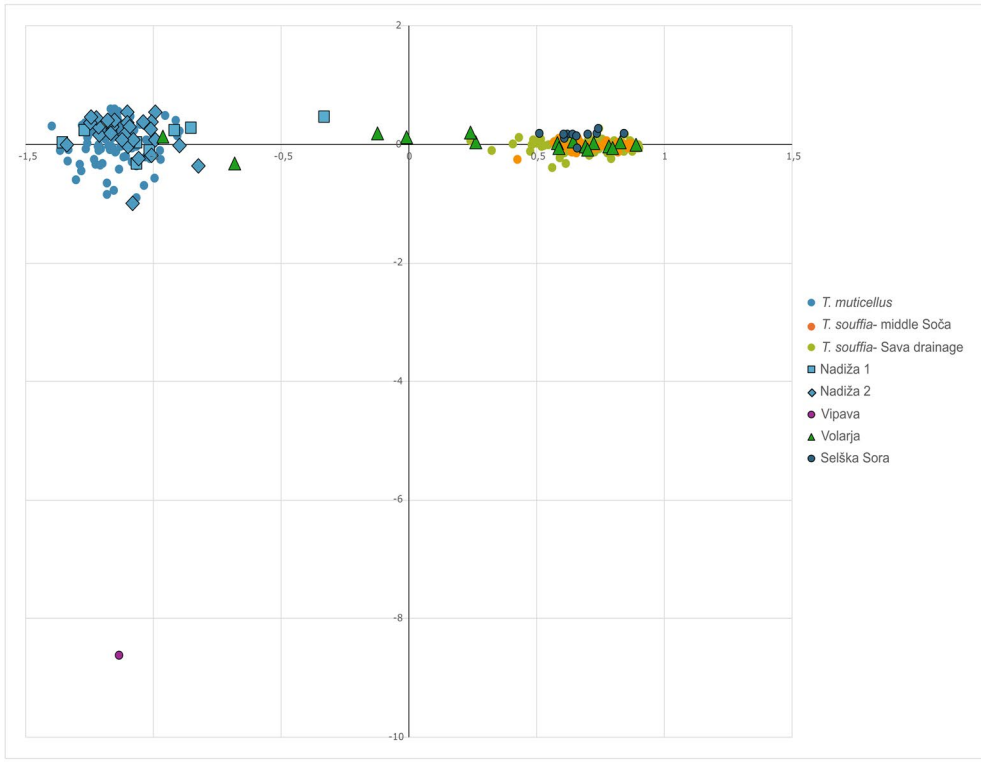
A consistent admixture signal was detected in the Volarja population, where individuals showed mixed assignment to *T. souffia*- and *T. muticellus*-associated clusters across all K values (K = 2–K = 8). A similar but weaker pattern occurred in the Nadiža 1 samples, where low-level assignment to the alternative species cluster persisted across all K values. One individual in particular—carrying a SOU haplotype—displayed an elevated level of inferred admixture (ca 50%), highlighting this case as the strongest admixture signal within the Nadiža (Fig. 4, Nadiža 1).

Additional population structure analysis based on AMOVA

AMOVA was performed to quantify how microsatellite variance is partitioned among hierarchical groupings (Table 3). Four comparisons were conducted:

(i) *T. souffia* vs. *T. muticellus*,
(ii) *T. souffia* from the Soča vs. Sava drainages,
(iii) The same comparison excluding the Volarja population, and.
(iv) *T. souffia* grouped into three regional clusters (Soča; Idrijca + Sora; Sava).

A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-0,5
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-2
-4
-6
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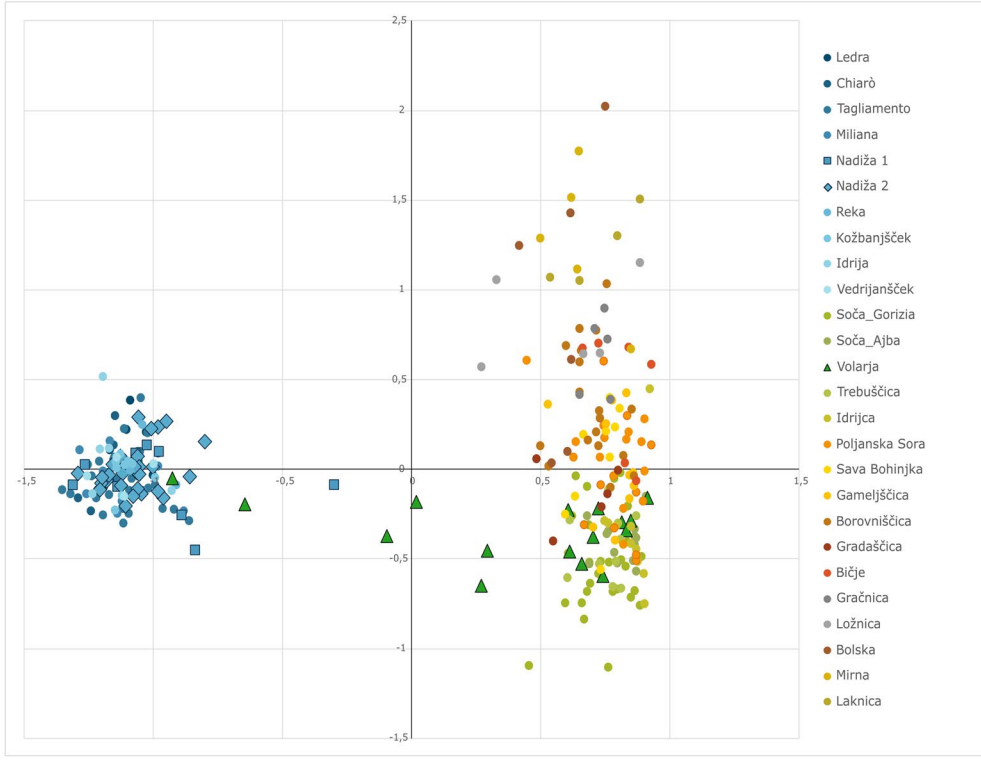
-10
T. muticellus
T. souffia- middle Soča
T. souffia- Sava drainage
Nadiža 1
Nadiža 2
Vipava
Volarja
Selška Sora

B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Chiarò
Tagliamento
Miliana
Nadiža 1
Nadiža 2
Reka
Kožbanjšček
Idrija
Vedrijanšček
Soča_Gorizia
Soča_Ajba
Volarja
Trebuščica
Idrijca
Poljanska Sora
Sava Bohinjka
Gameljščica
Borovniščica
Gradaščica
Bičje
Gračnica
Ložnica
Bolska
Mirna
Laknica

◂**Fig. 3** Factorial correspondence analysis (FCA) of multilocus microsatellite genotypes, showing the distribution of samples along the first and second axes. **a.** FCA of all sampled individuals. The first axis separates two species-level clusters corresponding to *Telestes muticellus* (Italian rivers, Brda, and most Nadiža individuals) and *T. souffia* (Sava drainage and middle Soča, excluding Volarja). Volarja individuals mostly cluster with *T. souffia* but include intermediate positions and one individual near the *T. muticellus* cluster. A single Nadiža individual lies between clusters. The Vipava (Močilnik) individual aligns with *T. muticellus* along the first axis but diverges along the second. **b.** FCA excluding the Vipava (Močilnik) and Selška Sora individuals. The separation of the two species-level clusters remains unchanged, while resolution among *T. souffia* populations increases along the second axis

Across all analyses, the majority of genetic variance resided within populations (76.9–96.0%). Among-group variance was substantial only in the interspecific comparison (19.8%) and was comparatively low in all within-*T. souffia* analyses. Excluding the admixed Volarja population reduced among-group variance to 0.37%, indicating that this locality disproportionately influenced differentiation estimates.

These AMOVA patterns are consistent with weak genetic structuring within *T. souffia* relative to the stronger species-level separation from *T. muticellus*.

Discussion

Telestes souffia across the Adriatic–Sava drainage boundary

Our microsatellite dataset provides a coherent biogeographic framework for interpreting the presence, structuring, and divergence of *Telestes* lineages across the Adriatic–Sava drainage boundary. Unlike many freshwater taxa for which stocking, translocations, or incomplete datasets obscure historical patterns, *T. souffia* in Slovenia has remained largely unmanaged, making it an informative model for natural drainage-crossing processes. A long-standing question is whether the occurrence of *T. souffia* in the middle Soča is of natural origin or reflects past introductions. Although early reports listed *T. muticellus* in this region (Munda, 1926; Povž, 1988), subsequent morphological, allozyme, and mitochondrial analyses have consistently identified these populations as *T. souffia* (Machordom et al., 1999; Gilles et al., 2010; Dubut et al., 2012; Buj et al., 2017; Bertoli et al., 2024), a conclusion corroborated by our mtDNA and microsatellite data.

Our results show that *T. souffia* in the middle Soča bears genetic signatures of long-term natural persistence. Although overall mtDNA diversity differs only slightly between the middle Soča (excluding Volarja) and the Sava drainage, the shallow *cyt-b* divergence typical of within-species Leuciscinae lineages (Perea et al., 2010) limits the power of nucleotide diversity to distinguish between historical separation and recent founder events. More informative is the structure of haplotype variation: the middle Soča exhibits a reduced and regionally shifted haplotype spectrum, including dominance of SOU7, the absence of several haplotypes common in the Sava drainage, and the presence of Soča-specific variants. Such a pattern is consistent with an older founder event followed by drift rather than with recent human-mediated introduction, which would be expected to replicate the haplotype composition of Sava populations more closely.

Nuclear markers reinforce this interpretation. The subtle but geographically coherent west–east differentiation in *T. souffia*, observed in both FCA and STRUCTURE analyses, suggests postglacial dispersal followed by restricted connectivity across the drainage boundary. Within the Sava drainage, upper tributaries retain relatively continuous connectivity, whereas central and eastern tributaries show stronger drift-driven differentiation, consistent with dendritic hydrology and variation in effective population sizes. Despite these drainage-internal contrasts, *T. souffia* across the upper Sava and middle Soča forms a shallow but spatially structured continuum.

Although STRUCTURE and FCA consistently detect this west–east pattern, AMOVA reveals only weak between-drainage variance. This discrepancy reflects the limited sensitivity of AMOVA to subtle, recent differentiation (Excoffier et al., 1992; Meirmans, 2006), whereas clustering and ordination approaches better resolve gradual, spatially patterned differentiation (Pritchard et al., 2000; Jombart et al., 2010).

Microsatellite-based diversity metrics (Table S1) varied among populations but are not discussed in detail due to uneven and generally small sample sizes. Similarly, measures of genetic differentiation (e.g., FST) were not included, as they would not yield robust or interpretable results.

Fig. 4 Bayesian clustering results inferred with STRUCTURE. At K = 2, individuals are assigned to two primary clusters corresponding to the species-level division between *Telestes muticellus* and *T. souffia*. At K = 3, the *T. souffia* cluster splits into middle Soča and Sava drainage components. At K = 4–6, additional clusters are increasingly localized to individual tributaries, reflecting fine-scale structure. Volarja individuals show mixed assignment across all K values, whereas the Vipava (Močilnik) individual remains predominantly assigned to *T. muticellus*

Table 3 Partitioning of genetic variance (%) among hierarchical levels based on AMOVA of microsatellite loci

Comparison	Among groups (%)	Among populations within groups (%)	Within populations (%)
T. souffia vs. *T. muticellus*	19.80	3.24	76.96
T. souffia (Soča vs. Sava)	1.37	3.19	95.44
T. souffia, Volarja excluded	0.37	3.64	96.00
T. souffia in 3 groups	1.22	4.06	94.72

The variation distribution was quantified among the following hierarchical groupings: (i) *Telestes souffia* vs. *T. muticellus*; (ii) *T. souffia* grouped by drainage (Soča vs. Sava); (iii) the same comparison excluding the Volarja population; and (iv) *T. souffia* grouped into three regional clusters (Soča; Idrijca + Sora; Sava). Values represent the percentage of total genetic variance among groups, among populations within groups, and within populations

Taken together, mtDNA haplotype structure and nuclear allele-frequency patterns point to the same conclusion: *T. souffia* in the middle Soča represents a naturally colonized lineage that has persisted since postglacial times, with limited subsequent connectivity to the Sava drainage. Only the Volarja population deviates from this broad pattern and is considered separately in the hybridization section.

Native and non-admixed populations of *T. muticellus* in the Brda region

The distribution of *T. muticellus* in Slovenia has long been uncertain, with inconsistent reports and limited genetic confirmation (Povž, 1988; Povž et al., 2015; Semrajc, 2021). Our microsatellite dataset resolves this ambiguity by identifying several non-admixed populations within the Torre–Soča drainage. In addition to confirming the species in the Nadiža—a tributary of the Torre—we document, for the first time, its occurrence in the Brda watercourses (Idrija, Reka, Kožbanjšček, Vedrijanšček).

These results extend the known eastern range limit of *T. muticellus* and are fully consistent with the presence of the species in adjacent north-eastern Italian systems, including the Tagliamento, Chiarò, and Grado–Maranoriver systems, from which our Italian reference samples were obtained (see also Bertoli et al., 2024).

No *T. souffia* ancestry were detected in any population from the Brda watercourses, and *T. souffia* was absent from all sites in this region. This indicates a clear spatial delineation between the two species within the Soča drainage: *T. muticellus* dominates the western tributaries flowing toward the Torre–Soča, whereas *T. souffia* is confined to middle Soča. The Močilnik Stream in the Vipava river system likewise harbors apparently non-introgressed *T. muticellus*, further supporting its native status in the lower Soča drainage in Slovenia.

These non-admixed populations are biogeographically significant and carry direct conservation implications. Although *T. muticellus* is not currently included in Slovenian conservation or Natura 2000 frameworks (Semrajc, 2021), its confirmed native presence in the Brda watercourses provides a strong basis for future assessments and for considering this river system in national biodiversity inventories.

Hybridization and introgression

Putative contact between *T. souffia* and *T. muticellus* has been proposed at several sites within the Soča–Torre drainage, largely based on mitochondrial DNA indicating the co-occurrence of divergent mitochondrial lineages (Salzburger et al., 2003; Bertoli et al., 2024). However, the extent, direction, and spatial distribution of nuclear genetic exchange have remained unresolved given the inherent limitations of uniparental markers for inferring nuclear gene flow.

Our dataset provides the first drainage-wide assessment of these putative contact areas—including the Nadiža, the Volarja Stream, the lower Soča, and the Vipava system—and resolves their genetic structure using nuclear markers, enabling a level of inference not previously possible.

The Nadiža river system: native *T. muticellus* with rare and localized mtDNA introgression

The Nadiža river system has been suggested as a potential hybrid zone between *T. souffia* and *T.*

muticellus based on detections of *T. souffia* mtDNA (Salzburger et al., 2003; Bertoli et al., 2024). Our multilocus data instead confirm *T. muticellus* as the native species, consistent with regional biogeography. This interpretation is supported by the river's hydrological setting: the Nadiža is isolated from the upper and middle Soča, with separate glaciation histories and no evidence of postglacial reconnection (Diercks et al., 2021, 2022). Crayfish biogeography mirrors this drainage boundary, as *Austropotamobius torrentium* (Schrank, 1803) (Danubian) occurs only in the Idrijca, whereas *Austropotamobius pallipes* (Lereboullet, 1858) (Adriatic) is restricted to the Nadiža (Govedič, 2013).

Within this setting, *T. souffia* mtDNA has been detected only sporadically—once in the Cosizza tributary (Salzburger et al., 2003) and once farther downstream near Orsaria (Bertoli et al., 2024; this study). The latter individual also displays nuclear signatures consistent with partial introgression. In contrast, our extensive upper-Nadiža sampling revealed no *T. souffia* haplotypes, and nuclear markers show non-introgressed *T. muticellus* genotypes.

Taken together, the evidence does not support a broad or persistent hybrid zone. Instead, the Nadiža represents a system, where *T. muticellus* is native and where rare, localized maternal-line introgression can occur—whether through sporadic natural processes or occasional human-mediated fish movements—without giving rise to a stable hybrid population.

Volarja stream: a localized and likely anthropogenic admixture hotspot

The Volarja Stream is the only site within the Soča drainage that shows clear multilocus evidence of admixture between *T. souffia* and *T. muticellus*. The ancestry profiles of its individuals range from predominantly *T. souffia* to strongly *T. muticellus*-like, consistent with recent gene flow. The presence of both SOU and the deeply divergent MUT haplotypes supports genuine maternal-line introgression rather than incomplete lineage sorting.

The most parsimonious explanation for this pattern is human-mediated translocation. Records from the Tolmin Angling Club document repeated transfers of *T. muticellus* from the Nadiža to the middle Soča during severe droughts in 2003 and 2006, and the multilocus signatures observed in Volarja are fully consistent with such introductions followed by several generations of mixing. Local environmental conditions may have reinforced this pattern. Volarja exhibits slightly warmer water temperatures than the main Soča channel, which may have facilitated the persistence of introduced fish. In contrast, *Telestes* apparently does not occur in the main Soča channel, likely because lower temperatures there limit its persistence.

Viewed in this context, Volarja represents a localized and likely anthropogenic admixture hotspot within an otherwise genetically cohesive *T. souffia* lineage in the middle Soča.

Similar human-mediated admixture events have been documented in other non-managed freshwater fishes. In European sculpins (*Cottus* spp.), anthropogenic hydrological modifications and accidental translocations have facilitated secondary contact and even lineage replacement (Nolte et al., 2005).

In *Phoxinus*, several studies have suggested human influence on lineage distributions (Museth et al., 2007; Miró & Ventura, 2015; Sternberg et al., 2025), and recent ecohydrological work in karstic river systems emphasizes how natural hydrological complexity may obscure the distinction between natural dispersal and human-mediated processes (Reier et al., 2022).

The lower Soča drainage: fragmented habitats and constrained detection of introgression

Previous studies of *Telestes* in the lower Soča drainage reported *T. muticellus* downstream near Gradisca (Ketmaier et al., 1998, 2004) and *T. souffia* upstream near Gorizia (Bertoli et al., 2024), suggesting that the two species occur in geographic proximity, although the extent of any direct contact remains unknown. Our data confirm *T. souffia* in Gorizia but reveal only weak, low-level *T. muticellus* nuclear ancestry (~ 8%) restricted to a single individual.

However, interpreting such patterns requires caution. A series of long-standing hydropower dams at Gorizia (1886), Gradisca (1905), Podselo (1939), Ajba (1939), and Solkan (1984)—have fragmented the lower Soča for more than a century. These barriers strongly limit upstream movement in riffle-dwelling cyprinids and would rapidly erode any historical admixture signal, making contemporary detection

difficult. Thus, the apparent absence of introgression in Gorizia does not rule out prior contact.

The individual detected in the Močilnik Stream (Vipava river system) provides an additional but limited data point on the distribution of *T. muticellus* in the lower Soča drainage. While STRUCTURE consistently assigns this individual to the *T. muticellus* cluster, minor *T. souffia*-like ancestry appears at higher K values. Given the single-individual sample size and the known tendency of STRUCTURE to distribute small ancestry fractions among available clusters at high K, this signal should be interpreted with caution, and it does not allow any reliable inference regarding possible admixture. Nevertheless, *T. muticellus* in the Vipava system indicates that the species historically occupied parts of the Soča between Gorizia and Gradisca, a section lacking contemporary samples and where past species turnover may have gone unnoticed.

Synthesis: restricted hybridization and contact processes at the Soča–Sava drainage boundary

Hybridization between *T. souffia* and *T. muticellus* around the Adriatic–Sava drainage boundary is spatially restricted and reflects three distinct processes:

(i) Isolated introgression in the Soča–Torre–Nadiža network, potentially arising via limited natural hydrological connectivity or unrecorded human-mediated fish movements.
(ii) A zone of geographic proximity blurred by century-old migration barriers in the lower Soča, where historical contact is plausible but any genomic signal is difficult to recover; and.
(iii) Anthropogenic translocations, which have created a localized admixture hotspot in the Volarja stream.

Based on the material available, we detected no evidence for a broad or persistent hybrid zone within the sampled sections of the Soča drainage. Historical contact between *T. souffia* and *T. muticellus* is expected somewhere between Gradisca (*T. muticellus*) and Gorizia (*T. souffia*), but this reach of the Soča is difficult to sample and heavily fragmented by dams, making potential introgression signals challenging to recover.

Despite observed localized introgression events, multilocus evidence consistently supports the long-term integrity of *T. souffia* and *T. muticellus* as distinct evolutionary lineages. Both STRUCTURE and FCA robustly recover two cohesive species-level clusters, and admixture remains spatially restricted and low in magnitude. No signal of genome-wide homogenization is detected, even in regions of geographic proximity.

Taken together, these patterns clarify the distributional limits of both species, refine the biogeographic interpretation of the Adriatic–Sava drainage boundary, and distinguish between natural processes and human-mediated admixture—information directly relevant for regional conservation planning and for interpreting species delineation.

Conclusions

Using a multilocus genetic approach, we show that *T. souffia* in Slovenia forms a genetically coherent lineage characteristic of the Sava drainage, with the middle Soča population representing a consistently differentiated offshoot derived through natural postglacial colonization rather than recent anthropogenic translocation. This pattern provides evidence for historical permeability of the Adriatic–Danube drainage divide following the last glaciation.

Despite the geographic proximity of *T. souffia* and *T. muticellus* in the lower Soča, no extant hybrid zone was detected. The absence of a clear admixture signal does not exclude past contact or introgression, as long-term river fragmentation by dams likely accelerated genetic drift and eroded historical genomic signatures in this reach.

Our analyses further confirm the native status of *T. muticellus* in the Brda watercourses, resolving long-standing taxonomic uncertainty and providing a basis for refining Natura 2000 site delineations in western Slovenia. In contrast, mismatches between expected and observed species occurrence at a few localities—notably Volarja and, to a lesser extent, the Nadiža—are best explained by localized, likely human-mediated translocations rather than natural

range dynamics, as indicated by their sporadic occurrence and the absence of stable hybrid populations.

Overall, our results disentangle natural postglacial colonization from recent anthropogenic admixture, sharpen species boundaries across a biogeographically complex region, and underscore the importance of multilocus data for conservation-relevant species delimitation in fragmented freshwater systems.

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Author contributions A.S. developed the conceptual framework of the manuscript, integrated and interpreted the results in a broader biogeographic and evolutionary context, and wrote the manuscript. D.Z. commented on the manuscript during its preparation, contributed to discussions on the studied topics, and coordinated sampling in Slovenia. A.J. contributed to discussions on the studied topics and prepared the cartographic material. C.M. contributed to the analysis of mitochondrial DNA from Italian samples, assisted in integrating the Italian data into the broader framework of the study, and commented on the manuscript during its preparation. M.B. performed laboratory work and mitochondrial DNA sequence analyses on Italian samples. S.S.B. performed laboratory work on the entire sample set, conducted data processing and analyses, prepared data visualizations, contributed to discussions on the studied topics, and commented on the manuscript during its preparation.

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Data availability Novel mitochondrial *cyt-b* sequences generated in this study have been deposited in GenBank under accession numbers PV792936–PV792959. All other data are included in the article and its Supplementary Information.

Declarations

Conflict of interests The authors declare that they have no competing interests.

Ethical approval All sampling was conducted under a permit issued by the Ministry of Natural Resources and Environment, authorizing the Fisheries Research Institute of Slovenia (FRIS) to handle and sample live fish for scientific purposes, in accordance with relevant national and institutional guidelines.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Bandelt, H.-J., P. Forster & A. Röhl, 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste & F. Bonhomme, 1996–2004. GENETIX v.4.04: Logiciel pour la génétique des populations. Université de Montpellier II, Montpellier.

Bertoli, M., C. Manfrin, P. G. Giulianini, A. Pallavicini, P. Pastorino & E. Pizzul, 2024. New insights into the distribution areas of *Telestes souffia* and *Telestes muticellus*: changes and overlapping zones for community interest fish species. Diversity 16: 713. https://doi.org/10.3390/d16120713.

Bianco, P. G., 1994. L'ittiofauna continentale dell'Appennino umbromarchigiano, barriera semipermeabile allo scambio di componenti primarie fra gli opposti versanti dell'Italia centrale. Biogeographia 17: 427–485.

Buj, I., Z. Marčić, M. Ćaleta, R. Šanda, M. F. Geiger, J. Freyhof, A. Machordom & J. Vukić, 2017. Ancient connections among European rivers and watersheds revealed from the evolutionary history of the genus *Telestes* (Actinopterygii, Cypriniformes). PLoS ONE 12: e0187366. https://doi.org/10.1371/journal.pone.0187366.

Costedoat, C. & A. Gilles, 2009. Quaternary patterns of freshwater fishes in Europe: comparative phylogeography and conservation perspectives. The Open Conservation Biology Journal 3: 36–48.

Crivelli, A. J. & P. G. Bianco, 2006. *Telestes muticellus*. IUCN Red List of Threatened Species. 2006: T61389A12458554. https://doi.org/10.2305/IUCN.UK.2006.RLTS.T61389A12458554.en.

Diercks, M., C. Grützner, M. Vrabec & K. Ustaszewski, 2021. A model for the formation of the Pradol (Pradolino) dry valley in western Slovenia and north-eastern Italy. Geologija 64: 21–33. https://doi.org/10.5474/geologija.2021.002.

Diercks, M., C. Grützner, M. Vrabec & K. Ustaszewski, 2022. Addendum to Diercks et al, 2022: A model for the formation of the Pradol (Pradolino) dry valley in W Slovenia and NE Italy. Geologija 65(1): 93–99.

Dubut, V., A. Fouquet, A. Voisin, C. Costedoat, R. Chappaz & A. Gilles, 2012. From Late Miocene to Holocene: processes of differentiation within the genus *Telestes* (Actinopterygii, Cyprinidae). PLoS ONE 7: e34423. https://doi.org/10.1371/journal.pone.0034423.

Evanno, G., S. Regnaut & J. Goudet, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier, L. & H. E. L. Lischer, 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Excoffier, L., P. E. Smouse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes. Genetics 131: 479–491.

Ford, M., 2024. *Telestes souffia*. IUCN Red List of Threatened Species 2024: eT61397A137342065. https://doi.org/10.2305/IUCN.UK.2024-2.RLTS.T61397A137342065.en.

Gilles, A., R. Chappaz, L. Cavalli, M. Lörtscher & E. Faure, 1998. Genetic differentiation and introgression between putative subspecies of *Leuciscus souffia* (Teleostei, Cyprinidae) in the Mediterranean Alps. Canadian Journal of Fisheries and Aquatic Sciences 55: 2341–2354.

Gilles, A., C. Costedoat, B. Barascud, A. Voisin, P. Banarescu, P. G. Bianco, P. S. Economidis, D. Marić & R. Chappaz, 2010. Speciation pattern of the *Telestes souffia* complex in Europe inferred from morphological and molecular markers. Zoologica Scripta 39: 225–242. https://doi.org/10.1111/j.1463-6409.2010.00417.x.

Govedič, M., 2013. Potočni raki v naših večjih rekah. Ribič 7–8: 203–206.

Hamilton, P. B. & C. R. Tyler, 2007. Identification of microsatellite loci for parentage analysis in roach *Rutilus rutilus* and eight other cyprinid fish by cross-species amplification, and a novel test for detecting hybrids between roach and other cyprinids. Molecular Ecology Notes 8: 462–465.

Hewitt, G. M., 1999. Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68: 87–112.

Hewitt, G. M., 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907–913.

Jombart, T., S. Devillard & F. Balloux, 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11: 94.

Ketmaier, V., M. Cobolli, E. De Matthaeis & P. G. Bianco, 1998. Allozymic variability and biogeographic relationships in two *Leuciscus* species complexes (Cyprinidae) from southern Europe, with the rehabilitation of the genus *Telestes* Bonaparte. Italian Journal of Zoology 65: 41–48. https://doi.org/10.1080/11250009809386793.

Ketmaier, V., P. G. Bianco, M. Cobolli, M. Krivokapić, R. Caniglia & E. De Matthaeis, 2004. Molecular phylogeny of two lineages of Leuciscinae cyprinids (*Telestes* and *Scardinius*) from the peri-Mediterranean area. Molecular Phylogenetics and Evolution 32: 1061–1071. https://doi.org/10.1016/j.ympev.2004.04.008.

Kottelat, M. & J. Freyhof, 2007. Handbook of European freshwater fishes. Cornol & Berlin, 646 pp

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547–1549.

Leigh, J. W. & D. Bryant, 2015. PopART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6: 1110–1116.

Li, Y. L. & J. X. Liu, 2018. StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. Molecular Ecology Resources 18: 176–177.

Librado, P. & J. Rozas, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Machordom, A., Y. Nicolas & A. J. Crivelli, 1999. Genetic variability and differentiation in Leuciscus (Telestes) souffia Taxonomic and conservation inferences. Comptes Rendus De l'académie des Sciences-Serie III 322(1): 15–28.

Marchetto, F., S. Zaccara, F. M. Muenzel & W. Salzburger, 2010. Phylogeography of the Italian vairone (*Telestes muticellus*, Bonaparte 1837) inferred by microsatellite markers: evolutionary history of a freshwater fish species with a restricted and fragmented distribution. BMC Evolutionary Biology 10: 111. https://doi.org/10.1186/1471-2148-10-111.

Meirmans, P. G., 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60: 2399–2402.

Miller, S. A., D. D. Dykes & H. F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16: 1215.

Miró, A. & M. Ventura, 2015. Evidence of exotic trout-mediated minnow invasion in Pyrenean high mountain lakes. Biological Invasions 17: 791–803.

Munda, A., 1926. Ribe v slovenskih vodah. Slovensko ribarsko društvo, Ljubljana, 63 pp.

Museth, J., T. Hesthagen, O. T. Sandlund, E. B. Thorstad & O. Ugedal, 2007. The history of the minnow *Phoxinus phoxinus* (L.) in Norway: from harmless species to pest. Journal of Fish Biology 71: 184–195. https://doi.org/10.1111/j.1095-8649.2007.01673.x.

Nolte, A. W., J. Freyhof, K. C. Stemshorn & D. Tautz, 2005. An invasive lineage of sculpins (*Cottus* sp.) in the Rhine has originated from hybridization between old

phylogeographic groups. Proceedings of the Royal Society B 272: 2379–2387. https://doi.org/10.1098/rspb.2005.3231.

Perea, S., M. Böhme, P. Zupančič, J. Freyhof, R. Šanda, M. Özuluğ, A. Abdoli & I. Doadrio, 2010. Phylogenetic relationships and biogeographical patterns in circum-Mediterranean Leuciscinae (Teleostei, Cyprinidae) inferred from mitochondrial and nuclear data. BMC Evolutionary Biology 10: 265.

Podgornik, S., N. Pajk, B. Bric, B. Marčeta & R. Hamzič, 2015. Monitoring populacij izbranih ciljnih vrst rib: blistavec (*Telestes souffia*) in primorski blistavec (*Telestes muticellus*). Poročilo, Zavod za ribištvo Slovenije, Ljubljana, 67 pp.

Povž, M., A. Gregori & M. Gregori, 2015. Sladkovodne ribe in piškurji v Sloveniji. Zavod Umbra, Ljubljana, 2005.

Povž, M., 1988. Favna Slovenije (Pisces, Decapoda), njena ogroženost in varstvo: Leuciscus souffia Risso 1826. Zavod za ribištvo Ljubljana, 42 pp.

Pritchard, J. K., M. Stephens & P. Donnelly, 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Puechmaille, S. J., 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources 16: 608–627.

Reier, S., L. Kruckenhauser, A. Snoj, P. Trontelj & A. Palandačić, 2022. The minnow *Phoxinus lumaireul* (Leuciscidae) shifts the Adriatic-Black Sea basin divide in the north-western Dinaric Karst region. Ecohydrology 15: e2449. https://doi.org/10.1002/eco.2449.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins & A. Sánchez-Gracia, 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34: 3299–3302.

Salzburger, W., A. Brandstätter, A. Gilles, W. Parson, M. Hempel, C. Sturmbauer & A. Meyer, 2003. Phylogeography of the vairone (*Leuciscus souffia*) in Central Europe. Molecular Ecology 12: 2371–2386. https://doi.org/10.1046/j.1365-294X.2003.01911.x.

Semrajc, B., 2021. Monitoring populacij izbranih vrst rib: primorski blistavec (*Telestes muticellus*). Poročilo, Zavod za ribištvo Slovenije, Ljubljana, 22 pp.

Stefani, F., P. Galli, S. Zaccara & G. Crosa, 2004. Genetic variability and phylogeography of the cyprinid *Telestes muticellus* within the Italian peninsula as revealed by mitochondrial DNA. Journal of Zoological Systematics and Evolutionary Research 42: 323–331. https://doi.org/10.1111/j.1439-0469.2004.00272.x.

Sternberg, N., A. Bodenheim, T. O. Oriowo, L. Podsiadlowski & M. Stange, 2025. Human impacts on the distribution and genetic diversity of Eurasian minnows (*Phoxinus*: Leuciscidae) in the Rhenish Massif. Knowledge and Management of Aquatic Ecosystems 2025: 15. https://doi.org/10.1051/kmae/2025015.

Sušnik Bajec, S., G. Pustovrh, D. Jesenšek & A. Snoj, 2015. Population genetic SNP analysis of marble and brown trout in a hybridization zone of the Adriatic watershed in Slovenia. Conservation Genetics 148: 239–250.

Sušnik Bajec, S. & L. Mrzelj, 2022. Genetske analize tujerodnih in domorodnih vrst rib (Podnebni sklad). Poročilo, Zavod za ribištvo Slovenije, Ljubljana, 48 pp.

Thompson, J. D., D. G. Higgins & T. J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4637–4680.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills & P. Shipley, 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Veenvliet, P. & J. Kus Veenvliet, 2006. Ribe slovenskih celinskih voda: priročnik za določanje. Zavod Symbiosis, Grahovo.

Veličković, T. Z., V. M. Simić, R. Šanda, M. D. Radenković, A. M. Milošković, N. M. Radojković & S. P. Marić, 2020. New record of a population of *Telestes souffia* (Risso, 1827) (Actinopterygii, Cyprinidae) in Serbia. Acta Zoologica Bulgarica 72: 13–20.

Zaccara, S., F. Stefani & G. B. Delmastro, 2007. Phylogeographical structure of vairone *Telestes muticellus* (Teleostei, Cyprinidae) within three European peri-Mediterranean districts. Zoologica Scripta 36: 443–453. https://doi.org/10.1111/j.1463-6409.2007.00294.x.

Zardoya, R. & I. Doadrio, 1998. Phylogenetic relationships of Iberian cyprinids. Proceedings of the Royal Society B 265: 1365–1372.