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Research Article

Small, isolated, and critically endangered: conservation genetics of the European mudminnow (*Umbra krameri*) in Austria

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Abstract

Floodplain specialist fishes have undergone disproportionate declines across Europe due to river regulation and groundwater alteration. The European mudminnow (*Umbra krameri*), now Critically Endangered in Austria, persists naturally in only two isolated populations (Fadenbach, Jesuitenbach). Field surveys were combined with microsatellite analyses to assess population structure and genetic diversity in both wild populations and in four *ex situ* stocks established from Fadenbach. Wild populations were strongly differentiated, indicating prolonged isolation without contemporary gene flow. Despite marked demographic fluctuations and recent habitat desiccation, both populations retained moderate levels of heterozygosity and showed no significant signal of a recent bottleneck. Whereas the estimated contemporary effective population size was critically low for the Fadenbach population, no robust estimate could be obtained for the Jesuitenbach population. All *ex situ* populations derived exclusively from Fadenbach and collectively retained their allelic diversity, yet showed significant divergence from one another due to founder effects and drift. These results thus identify two genetically distinct conservation units and reveal acute vulnerability masked by currently stable diversity metrics. Securing *U. krameri* in Austria will require hydrological restoration of suitable habitats, protection of both wild populations, and coordinated genetic management integrating *in situ* and *ex situ* conservation.

Key words: *ex situ* conservation, fish, floodplain species, microsatellites, population decline, Umbridae

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Introduction

Fishes associated with muddy, low-flow habitats, such as floodplain backwaters, oxbow lakes, marshes, and drainage channels, have suffered especially pronounced declines across Europe during the past century (Freyhof and Brooks 2011; Costa et al. 2021). These habitats, characterized by fine sediments, low current velocities, high organic content, and strong seasonal dynamics, have undergone disproportionate losses due to river regulation, wetland drainage, agricultural intensification, and the disconnection of floodplains from main river channels (Tockner et al. 2022). As a consequence, fish assemblages specialized to such environments represent some of the most

threatened components of European freshwater biodiversity (Šmejkal et al. 2025). This is also true for the Danube basin, where the historical reduction of floodplain area and the simplification of lateral habitats have resulted in severe fragmentation and isolation of remnant populations of mud-associated fishes, such as the European weatherfish (*Misgurnus fossilis*), crucian carp (*Carassius carassius*), sunbleak (*Leucaspius delineatus*), and European mudminnow (*Umbra krameri*). Together with competition with and predation by invasive non-native fish species, hybridization between native and non-native species, especially in *Carassius*, and potential pathogen transmission by non-native species, this increases their vulnerability to demographic stochasticity and local extirpation (Šmejkal et al. 2025).

The European mudminnow (*Umbra krameri*), a habitat specialist of stagnant and slow-flowing waters with typically fine sediments at the bottom and low oxygen content (Wanzenböck 1995), exemplifies these broader patterns of decline. The species is currently classified as Vulnerable at the European scale, with population declines primarily attributed to habitat loss, hydrological alteration, and fragmentation across the Pannonian and Danube regions (Freyhof and Brooks 2011; Ford 2024). It is also listed in the Habitats Directive Annex II (European Commision 2013). Its current distribution is patchy across its entire range, the lowland drainage of the Danube from Vienna to the delta, as well as the southern reaches of the Dniestr basin (Lelek 1987; Kottelat and Freyhof 2007; Freyhof 2013; Bănăduc et al. 2022; Ford 2024). In eastern Austria, the species was historically widespread, with confirmed records from the Danube floodplains east of Vienna, the Leitha floodplains, wetlands near Moosbrunn, and Lake Neusiedl (Wanzenböck and Spindler 1995). Additional historical occurrences are assumed for several lowland river floodplains, e.g., Morava-Dyje system, Mur, Lafnitz, Feistritz, Rába, Strem, and Rabnitz, supported by the proximity of extant populations in neighboring countries (Woschitz 2006; Bănăduc et al. 2022; Wolfram et al. 2022).

Today, the species presumably occurs naturally in Austria at only two sites: the Fadenbach between Orth and Eckartsau within the Donau-Auen National Park and the spring area of the Jesuitenbach near Moosbrunn in the Natura 2000 site "Feuchte Ebene – Leithaauen" (Wanzenböck and Spindler 1995; Bănăduc et al. 2022) (Fig. 1). Extensive surveys, conducted across the species' former range in Austria within the Habitats Directive monitoring programs, and additional investigations over the past three decades indicate that the existence of further, as yet undiscovered, populations in Austria is highly unlikely. Monitoring under the Austrian Water Framework Directive primarily targets main river channels and certain stagnant water bodies within adjacent floodplains and thus does not encompass the specific habitat types suitable for *U. krameri*. Consequently, this program has not yielded, nor is it expected to yield, records of the species.

As a consequence of this dramatic range contraction, *U. krameri* is considered the most threatened fish species in Austria. Between 1975, when the species was last recorded in the Danube floodplains near Orth, and its rediscovery in the Fadenbach in 1992, it was even regarded as nationally extinct (Hacker 1983; Herzig-Straschil 1991; Wanzenböck 1992; Wanzenböck and Spindler 1995). The current Red Lists classify the species as Critically Endangered in Austria (Wolfram and Mikschi 2007; Ratschan et al. 2026).

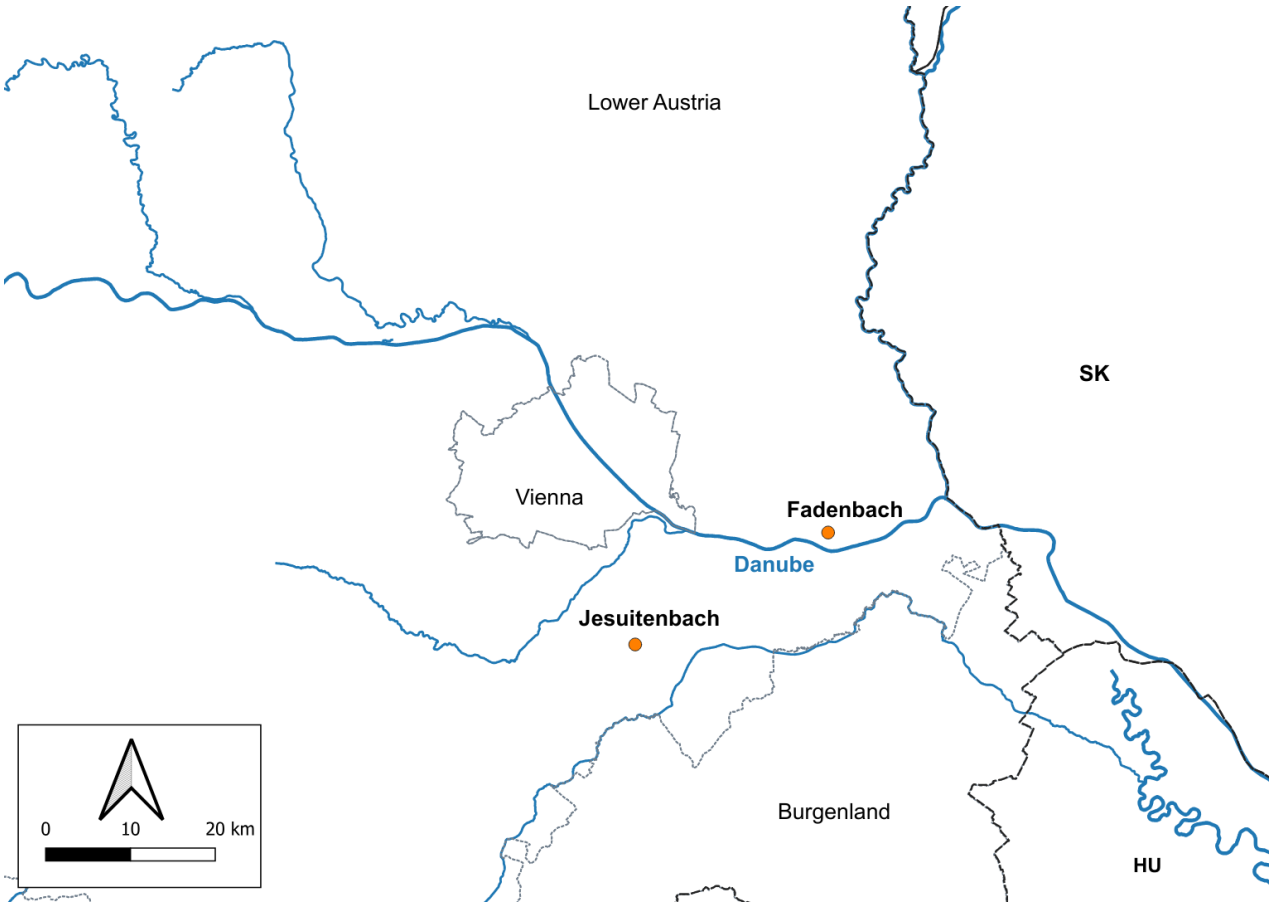

Figure 1. Location of the last two wild Austrian populations of *Umbra krameri*.

Following its rediscovery in 1992, the Fadenbach population became the focus of intensive research and targeted conservation measures. The Fadenbach is a former Danube side arm with a meandering course that has undergone substantial siltation and hydrological alteration, including partial disconnection by a flood protection dam. The species now inhabits only the landward section of the system, where water levels are primarily controlled by groundwater dynamics. During periods of high groundwater levels, the Fadenbach forms a largely continuous, weakly flowing channel system, whereas low groundwater levels result in fragmentation into a chain of isolated ponds. In addition to *U. krameri*, the Fadenbach is populated by other typical fish of the Danube floodplain, such as weatherfish (*Misgurnus fossilis*), rudd (*Scardinius erythrophthalmus*), crucian carp (*Carassius carassius*), bitterling (*Rhodeus amarus*), sunbleak (*Leucaspius delineatus*), tench (*Tinca tinca*), bleak (*Alburnus alburnus*), white bream (*Blicca bjoerkna*), pike (*Esox lucius*), tubenose goby (*Proterorhinus similunaris*), and non-native gibel carp (*Carassius gibelio*). Occasionally, other fish species immigrate from the main river during high water levels, increasing the number of recorded species to 29. Results from six electrofishing surveys from 1993 to 2025 are summarized in Jung et al. (2025a). To counteract progressive siltation, desiccation, and habitat fragmentation, a series of restoration measures were implemented from the late 1990s onward, including the deepening of connecting channels, the creation of refuge pools, and the general restoration of formerly infilled aquatic habitats to improve connectivity and persistence

during drought periods (Spindler 2006). In parallel with *in situ* habitat management, *ex situ* breeding of mudminnows originating from the Fadenbach population was initiated approximately 2000. Offspring from these breeding efforts were used in several reintroduction attempts in the Austrian Hanság wetlands (Benesch 2004), the Lobau floodplains, and areas adjacent to the Fadenbach on the Danube side of the flood protection dam (Spindler 2006). With the exception of the Hanság population, which persisted for at least 15 years after stocking but is currently considered extinct (Jung et al. 2024), these reintroduction efforts were likely unsuccessful, mainly due to desiccation or complete freezing during the winter months.

In contrast to the extensive management of the Fadenbach population, no targeted conservation measures have been implemented for the Jesuitenbach population. This population inhabits only the lentic spring area of the watercourse, extending for approximately 0.6 km. The habitat is surrounded by fens and characterized by much lower fish diversity than the Fadenbach system. The only accompanying fish species are roach (*Rutilus rutilus*) and pike (*Esox lucius*). Downstream of the spring area, in a more lotic section, more rhithral species such as brown trout (*Salmo trutta*), bullhead (*Cottus gobio*), gudgeon (*Gobio* sp.), minnow (*Phoxinus* sp.), and chub (*Squalius cephalus*) are also present (Jung et al. 2025a). Species composition indicates low summer temperatures, as would be expected for groundwater-fed water bodies, but detailed temperature data is lacking. The lotic section of the watercourse as well as nearby drainage ditches and streams is not populated by *U. krameri*.

Initially, conservation measures at the Fadenbach appeared effective, with the species expanding its range within the system and occurring locally in high densities (Sehr and Keckeis 2017). However, declining groundwater levels in recent years (Jung et al. 2025b) have resulted in a severe population decline. After extensive desiccation of the Fadenbach system in winter 2022 and spring 2023, no European mudminnows were detected during EU Habitats Directive monitoring in summer 2023. Emergency measures aimed at mitigating desiccation, including episodic water supplementation from Danube side arms during high-discharge events, may further threaten the species by introducing competitor fish species and elevated sediment loads. Together, these developments underscore the extreme vulnerability of *U. krameri* in Austria and highlight the urgent need for integrative conservation strategies, including DNA-based assessments, to inform future management and recovery efforts.

The present study examines genetic diversity and differentiation in Austria's two remaining wild populations of *U. krameri*, Fadenbach and Jesuitenbach, as well as in four *ex situ* populations derived from Fadenbach, using microsatellite markers. Characterizing the wild populations provides insights into their recent demographic dynamics and establishes a reference for the genetic integrity of the *ex situ* populations, i.e., for evaluating whether the *ex situ* stocks accurately represent the Fadenbach gene pool and were not mixed with fish from other source populations. These data are important for future conservation measures, including the optimization of *ex situ* breeding and potential reintroduction programs, supporting the long-term conservation of Austria's most threatened fish species.

Methods

Sampling was conducted under permits NÖ LFV-E-14/2023, NÖ LFV-E-57/2024, NÖ LFV-E-063/2025, and KOL2-F-091/015. In 2024, electrofishing surveys covered nearly the entire Fadenbach system. In total, 15 sections of the Fadenbach were surveyed, covering 3.3 km. Sampling was conducted from a boat in late October 2024, as water levels were sufficiently high, ensuring continuous flow throughout the system. In August 2025, sampling in Fadenbach focused on areas where mudminnows were detected in 2024. At this time, the Fadenbach was reduced to isolated pools, and all sampling was conducted by wading. Electrofishing in the Jesuitenbach near Moosbrunn was conducted mainly by boat in October 2024 and November 2025.

Sampling methods varied depending on water body type and water levels. Surveys were conducted either by wading or from a boat. Electrofishing equipment included a backpack unit (EFKO FEG 2000, 2.0 kW, 500 V DC idle voltage) for wading and a stationary unit (EFKO FEG 3000s, 3.0 kW, 620 V DC idle voltage) for boat-based sampling. The anode consisted of a metal ring (32 cm diameter) covered with net material (8 mm mesh size), while the cathode was a trailing copper band.

For all individuals captured in Fadenbach and most individuals from Jesuitenbach, total length (TL) was measured.

Mudminnows were also collected from four *ex situ* populations in garden ponds and the aquarium at the Vienna Zoo using electrofishing, traps, or dip nets.

In total, 191 DNA samples (fin clips) were taken between October 2024 and November 2025, 50 of which were collected from the Fadenbach, 61 from the Jesuitenbach, and the other 80 from the *ex situ* populations (Vienna Zoo, N = 20; pond and aquarium Pekny, N = 20; pond Engelhartszell, N = 9 — this is the whole population; pond Wanzenböck, N = 31). Samples were stored in 96% undenatured ethanol. The 47 individuals captured in Fadenbach in 2025 were transferred to the Donau-Auen National Park's captive breeding program and stocked into a dedicated conservation pond established for this purpose. A second conservation pond there was stocked with fish from the two *ex situ* populations "pond Engelhartszell" and "pond Wanzenböck" (Jung et al. 2025a).

Genetic analyses were conducted at the Institute of Biology of the University of Graz. Whole genomic DNA was extracted using the Quick-DNA Miniprep Plus Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's protocol. Eight microsatellite loci (UKTet1, UKTet3, UKTet4, UKTet5, UKTet6, UKTet7, UKTet8, UKTet9; Winkler and Weiss 2009) that are commonly used for *Umbra krameri* (Marić et al. 2015, 2017, 2019; Takács et al. 2015) were amplified in multiplex and single polymerase chain reactions (PCRs). Reaction cocktails with a final volume of 6 µL consisted of 1 µL DNA extract, 2.5 µL Type-it Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 0.5 µL primer mix (see Suppl. material 1: table S1), and 2 µL HPLC water. PCR success was checked on a 2% agarose gel prior to genotyping on an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Allele sizes were scored in GeneMapper 3.7 (ThermoFisher Scientific, Waltham, MA, USA). For a total of 189 out of the 191 initial samples, data of sufficient quality for further analysis could be generated (Suppl. material 1: table S2). The data were checked with Micro-Checker 2.2.3 (Van Oosterhout et al. 2004) for

potential null alleles and scoring errors. There was no indication of either null alleles or scoring errors in the dataset. Arlequin 3.5 (Excoffier and Lischer 2010) was used to calculate the number of alleles (N_A), observed (H_O), and expected heterozygosity (H_E) for each locus and population. Further, Arlequin was applied to test for Hardy-Weinberg equilibrium (with a Markov chain of 100,000 steps following 1000 dememorization steps), linkage disequilibrium (with 10,000 permutations), and the inbreeding coefficient (F_{IS}) for each population. Arlequin was also used to infer pairwise population genetic differentiation by means of F_{ST}. *P*-values were corrected for multiple testing following the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

Population genetic structure was also assessed using a Bayesian clustering method in STRUCTURE 2.3 (Pritchard et al. 2000), in which each sample was assigned to *K* clusters (correlated allele frequency model (Falush et al. 2003), burn-in 100,000, and run time 1,000,000 steps). For each number of clusters (*K* = 1–7), 10 runs were performed, and the most likely number of clusters was determined based on the Delta *K* statistic (Evanno et al. 2005) with the R package pophelper (Francis 2017). Pophelper was also used to summarize and visualize STRUCTURE results. In addition, population genetic structure was analyzed by principal component analysis (PCA) and discriminant analysis of principal components (DAPC) using the R package adegenet 2.1.11 (Jombart et al. 2010; Jombart and Ahmed 2011). Groups were defined *a priori* based on population of origin. To avoid overfitting, the optimal number of principal components (PCs) used for the DAPC was determined by cross-validation. The lowest number of PCs with the greatest discriminating power was used (35 PCs were retained).

The contemporary effective population size (N_e) of the two wild populations was estimated by means of the linkage disequilibrium (LD) method (Waples and Do 2010) in NeEstimator v2 (Do et al. 2014), a robust method across a wide range of potential demographic scenarios (Gilbert and Whitlock 2015). Alleles present only in a single heterozygote were excluded (Waples 2024).

To test the theoretical prediction that recent bottlenecks cause allelic diversity to decline more rapidly than heterozygosity, resulting in a transient excess of heterozygotes (Cornuet and Luikart 1996), the two-phase mutation model (TPM) implemented in Bottleneck 1.2 (Piry et al. 1999) was employed. The TPM is regarded as most appropriate for microsatellite data because it better reflects observed allele frequency distributions than either the stepwise mutation model (SMM) or the infinite alleles model (IAM) (Di Rienzo et al. 1994). For the TPM, it was assumed that 95% of mutations follow the SMM, while the remaining 5% follow the IAM, with the number of repeat units per multistep mutation drawn from a geometric distribution with variance = 12, following the recommendation of Piry et al. (1999). Statistical significance was assessed based on 10,000 coalescent simulations and one-tailed Wilcoxon signed-rank tests (Piry et al. 1999).

To detect a potential reduction in genetic diversity in the Fadenbach population over the last ~10 years, present-day diversity was compared with that reported in Marić et al. (2017) for individuals sampled between 2011 and 2015, based on the same seven loci as used by Marić et al. (2017) and by inferring rarefied allelic richness in HP-RARE (Kalinowski 2005), standardized to a sample size of 20 individuals to match Marić et al. (2017).

Results

In the course of this study, 640 individual *U. krameri* were captured in the Fadenbach (*N* = 81) and Jesuitenbach (*N* = 559). Length–frequency distributions were multimodal in the Fadenbach and bimodal in the Jesuitenbach, and this pattern was consistent across both sampling years. Larger specimens were more common in the Fadenbach, and young-of-the-year fish were very rare in the Fadenbach sample from 2024 (Fig. 2).

Microsatellite data suitable for downstream analyses were obtained for 189 of the 191 samples; for only two samples from Vienna Zoo, insufficient data were generated. The microsatellite markers showed low to moderate levels of polymorphism, with 2–15 alleles per locus across all populations and 1–11 alleles per locus and population (Table 1). Observed (H_O) and expected (H_E) heterozygosities ranged from 0 to 0.944 and from 0 to 0.881, respectively (Table 1). After correcting for multiple testing, no significant deviations from Hardy-Weinberg expectations and no significant linkage disequilibrium between any pair of loci were detected in any population. None of the populations showed a significant inbreeding coefficient, F_{IS} (Table 2).

Pairwise population comparisons revealed significant F_{ST} values among all six studied populations (Table 3), including comparisons involving the *ex situ* population from pond Engelhartszell. Although sample size for this population is small

Table 1. Microsatellite diversity in Austrian wild and *ex situ* populations of *Umbra krameri*.

Population		Locus								Mean (SD)
		UkrTet1	UkrTe3	UkrTet4	UkrTet5	UkrTet6	UkrTet7	UkrTet8	UkrTet9	
FB (*N* = 50)	N_A	6	11	3	3	2	5	3	9	5.250 (3.240)
	H_O	0.660	0.894	0.120	0.140	0.622	0.740	0.592	0.702	0.559 (0.280)
	H_E	0.792	0.881	0.115	0.200	0.505	0.678	0.670	0.779	0.578 (0.283)
JB (*N* = 61)	N_A	8	6	4	5	2	5	4	5	4.875 (1.727)
	H_O	0.738	0.475	0.246	0.350	0.255	0.443	0.689	0.667	0.481 (0.197)
	H_E	0.749	0.584	0.265	0.395	0.243	0.441	0.692	0.680	0.506 (0.198)
VZ (*N* = 18)	N_A	3	6	1	1	2	3	2	2	2.500 (1.604)
	H_O	0.944	0.500	0.000	0.000	0.625	0.647	0.611	0.167	0.437 (0.343)
	H_E	0.675	0.610	0.000	0.000	0.508	0.533	0.475	0.157	0.370 (0.274)
PE (*N* = 20)	N_A	4	4	1	2	2	3	2	4	2.750 (1.165)
	H_O	0.750	0.700	0.000	0.350	0.091	0.500	0.500	0.750	0.455 (0.290)
	H_E	0.665	0.622	0.000	0.296	0.091	0.417	0.507	0.665	0.408 (0.258)
EZ (*N* = 9)	N_A	3	2	1	2	2	3	2	4	2.375 (0.916)
	H_O	0.667	0.222	0.000	0.556	0.429	0.375	0.556	0.889	0.461 (0.258)
	H_E	0.621	0.209	0.000	0.425	0.363	0.508	0.503	0.752	0.422 (0.236)
WB (*N* = 31)	N_A	6	5	4	2	2	3	2	4	3.500 (1.512)
	H_O	0.935	0.633	0.100	0.032	0.154	0.433	0.287	0.233	0.351 (0.304)
	H_E	0.756	0.615	0.098	0.032	0.145	0.481	0.355	0.292	0.347 (0.257)
Mean	N_A	5.000	5.667	2.333	2.500	2.000	3.667	2.500	4.667	3.542 (1.694)
(SD)		(2.000)	(3.011)	(1.506)	(1.378)	(0.000)	(1.033)	(0.837)	(2.338)	
Total (SD)	N_A	9	15	5	5	2	6	6	10	7.250 (3.991)

N, sample size; N_A, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; FB, Fadenbach; JB, Jesuitenbach; VZ, Vienna Zoo; PE, pond Pekny; EZ, pond Engelhartszell; WB, pond Wanzenböck.

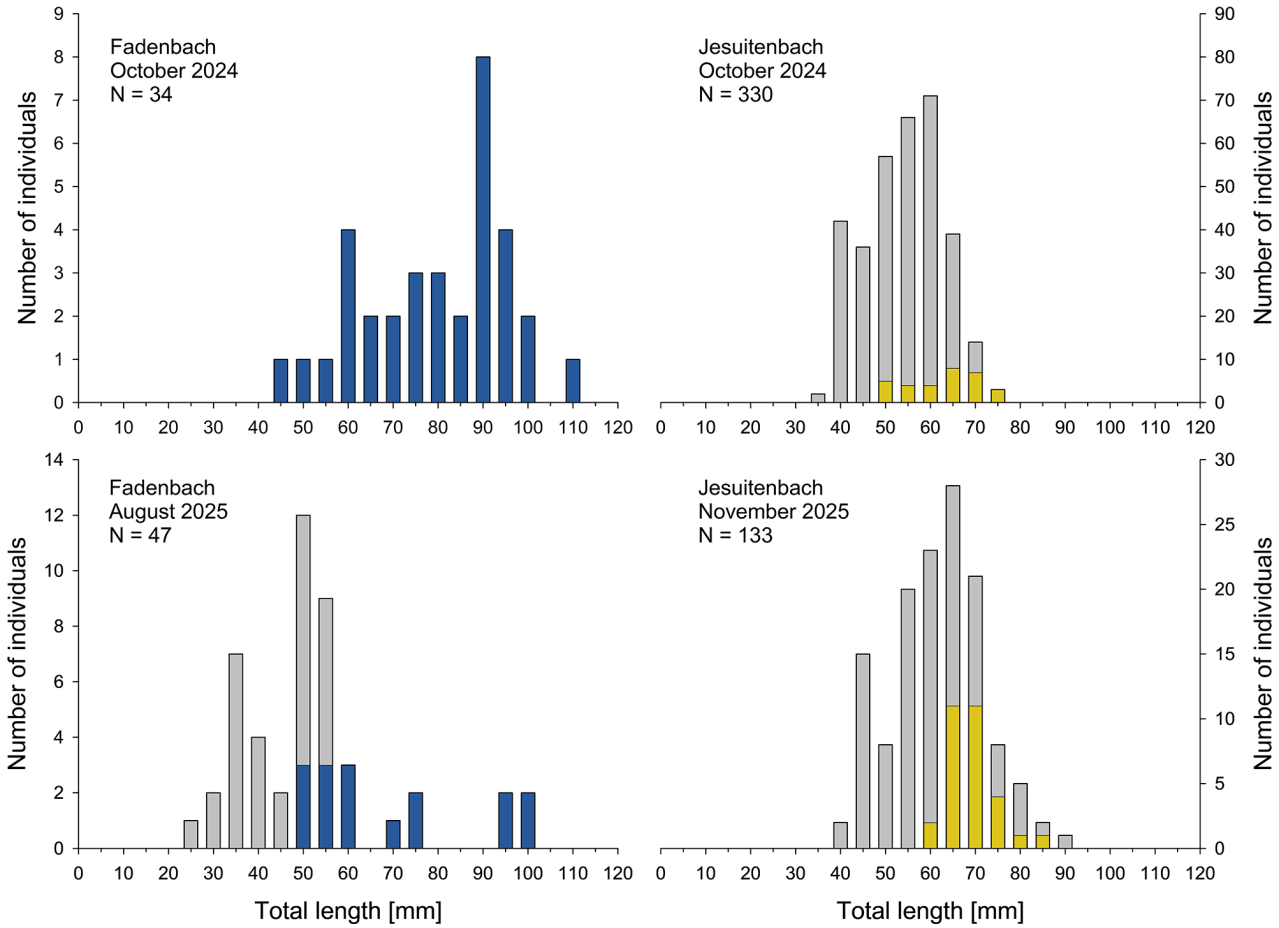

Figure 2. Length–frequency diagram of *Umbra krameri* from Fadenbach (left) and Jesuitenbach (right) in the two years of the study. Colored bars indicate the individuals used for genetic analysis.

Table 2. Inbreeding coefficient (F_{IS}) for Austrian wild and *ex situ* populations of *Umbra krameri*.

Population	F_{IS}	P
Fadenbach	0.0009	0.532
Jesuitenbach	0.0356	0.263
Vienna Zoo	−0.2222	0.987
Pond Pekny	−0.1287	0.960
Pond Engelhartszell	−0.1480	0.883
Pond Wanzenböck	−0.0762	0.891

(*N* = 9), this is the total census population size, such that estimates are not affected by subsampling within this population. The highest F_{ST} values were consistently observed in comparisons involving the Jesuitenbach population. Bayesian clustering analyses in STRUCTURE, evaluated using the Evanno method, identified *K* = 2 as the highest level of genetic structuring (see Suppl. material 1: fig. S1). At this level, the Jesuitenbach population was resolved as a distinct cluster, whereas the Fadenbach population and all *ex situ* populations grouped into a second cluster (Fig. 3A). When Jesuitenbach samples were excluded, the strongest support was obtained for *K* = 4 (see Suppl. material 1: fig. S2), with each *ex situ* population forming a largely distinct cluster, while the wild Fadenbach population showed as-

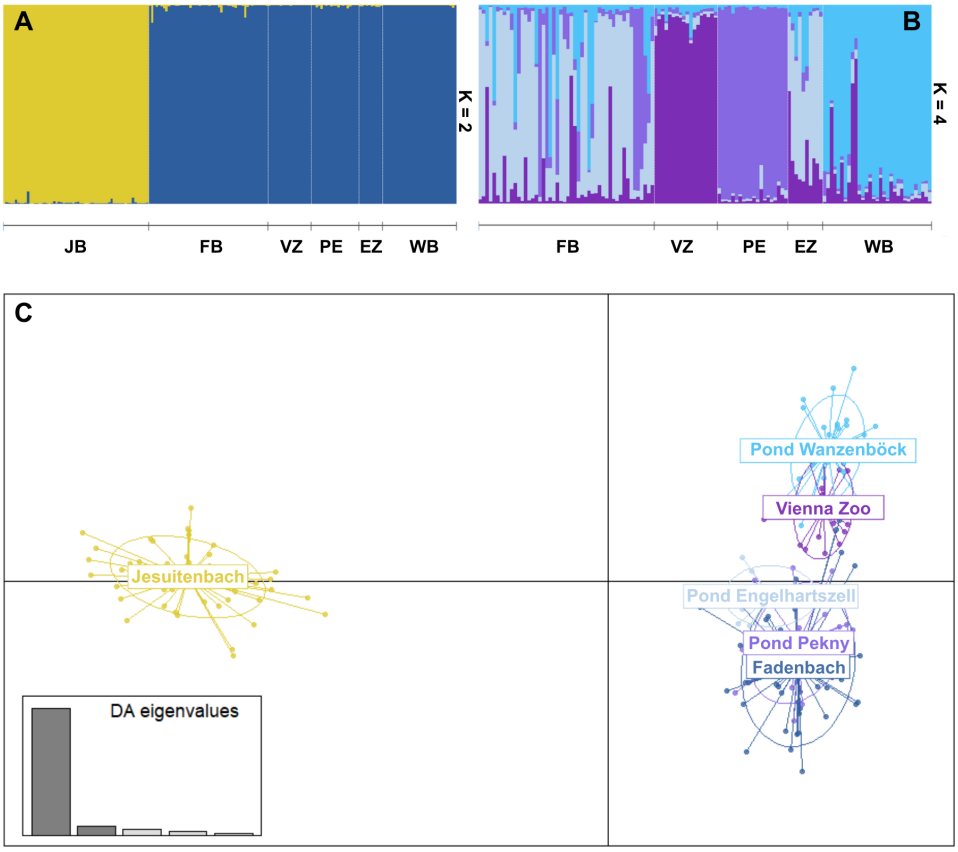

Figure 3. Results of hierarchical STRUCTURE analysis and discriminant analysis of principal components (DAPC) for Austrian *Umbra krameri*. **A.** First step of the hierarchical STRUCTURE analysis with all samples; **B.** Second step of the hierarchical STRUCTURE analysis, excluding the population from Jesuitenbach; **C.** Results of a DAPC based on 35 principal components. JB, Jesuitenbach; FB, Fadenbach; VZ, Vienna Zoo; PE, pond Pekny; EZ, pond Engelhartszell; WB, pond Wanzenböck.

Table 3. Pairwise population genetic differentiation between Austrian wild and *ex situ* populations of *Umbra krameri* based on F_{ST}.

	FB	JB	VZ	PE	EZ
JB	0.349				
VZ	0.145	0.466			
PE	0.117	0.422	0.185		
EZ	0.130	0.378	0.256	0.265	
WB	0.173	0.486	0.191	0.292	0.277

All estimates were significant after correcting for multiple testing (Benjamini and Hochberg 1995). FB, Fadenbach; JB, Jesuitenbach; VZ, Vienna Zoo; PE, pond Pekny; EZ, pond Engelhartszell; WB, pond Wanzenböck.

signment to all four clusters (Fig. 3B). Principal component analysis (PCA) and discriminant analysis of principal components (DAPC) corroborated the STRUCTURE results. The Jesuitenbach population was consistently separated from all other populations. In the PCA, all *ex situ* populations fully overlapped with the wild Fadenbach population, although occupying a reduced multivariate space (see Suppl. material 1: fig. S3). In the DAPC, pond Pekny and pond Engelhartszell overlapped with the wild Fadenbach population, whereas Vienna Zoo and pond Wanzenböck were more clearly separated from the source population (Fig. 3C).

Contemporary effective population size (N_e) estimated using the linkage disequilibrium method implemented in NeEstimator v2 was ∞ (95% CI: 220.0–∞) for the Jesuitenbach population and 36.9 (95% CI: 24.1–77.6) for the Fadenbach population. No significant heterozygote excess was detected in either population (one-tailed Wilcoxon tests, $p > 0.05$), indicating no evidence for a very recent, severe reduction in N_e. Consistent with this, levels of genetic diversity in the contemporary Fadenbach population were comparable to those reported approximately 10 years earlier (Marić et al. 2017). Rarefied present-day allelic richness for the Fadenbach population, standardized to a sample size of 20 individuals to match Marić et al. (2017), was estimated at 4.25.

Discussion

This study revealed pronounced genetic differentiation between the two remaining Austrian wild populations of *U. krameri* in the Fadenbach and Jesuitenbach. The consistent separation of these populations based on F_{ST} estimates, Bayesian clustering, and multivariate analyses indicates the absence of contemporary gene flow. Similar levels of population structuring have been reported across the species' range, including at relatively small spatial scales within the Pannonian Basin and Danube floodplains (Takács et al. 2015; Marić et al. 2017, 2019).

Genetic diversity in both wild populations was moderate to high and comparable to values reported for *U. krameri* populations elsewhere in the species' distribution (Takács et al. 2015; Marić et al. 2017, 2019). Expected heterozygosity in both Fadenbach and Jesuitenbach falls well within the range observed for other Central and Eastern European populations, indicating that severe long-term genetic erosion has not yet occurred. Importantly, standardized comparison of the data from this study with those reported by Marić et al. (2017) revealed no difference in allelic richness for the Fadenbach population, and heterozygosity remained similarly stable between sampling periods (Table 4). This suggests temporal stability of neutral genetic diversity over approximately the past decade, despite the pronounced demographic and hydrological deterioration documented in recent years. The absence of detectable changes in allelic richness or heterozygosity does not contradict recent population declines. Genetic diversity at neutral markers often responds with a time lag to reductions in census size, particularly when declines are recent or when populations persist at low size for only a limited number of generations (Cornuet and Luikart 1996; Hoban et al. 2013). *Umbra krameri* is expected to live up to 5–6 years, with first reproduction occurring after 1 year (Wanzenböck 1995). This rapid generational turnover might contribute to a temporal lag in the detection of population decline by means of neutral markers. Therefore, the apparent stability

Table 4. Change in diversity indices for the *Umbra krameri* population in the Fadenbach over the last decade, based on the same seven microsatellite loci.

	N	N_A	H_O	H_E
This study	50	4.25*	0.538	0.549
Marić et al. 2017	20	4.37	0.523	0.500

* Estimate standardized to the sample size in Marić et al. (2017).

of genetic diversity should be interpreted with caution and not overestimated. Accordingly, the lack of significant heterozygote excess in bottleneck tests does not preclude an ongoing reduction in N_e, given the limited power of such tests under moderate or gradual demographic change and the limited number of microsatellite loci and samples used (Peery et al. 2012; Hoban et al. 2013).

Despite the apparent stability of genetic diversity, the estimate of contemporary N_e for the Fadenbach population is alarmingly low. N_e for the Fadenbach population was estimated at approximately 37, clearly below thresholds commonly considered necessary to avoid short-term inbreeding depression. For the Jesuitenbach population, the point estimate of N_e was infinite. This should not be interpreted as evidence of an exceptionally large population, but rather as a methodological limitation of the LD approach—which is most powerful for small populations—when N_e exceeds the resolution provided by the available marker set and sample size (Waples and Do 2010). Importantly, even if Jesuitenbach currently maintains a larger N_e than Fadenbach, its highly restricted spatial extent renders it vulnerable to environmental stochasticity, and a larger N_e alone does not guarantee long-term persistence under continued habitat degradation. Both populations appear to have undergone pronounced fluctuations in census size in the recent past, as indicated by several non-standardized surveys. In the Fadenbach, population size was quite large in the 1990s but showed high fluctuation, mainly due to high winter mortality. The species was still abundant in 2013 and 2018 and had expanded its range within the Fadenbach system. Between 2018 and 2023, a sharp population decline occurred, and the species was not recorded in 2023. This was most likely due to droughts and falling groundwater levels during this period. In 2024 and 2025, a slight recovery was observed, but at a very low level (2024: 34 individuals captured, 2025: 47 individuals captured) (Jung et al. 2025b). In the Jesuitenbach, population size was reported to be relatively small in the 1990s (Wanzenböck and Spindler 1996) and again in 2018 (Jung et al. 2018), increasing substantially in 2023 (Jung et al. 2024) and 2024 (this study; 426 individuals captured), before declining again in 2025 (this study; 133 individuals captured). Effective population size is a key determinant of the rate of genetic drift, inbreeding accumulation, and loss of adaptive potential (Frankham 1995). Under both the classical 50/500 rule (Franklin 1980) and its more conservative revision to 100/1000 (Frankham et al. 2014), at least the Fadenbach population must be considered at high risk. These results underline that the absence of current genetic erosion should not be interpreted as demographic security. Rather, continued low N_e is likely to translate into genetic erosion if unfavorable conditions, e.g., low groundwater level and high density of competitors, persist.

At first glance, the combination of very small estimated N_e and the absence of significant inbreeding coefficients (F_{IS}) may appear contradictory. However, this pattern is well documented in small and recently declining populations (e.g., Addison and Diamond 2011; Perl et al. 2018) and can be explained by several non-exclusive mechanisms. First, F_{IS} reflects the deviation of the observed heterozygosity relative to the heterozygosity expected under random mating. Importantly, when a population is small, random mating can lead to matings between relatives, thus increasing average pedigree inbreeding, while F_{IS} will remain zero (Keller and Waller 2002). Second, despite being rather short-lived (max. 5–6 years; Wanzenböck 1995), *U. krameri* has overlapping generations,

a life-history trait that can buffer short-term increases in inbreeding even when N_e is low (Nunney 1993; Frankham et al. 2017). In theory, in such cases, heterozygosity and F_{IS} may remain stable for several generations following a decline, while N_e responds more rapidly to changes in variance in reproductive success or population size. However, considering that *U. krameri* population sizes in Austria have been small over the last few decades, this does not seem to apply as an explanation for the pattern observed in the Austrian wild *U. krameri* populations but might be a potential factor in the *ex situ* populations. Third, the absence of detectable inbreeding may also reflect limited statistical power. With a moderate number of loci and samples, small but biologically relevant increases in inbreeding can remain undetected (Keller and Waller 2002). Fourth, active inbreeding avoidance based on phenotype matching has been shown for other fish species (e.g., zebrafish; Gerlach and Lysiak 2006) but requires the establishment of an olfactory, visual, or acoustic template for kin during early development (Gerlach and Lysiak 2006). Whether this also applies to *U. krameri* is not known. Importantly, the absence of detectable inbreeding should therefore not be interpreted as evidence of demographic or genetic security. Instead, the low N_e value observed here for the Fadenbach population indicates a high susceptibility to future inbreeding accumulation and genetic erosion.

Field surveys (Wanzenböck et al. 2026) and genetic data jointly indicate that the situation of the Fadenbach population is particularly critical. The population now inhabits only a short stretch (~5 km) within the Fadenbach system. Increasingly frequent and prolonged desiccation events pose an immediate extinction risk, especially when combined with harsh winter conditions, because the remaining water bodies are then at a high risk of completely freezing through. The primary driver of this deterioration appears to be hydrological change. Declining groundwater levels in the Marchfeld region—resulting from drainage, intensive agriculture, riverbed incision of the Danube, and climate-driven changes in precipitation and evapotranspiration—directly reduce water availability in the Fadenbach system (Jung et al. 2025b). Additional impacts may arise from flood protection infrastructure, including sealing walls and pressure-relief measures associated with dam refurbishment. Emergency water supplementation from the Danube can temporarily enhance connectivity but simultaneously introduces numerous fish species, including non-native taxa such as pumpkinseed (*Lepomis gibbosus*) and topmouth gudgeon (*Pseudorasbora parva*). In some areas of its distribution, the introduction of non-native fish species is already assumed to be a major driver of population decline of *U. krameri* (Somogyi et al. 2023). Given the weak competitive ability and narrow trophic niche of *U. krameri* (Sehr and Keckeis 2017), increased competition, sediment input, and possibly also altered physicochemical conditions are likely to have negative consequences. Both remaining natural habitats of *U. krameri* in Austria are, or were, strongly groundwater-driven systems. Frequent or permanent supplementation with sediment-rich Danube water may therefore fundamentally alter habitat characteristics essential for the species. In contrast to the Fadenbach population, the Jesuitenbach population may have benefited from recent dry phases, which possibly reduced competition with other fish species. In this scenario, conditions might have been fatal for species with higher oxygen demand, such as roach (*Rutilus rutilus*) and pike (*Esox lucius*), but still suitable for *U. krameri*. Generally, the current situation and future prospects of groundwater levels in the Jesuitenbach area seem less critical than in the Fadenbach area. As

climate change presumably increases the number of dry phases, it is unclear how single local *U. krameri* populations will be affected. In general, it can be assumed that reduced habitat availability will lead to reduced or even extinct populations at some sites, whereas some populations might even benefit in the long run, as long as the decline is not too extreme and competition is reduced. Recently, beaver-induced impoundments may have further enhanced habitat suitability for *U. krameri* in the Jesuitenbach, although causal relationships remain unclear.

Genetic differentiation between the four *ex situ* populations and between these populations and their alleged source population from the Fadenbach was significant, though much lower than between these populations and the Jesuitenbach population. This pattern reflects founder effects and subsequent genetic drift. Each *ex situ* population was established between the late 1990s (pond Wanzenböck, pond Engelhartszell) and 2013 (Vienna Zoo) from a limited number of individuals originating exclusively from the Fadenbach population (pond Wanzenböck—15–20 fish, pond Engelhartszell—approximately 12 fish, pond Pekny—number of original fish unknown, Vienna Zoo—50 fish), leading to stochastic loss of alleles at the time of founding. In small, closed captive populations, genetic drift can rapidly amplify these differences across generations (Allendorf et al. 2013; Frankham et al. 2017). Despite this divergence, all *ex situ* populations clearly cluster with the Fadenbach population, confirming their origin and the absence of detectable admixture from other sources. According to unpublished information, this may not be the case for other Austrian *ex situ* populations, five of which are known to the authors and were not included in this study. The clear clustering of all *ex situ* populations with Fadenbach, and their distinct separation from Jesuitenbach, provides strong genetic support for the documented management history of these stocks and confirms their genetic integrity. This finding is particularly important in the context of conservation breeding, as undocumented admixture can compromise both evolutionary distinctiveness and the suitability of captive stocks for future reintroduction efforts. At the same time, the notable genetic differentiation among the four *ex situ* populations themselves, as well as between these populations and their source population, highlights the speed at which population genetic structure can arise. Even a few generations of spatial isolation, when coupled with small effective population sizes or pronounced demographic fluctuations, appear sufficient to generate considerable differentiation. Such dynamics are especially plausible for fish species specialized to floodplain habitats, where population sizes are strongly influenced by hydrological variability and habitat connectivity (Jopp et al. 2010; Górski et al. 2011). The results suggest that similarly rapid genetic differentiation may also have occurred historically in wild *U. krameri* populations and probably also in populations of other fish species with similar habitat requirements. Although floodplain systems of Central and Eastern European rivers likely facilitated connectivity over large spatial scales, this connectivity was probably intermittent and structured by isolation-by-distance, hydromorphological heterogeneity, including breakthrough sections without suitable habitats, or by temporary barriers during low-water phases. Consequently, even populations that were periodically connected via extensive floodplains may have experienced sufficient isolation to promote genetic divergence, which became more pronounced in the recent past due to river regulations and the establishment of permanent barriers to dispersal (also see Marić et al. 2017, 2019). From a conservation perspective,

this underscores the importance of maintaining or restoring dynamic floodplain connectivity while simultaneously accounting for the potential existence of fine-scale genetic structure when designing management units and conservation actions for this species. It should be noted that the microsatellite markers used in this study primarily reflect neutral genetic variation; genome-wide approaches would be required to assess adaptive genetic diversity and to fully resolve functional differentiation among populations of *U. krameri*.

Collectively, the four *ex situ* populations capture the full genetic diversity present in the Fadenbach population. This has direct implications for conservation breeding and potential reintroduction efforts. Rather than relying on a single captive line, future programs should integrate individuals from all *ex situ* populations to maximize genetic diversity and minimize inbreeding risk. Strategic mixing of captive lines is a well-established approach to counteract drift and maintain adaptive potential in conservation breeding programs (Lacy 2013; Frankham et al. 2017). Continued genetic monitoring of *ex situ* populations is essential to track genetic changes in diversity and inbreeding and to support adaptive management over successive generations.

Overall, this study highlights the urgent need for an integrated conservation strategy for *U. krameri* in Austria, combining hydrological restoration, habitat management, *ex situ* conservation, reintroduction programs, and genetic monitoring, ideally also including eDNA methods that are currently being established for this species (Rund et al. 2025). The Jesuitenbach population represents a genetically distinct and currently more viable conservation unit and should be treated as a separate priority, potentially including the establishment of a dedicated captive breeding population. *In situ*, priority measures should focus on improving hydrological conditions, particularly through the restoration of local groundwater levels, which are a key determinant of habitat availability. The creation of additional refuge ponds appears equally important, as such habitats have likely contributed substantially to the species' persistence in the system. At the same time, artificial water supplementation from the Danube should be minimized, as it may introduce non-native species and alter habitat characteristics. *Ex situ* conservation should aim to maintain and enhance genetic diversity by integrating individuals from multiple captive populations and facilitating exchange among them, thereby reducing the risk of genetic drift and inbreeding. In contrast, supportive stocking of the Fadenbach population is currently not recommended, as it is often ineffective and may entail ecological risks (Claussen and Philipp 2023). Instead, *ex situ* populations should primarily function as a genetic backup and as a source for potential future reintroduction efforts. Without substantial improvement of hydrological conditions, however, even well-designed genetic management cannot prevent local extinction. Nonetheless, integrating population genetics into adaptive conservation management frameworks will be critical to safeguarding Austria's most threatened fish species over both the short and long term.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

Regarding the use of AI in the preparation of this manuscript, the authors declare the following:
Grammar, syntax and spelling were checked using AI.

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Author contributions

Conceptualization: MJ, SK. Data curation: RD. Formal analysis: RD. Funding acquisition: SK, MJ. Investigation: CR, MJ, SK, RD. Project administration: MJ, SK. Resources: SK. Visualization: MJ, RD. Writing - original draft: RD, SK. Writing - review and editing: MJ, CR.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

Supplementary material 1

Additional information

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Data type: pdf

Explanation note: **table S1.** Microsatellite multiplex PCR-cocktail information. **table S2.** Microsatellite data of Austrian *Umbra krameri* from two wild and four ex situ populations used for population genetic analyses. **fig. S1.** Delta K statistics for the whole dataset. **fig. S2.** excluding the Jesuitenbach population. **fig. S3.** PCA of Austrian *Umbra krameri* from two wild and four ex situ populations, based on eight microsatellite loci.

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