scientific reports

OPEN

Molecular diversity patterns and introgression in alpine and Northern European populations of Arctic charr (*Salvelinus alpinus*)

Giulia Riccioni[1]✉, Mariantonietta Palazzo[1,10], Elisa Somenzi[1], Riccardo Negrini[1], Elisa Eufemi[1], Andrea Gandolfi[2], Fatima Chegdani[1,3], Elia Vajana[4], Ian D. McCarthy[5], Maria Stella Grando[2,8], Francesco Nonnis Marzano[6], Craig R. Primmer[7], John Lewis Williams[1], Paolo Ajmone-Marsan[1] & Licia Colli[1,9]

The origin of Arctic charr populations in the lakes of the Italian Alps is not well understood. While some studies have suggested that they are postglacial relics, others have proposed that these populations are the result of intentional stocking efforts dating back to the sixteenth century. Subsequent introductions of Arctic charr to these lakes have made it difficult to untangle the evolutionary history of these Alpine populations. In this study, we examined the distribution of genetic variation among Arctic charr collected throughout their Northern and Southern European ranges at the beginning of the twenty-first century, using nuclear (amplified fragment length polymorphisms) and mitochondrial (control region and cytochrome oxidase I) loci. These analyses revealed the impact of restocking activities, which have resulted in admixture and hybridisation with Brook charr (*Salvelinus fontinalis*) in Italian alpine lakes and provides clues on the native or pseudo-native origin of Trentino-Alto Adige populations. The lack of detailed historical information, however, makes it difficult to disentangle the postglacial history of the species and to determine how much of the current diversity pattern can be ascribed to the consequences of Pleistocene events or anthropogenic activities.

The Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), is a Holarctic salmonid adapted to cold Arctic waters[1]. It exhibits considerable physiological flexibility that allows it to thrive in a variety of habitats, from high-latitude rivers and lakes to isolated alpine water bodies, as long as ambient temperatures remain within its narrow and highly specialized thermal niche[2]. Following the last glacial period, the retreat of the ice allowed *S. alpinus* to colonize a variety of newly available habitats. This postglacial colonization, combined with extensive local adaptation, resulted in both genetic and morphological divergence among populations[1]. The Arctic charr is now widely distributed throughout northern Europe, with several isolated populations recorded on the Northern side of the Alps which are adapted to high altitude and considered by some authors[3] to be relics of the last Pleistocene glacial event.

While populations in northern latitudes are often anadromous, those in landlocked Alpine lakes are resident, leading to distinct evolutionary trajectories shaped by local environmental conditions[4]. The native range of *S. alpinus* in the Alps includes Switzerland, France, Austria and Germany. However, a few populations in Trentino-Alto Adige (Italy) are considered to be the only native examples located on the southern side of the Alps[5,6]

[1]Dipartimento di Scienze Animali, della Nutrizione e degli Alimenti, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del S. Cuore di Piacenza, via Emilia Parmense 84, 29122 Piacenza, Italy. [2]Research and Innovation Centre, Fondazione Edmund Mach, Via Edmund Mach, 1, 38098 San Michele all'Adige, TN, Italy. [3]Laboratory of Integrative Biology, Faculty of Sciences Ain Choch, University Hassan II of Casablanca, Casablanca, Morocco. [4]Institute of Biosciences and BioResources (IBBR), National Research Council (CNR), 50019 Sesto Fiorentino (Firenze), Italy. [5]School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey LL59 5AB, UK. [6]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, Parma, Italy. [7]Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki 00014, Finland. [8]Center Agriculture Food Environment (C3A), University of Trento, via E. Mach, 1, 38098 San Michele all'Adige, Italy. [9]BioDNA Centro di ricerca sulla Biodiversità e sul DNA Antico, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del S. Cuore di Piacenza, via Emilia Parmense 84, 29122 Piacenza, Italy. [10]Present address: CMP3 VdA, Istituto Italiano di Tecnologia, 11100 Aosta, Italy. ✉email: giulia.riccioni@unicatt.it

Lake	Country	ID	Origin	Latitude North	Longitude Est (Greenwich)	n. sampled	AFLPs					
							n. individuals	n. loci	n. polym. loci*	% polym. loci	H_E[§]	S.E.(H_E)
Achensee	Austria	ASE		47.464	11.708	20	18	94	52	55,3	0,176	± 0,019
Sankt Wolfgang See	Austria	AUS		47.745	13.415	37	29	94	16	17,0	0,070	± 0,013
Drachensee	Austria	DRS		47.358	10.934	5	4	94	45	47,9	0,134	± 0,017
Hintersteiner See	Austria	HIN		47.541	12.214	11	22	94	52	55,3	0,138	± 0,017
Kleiner Muhldorfer See	Austria	KMS		46.924	13.368	7	7	94	43	45,7	0,100	± 0,015
Lunzer See	Austria	LUZ		47.853	15.053	20	20	94	15	16,0	0,083	± 0,015
Oberer Plendeler See	Austria	OPI		47.203	11.039	6	6	94	6	6,4	0,057	± 0,012
Plansee	Austria	PLS		47.469	10.798	15	12	94	51	54,3	0,173	± 0,018
Rotfelsee	Austria	RFS		47.231	11.008	12	12	94	14	14,9	0,094	± 0,016
Stapnik See	Austria	SKS		46.949	13.340	20	20	94	21	22,3	0,105	± 0,016
Spullersee	Austria	SPS		47.158	10.098	2						
Stappitzer See	Austria	STS		47.018	13.194	5	5	94	48	51,1	0,158	± 0,018
Zurser See	Austria	ZUS		47.175	10.139	12	11	94	17	18,1	0,105	± 0,017
Saimaa	Finland	ENO		61.250	27.583	20	13	94	48	51,1	0,145	± 0,019
Toskaljärvi	Finland	TOS		69.183	21.467	20	16	94	22	23,4	0,114	± 0,016
Bodlyn	Wales	BYN		52.796	-4.006	8	7	94	13	13,8	0,094	± 0,016
Cowlyd	Wales	COW		53.143	-3.904	8	4	94	52	55,3	0,127	± 0,014
Cwellyn	Wales	CWE		53.072	-4.151	8	8	94	53	56,4	0,126	± 0,016
Ffynnon Llugwy	Wales	FFY		53.145	-3.957	8	8	93	54	58,1	0,129	± 0,016
Bodensee	Germany	BOD		47.599	9.432	9	8	94	64	68,1	0,261	± 0,019
Barco	Italy	BAR	introduced	46.274	10.706	8	8	94	17	18,1	0,087	± 0,015
Bombasel (VI)	Italy	BOM	introduced	46.226	11.511	15	15	94	11	11,7	0,080	± 0,016
Brutto	Italy	BRU		46.277	11.617	13	13	94	11	11,7	0,066	± 0,013
Caserina	Italy	CAS		46.275	11.669	10	9	94	6	6,4	0,040	± 0,011
Lago di Cavazzo	Italy	CAV		46.326	13.072	3	3	93	7	7,5	0,075	± 0,013
Cece	Italy	CEC	introduced	46.281	11.669	13	10	94	47	50,0	0,138	± 0,018
Costabrunella	Italy	COB		46.132	11.579	22	22	94	10	10,6	0,066	± 0,013
Corvo maggiore	Italy	COM	native	46.442	11.813	24	23	94	11	11,7	0,063	± 0,015
Cornisello (inf. + sup.)	Italy	COR		46.221	10.729	7						
Colbricon	Italy	COS		46.282	11.765	2						
Erdemolo	Italy	ERD	introduced	46.110	11.376	28	28	94	53	56,4	0,204	± 0,021
S. Giuliano	Italy	GIU	introduced	46.147	10.701	18	6	94	11	11,7	0,090	± 0,016
Grande (Valsugana)	Italy	GRA		46.084	11.411	18	18	94	25	26,6	0,135	± 0,018
Lago d'Iseo	Italy	ISE		45.735	10.070	8	6	94	56	59,6	0,207	± 0,018
Iuribrutto	Italy	IUR		46.343	11.769	20	20	94	49	52,1	0,138	± 0,018
Lagorai Maggiore	Italy	LAM		46.213	11.524	30	21	94	14	14,9	0,079	± 0,015
Molveno	Italy	MOL		46.125	10.954	2						
Morgex	Italy	MOR	hatchery	45.758	7.034	39	33	94	58	61,7	0,202	± 0,019
Nero di Cornisello	Italy	NER		45.764	10.057	14						
Lago Santo	Italy	SAN	Introduced	44.402	10.007	11	8	94	10	10,6	0,067	± 0,013

Lake	Country	ID	Origin	Latitude North	Longitude Est (Greenwich)	n. sampled	AFLPs					
							n. individuals	n. loci	n. polym. loci*	% polym. loci	H_E[§]	S.E.(H_E)
Continued												
Santo Stefano	Italy	SST		46.110	9.992	4	4	94	50	53,2	0,194	± 0,019
Stellune	Italy	STE		46.194	11.499	7	7	94	9	9,6	0,062	± 0,012
Tovel	Italy	TOV	native	46.261	10.952	30	28	94	11	11,7	0,054	± 0,012
Valbona	Italy	VAL	introduced	46.047	10.184	19						
Buevattnet	Norway	BUE		70.617	30.083	20	19	94	64	68,1	0,194	± 0,018
Haukejavr	Norway	HAU		69.950	29.233	20	19	94	17	18,1	0,090	± 0,015
Riasten nord/sud	Norway	RIAN/RIAS		62.845	11.766	20						
Somasjärvi	Norway	SOM		69.283	21.550	20	18	94	53	56,4	0,158	± 0,019
Sankt Moritz	Switzerland	MZL		46.494	9.846	5						
Sils Maria	Switzerland	SMA		46.418	9.731	5	3	94	50	53,2	0,213	± 0,019
Lago di Zugo	Switzerland	ZUG		47.123	8.481	3	3	94	52	55,3	0,242	± 0,019

Lake	Control Region				COI				CR + COI			
	n. individuals	n. haplotypes	*h*	π	n. individuals	n. haplotypes	*h*	π	n. individuals	n. haplotypes	*h*	π
Achensee												
Sankt Wolfgang See	7	2	0.48	0.001	7	1	0	0	7	2	0.47	0
Drachensee	3	1	0	0	3	2	0.66	0.00109	3	2	0.66	0.00061
Hintersteiner See	3	1	0	0	3	1	0	0	3	1	0	0
Kleiner Muhldorfer See	4	1	0	0	3	1	0	0	3	1	0	0
Lunzer See	3	1	0	0	3	1	0	0	3	1	0	0
Oberer Plendeler See												
Plansee	3	1	0	0	3	1	0	0	3	1	0	0
Rotfelsee	3	1	0	0	3	1	0	0	3	1	0	0
Stapnik See	3	1	0	0	3	2	0.66	0.00109	3	2	0.66	0.00061
Spullersee	2	1	0	0	1	1	0	0	1	1	0	0
Stappitzer See	3	1	0	0	3	1	0	0	3	1	0	0
Zurser See	3	1	0	0	2	2	1	0.00164	2	2	1	0.00092
Saimaa	4	1	0	0	3	1	0	0	3	1	0	0
Toskaljärvi	3	1	0	0	3	1	0	0	3	1	0	0
Bodlyn	8	2	0.53	0.00112	8	1	0	0	5	1	0	0
Cowlyd	5	1	0	0	8	1	0	0	2	1	0	0

Lake	Control Region				COI				CR + COI			
	n. individuals	n. haplotypes	*h*	π	n. individuals	n. haplotypes	*h*	π	n. individuals	n. haplotypes	*h*	π
Cwellyn	8	1	0	0	8	2	0.25	0.00164	6	1	0	0
Ffynnon Llugwy	8	1	0	0	8	1	0	0	5	1	0	0
Bodensee	5	1	0	0	5	1	0	0	5	1	0	0
Barco	3	1	0		3	2	0.66	0.00109	3	2	0.66	0.00061
Bombasel (VI)												
Brutto	3	2	0.66	0.00139	3	1	0	0	3	2	0.66	0.00092
Caserina												
Lago di Cavazzo	3	1	0	0	3	1	0	0	3	1	0	0
Cece	2	2	1	0.00209	2	1	0	0	2	2	1	0.00092
Costabrunella	3	1	0	0	8	1	0	0	3	1	0	0
Corvo maggiore												
Cornisello (inf. + sup.)	7	2	0.28	0.0006								
Colbricon												
Erdemolo	6	2	0.33	0.0007	6	2	0.33	0.00055	6	3	0.33	0.00061
S. Giuliano	5	1	0	0	4	1	0	0	4	1	0	0
Grande (Valsugana)	4	1	0	0	4	1	0	0	4	1	0	0
Lago d'Iseo	3	1	0	0	3	1	0	0	3	2	0.66	0.00061
Iuribrutto	6	1	0	0	6	1	0	0	6	1	0	0
Lagorai Maggiore	1	1	0	0								
Molveno												
Morgex	36	2	0.38	0.00081	31	2	0.06	0.00011	28	3	0.23	0.00042
Nero di Cornisello	13	2	0.28	0.00059	7	1	0	0	7	1	0	0
Lago Santo	3	1	0	0	3	1			3	1	0	0
Santo Stefano	3	1	0	0	3	1	0	0	3	1	0	0
Stellune	2	1	0	0	3	2	0.66	0.00109	2	2	1	0.00092
Tovel	3	1	0	0	3	1	0	0	3	1	0	0
Valbona	12	3	0.42	0.00266	9	1	0	0	9	3	0.50	0.00138
Buevattnet	5	2	0.66	0.00251	6	1	0	0	5	2	0.40	0.0011
Haukejavr	2	1	0	0	3	1	0	0	2	1	0	0
Riasten nord/sud												

	Control Region				COI				CR + COI			
Lake	**n. individuals**	**n. haplotypes**	***h***	**π**	**n. individuals**	**n. haplotypes**	***h***	**π**	**n. individuals**	**n. haplotypes**	***h***	**π**
Somasjärvi	3	1	0	0	3	1	0	0	3	1	0	0
Sankt Moritz	5	1	0	0	5	2	0.4	0.00066	5	2	0.40	0.00037
Sils Maria	5	1	0	0	5	1	0	0				
Lago di Zugo	3	1	0	0	2	1	0	0	2	1	0	0

Table 1. Sampling data and summary statistics for the genetic markers used (*AFLPs* amplified fragment length polymorphisms, *CR* control region, *COI* cytochrome oxidase I, *CR + COI* concatenated dataset, H_E expected heterozygosity, *h* haplotype diversity, π nucleotide diversity). *Polymorphic loci at the 5% level. $^{\$}$Following Nei (Nei's gene diversity).

although this is still a matter of debate. While some studies propose that these represent relict populations dating back to the initial postglacial colonization[5,6], others do not consider them to be native. Most of the present-day Trentino-Alto Adige populations are landlocked, inhabiting small high-altitude alpine lakes that lack connections with the surrounding river basins, suggesting that the species may have been introduced before the sixteenth century[6–8]. While recent studies have highlighted significant geographic divergence among populations of Arctic charr, driven largely by postglacial recolonization patterns and local adaptation[9,10], the genetic diversity of this species in the Alpine region is further complicated by continuous restocking and possible hybridisation with closely related species such as the introduced Brook charr, *Salvelinus fontinalis*[11,12] (Mitchill, 1814).

Translocations of *S. alpinus* have been documented since the sixteenth century, during the reign of Maximilian I of the Holy Roman Empire, and over the centuries the species has been introduced into several Italian and Austrian lakes to support fisheries and aquaculture[6,13]. These interventions have likely led to genetic admixture between native and introduced stocks, blurring the historical biogeography of the species.

In a preliminary study[10] the genetic structure and diversity in *S. alpinus* revealed that individuals generally clustered according to their country of origin, with some populations showing clear signals of extensive gene flow, particularly across the Alps between Italy and Austria. These Arctic charr populations overall showed low levels of genetic variation, which is likely to be due to isolation, demographic bottlenecks and possibly anthropogenic related pressures including overfishing, climate change, habitat loss and habitat fragmentation. No comprehensive genetic analysis of *S. alpinus* biodiversity have been carried out in the Palearctic region including the Italian Alps, therefore the present study investigated the genetic structure of the species across the Alpine region and Northern Europe. Here a comprehensive study of mitochondrial (Control Region and Cytochrome Oxidase I) and nuclear AFLP (Amplified Fragment Length Polymorphism) variation was carried out to explore the genetic structure of these populations and to detect signals of introgression from the *Salvelinus fontinalis*. Data were obtained from a sample collection representing the species variation around the beginning of the twenty-first century from 41 European alpine and 10 Northern European lakes from eight countries (Austria, Italy, Germany, Norway, Finland, United Kingdom (Wales), Switzerland and Denmark). These data were used to assess hybridisation with *S. fontinalis*, quantify the genetic variation within and among populations and identify patterns of haplotype distribution and nuclear variability. This enabled the impact of historical restocking to be determined by assessing genetic signatures associated with human-mediated introductions in Italian lakes. The study assessed the likely evolutionary history and ecological dynamics of *S. alpinus*, offering critical insights for conservation and management strategies and providing a comprehensive baseline for further studies to evaluate the present-day genomic variation of the species particularly in regions where the native status of Arctic charr remains unresolved.

Results

The final dataset for the mtDNA control region contained 401 sequences, comprised of 234 new sequences from *S. alpinus* (n = 221), *S. fontinalis* (n = 7) and *S. alpinus* x *S. fontinalis* hybrids (n = 6, see MM, Table 1 and Table S1), and 167 reference sequences retrieved from the NCBI database (Table S2). In addition, 230 new Cytochrome Oxidase I (COI) sequences were obtained for *S. alpinus* (n = 200), *S. fontinalis* (n = 12) and *S. alpinus* x *S. fontinalis* hybrids (n = 18) which were joined with 28 reference sequences available in the NCBI database, giving a final COI dataset of 258 sequences. Seven and 10 haplotypes were identified among the newly produced CR and COI sequences respectively, which had mean haplotype and nucleotide diversity values of $h = 0.350$ and $\pi = 0.0014$, and $h = 0.146$ and $\pi = 0.0003$, respectively. The expected heterozygosity (He) ranged from 0.000 to 0.660 for both the CR and COI sequences with average values of $He_{CR} = 0.116$ and $He_{COI} = 0.127$. Tajima's D for both loci was negative but was only statistically significant for COI (-2.05293, p-value < 0.05,). The Analysis of MOlecular VAriance (AMOVA) of CR and COI haplotypes grouped by country, which corresponded to the division between the main drainage systems, detected a statistically significant partitioning of genetic variance among and within populations but not among countries (Table 2). For the concatenated dataset of CR and COI sequences 16 haplotypes were identified showing a mean haplotype diversity of $h = 0.446$ and nucleotide diversity of $\pi = 0.0007$. The expected heterozygosity had an average value of He = 0.232, ranging from 0.000 to 0.660. Tajima's D was negative and significant (-1.89765, p-value < 0.05). The AMOVA analysis detected a significant genetic variance only within groups and within populations (Vb = 57.39%, p-value < < 0.01; Vc = 48.69%, p-value < < 0.01) as observed for the separate analysis of CR and COI (Table 2).

A predominant CR haplotype was found in most sampling sites (Fig. S1), with some private haplotypes occurring in one population from Wales (Fig. S1, Hap_4) and two Italian populations (Fig. S1, Hap_7 and Hap_6). The Italian Valbona population shared two CR haplotypes with a Finnish and a Norwegian population (Fig. S1, Hap3 and Hap_5). Austrian CR haplotypes (Fig. S1, Table 1) were found in some Italian populations (Fig. S1 and Table 1). Analysis of COI sequences (Fig. S2) identified private haplotypes in Italian, Austrian and Swiss samples.

AMOVA	AFLPs	dloop	COI
Among countries (Va)	22.93%**	-2.98%	-7.32%
Among populations within countries (Vb)	44.07%**	61.66%**	40.64%**
Within populations (Vc)	33.00%**	41.32%**	66.68%**

Table 2. Analysis of molecular variance computed on all the different genetic markers used. ** = p-value < < 0.001.

The haplotype network constructed using the *S. alpinus* CR sequences produced in this study (Fig. S3) clearly identified one core haplotype shared by the populations of all countries (Hap_1), with additional variants corresponding to haplotypes occurring at lower frequencies shared by Italy and Austria (Hap_2), Italy and Finland (Hap_5) and Italy and Norway (Hap_3). There were three haplotypes that were private to either Italian (Hap_6 and Hap_7), or to Welsh populations (Hap_4). The haplotype network including *S. alpinus* hybrids individuals (*S. alpinus* x *S. fontinalis*) and *S. fontinalis* CR sequences (Fig. S4) showed a clear differentiation from *S. fontinalis* (Fig. S4, Hap_7) whereas sequences from hybrids were included within the two main nodes (Fig. S4). All sequences from *S. alpinus* clustered within the *S. alpinus* haplogroup.

When CR reference sequences obtained from NCBI database were included in the network analyses (Fig. 1A) three main haplotype clusters appeared: one mostly represented by European and NCBI sequences assigned to the Atlantic haplogroup (*sensu*[9]); a second cluster including NCBI sequences collected in Russia and Siberia (Siberian haplogroup *sensu*[9]) and one haplotype representing individuals from the Acadian and Arctic haplogroups (*sensu*[9]).

The haplotype network constructed using the new COI sequences of *S. alpinus* (Fig. S5) showed a star-like shape, with the presence of a very frequent core haplotype (Hap_1) and derived haplotypes which were private to individual countries. The only exception was a low-frequency haplotype shared by Austria and Wales (Fig. S5, Hap_3). Sequences from hybrid and *S. fontinalis* samples confirmed the results obtained with the analysis of the CR: *S. fontinalis* was well differentiated from *S. alpinus* (Fig. S6, Hap_12 and Hap_13), whereas sequences from hybrids clustered with the *S. alpinus* sequences, either matching the core haplotype (Fig. S6, Hap_1) or forming a derived variant (Fig. S6, Hap_11). The network analysis including public sequences from NCBI confirmed the presence of a main haplotype shared by Swedish and Russian samples, whereas sequences from Canada showed a higher degree of differentiation (Fig. 1B). The haplotype network of the *S. alpinus* concatenated CR and COI sequences showed the same topology as CR sequences (Fig. S7) with a main haplotype shared by all countries, a haplotype found in both Austrian and Italian sequences and other private haplotypes.

We obtained AFLP profiles from 563 *S. alpinus*, 19 hybrids and 57 *S. fontinalis* individuals (639 individuals in total). Overall, the four primer combinations detected a total of 94 polymorphic loci specific to *S. alpinus* out of about 400 amplified AFLP bands, with an average of 23 variants per primer pair. Samples and variants with a level of missing data above 5% were removed from the definitive dataset. The working dataset used to assess *S. fontinalis* introgression included a total of 632 individuals and 47 *S. fontinalis*-specific loci detected by the four primer combinations.

The supervised STRUCTURE analysis of the 12 hybrid individuals retained after quality check showed signals of *S. fontinalis* ancestry varying between 1.3% and 98.6% (Fig. S8). Among the putatively pure Arctic charr populations, the Valbona samples (Italy) had an average of 50.7% *S. fontinalis* introgression. All samples showing *S. fontinalis* introgression above 2% were excluded from the subsequent diversity analyses carried out on the *S. alpinus* dataset.

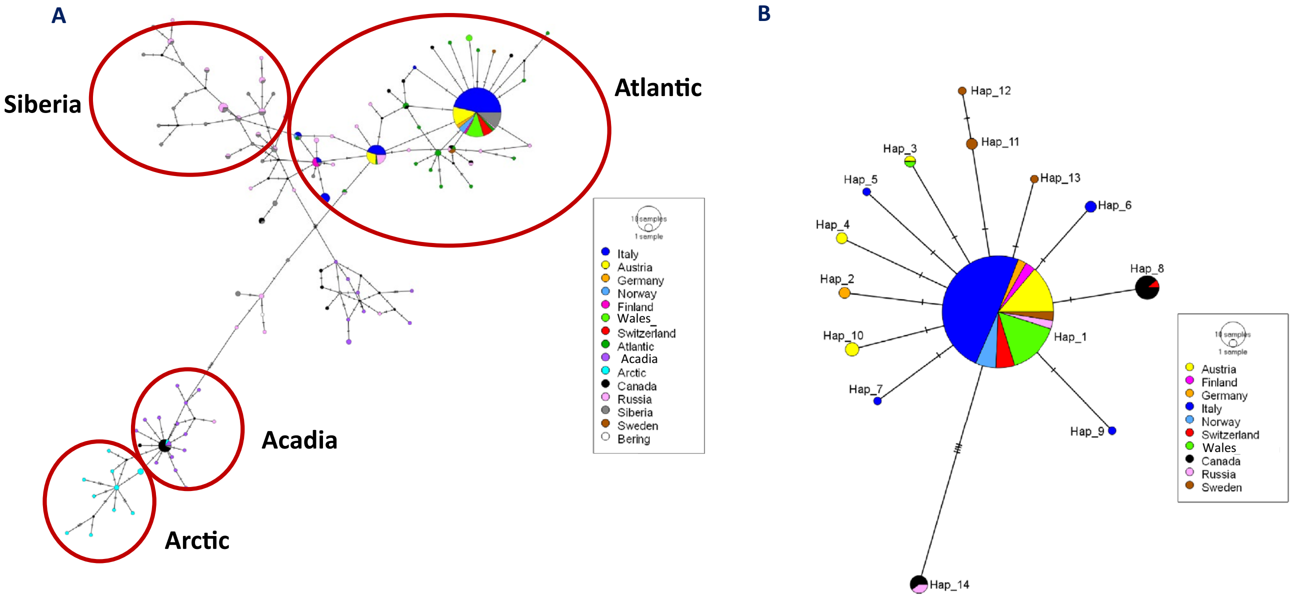

Fig. 1. TCS dloop and COI haplotype networks. The dloop haplotype network (**A**) was constructed using the mitochondrial control region sequences obtained in this study and the sequences retrieved from NCBI database (Acadia, Arctic, Atlantic, Bering, Canada, Russia, Siberia, Sweden): circles highlight the main haplogroups described in the literature (Brunner et al. 2001). The COI haplotype network (**B**) was constructed using COI sequences of *S. alpinus* produced in this study and the sequences retrieved from NCBI database (Canada, Russia and Sweden).

The final working dataset assembled to study Arctic charr diversity thus included had 563 *S. alpinus* individuals from 43 populations and 88 AFLP markers.

The expected heterozygosity ranged between 0.029 (Caserina lake, Standard Error 0.017) and 0.200 (Zugo lake, SE 0.150) with a mean value of 0.093 (Fig. S8). The AMOVA analysis (Table 2) detected significant partitioning of genetic variance between countries (Va = 22.93%, p-value < < 0.01) and also between populations within country and within populations (Vb = 44.07% p-value < < 0.01; Vc = 33.00% p-value < < 0.01), thus indicating that the 22.9% of the total genetic variance was explained by the country of origin, the 44% by the population and 33% by individual genetic variance.

The first and second axes of the Factorial Correspondence Analysis (FCA, Fig. 2) accounted for 25.60% and 14.82% of the total variance, respectively, and identified three main clusters, grouping most individuals and populations according to their geographical origin. The first dimension represented a North to South gradient, with Norwegian and Finnish clusters on the right side and a cluster formed by 10 Italian populations on the left side. The second dimension separated the Austrian samples from the others. There was a clear connection between Italy and Austria with one Austrian population (OPI) falling with in the Italian cluster, five Italian populations falling within the Austrian cluster and one Austrian sample (ASE) placed in an intermediate position (Fig. 2). A sparse cloud of Italian samples was scattered between the Italian-Northern European and the Austrian-Northern European clusters in the central part of the graph, suggesting restocking and admixture with central/northern European fish stocks. Notably, the samples collected from the hatchery of Morgex (MOR) occupied an intermediate positioning between the Austrian and the Northern European poles.

Population structure analysis based on the STRUCTURE software revealed a subdivision between an Italian and a Central-Northern European cluster at K = 2 (Fig. 3). K = 3 was suggested as the most probable population structure by both Likelihood and ΔK methods (Fig. 3A,B), which identified a Northern European (purple color, Figs. 3 and S10) an Italian (light blue color, Fig. 3C and S10), and a central European gene pool (orange color, Fig. 3C and S10). Signals of a Northern European ancestry were detected in a German, some Italian and a Swiss population (Figs. 3C and S10). Several populations from Austria, Italy and Switzerland shared a central European ancestry. (orange color, Fig. 3C), while the third Italian component (light blue, Fig. 3C and S10) was shared also by three Austrian populations. K = 4 assigned two Austrian populations and three Italian populations to a different cluster (green colour, Fig. 3C and 4), while K = 5 detected a distinct component for a Finnish population (dark magenta color, Fig. 3C). Neighbor-net analysis supported these results, confirming the cluster of Italian and Austrian populations revealed by STRUCTURE at K = 4 (Fig. S11).

The population structure analysis performed using an alternative algorithm, i.e., the sparse non-negative matrix factorisation algorithm (sNMF), in the LEA R package, provided results similar to STRUCTURE, with the PCA (Fig. S12A) clearly showing at least three main clusters and a sparse cloud of Italian and Austrian samples

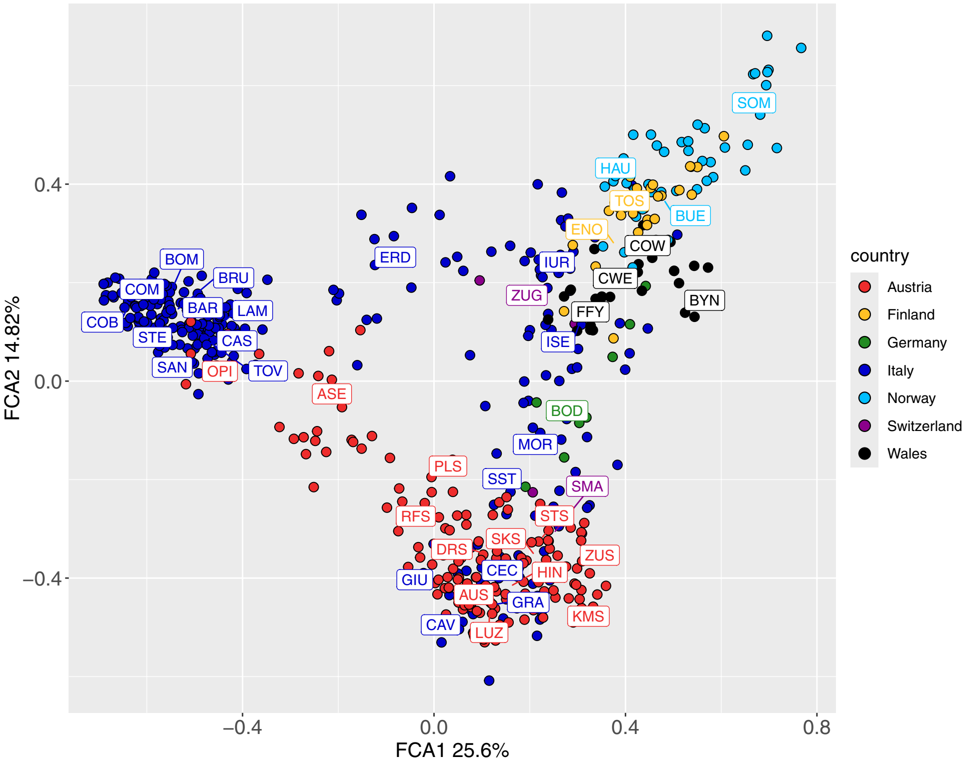

Fig. 2. FCA analysis plot computed using AFLPs data. All populations in the dataset were used to perform the FCA. Values in brackets correspond to the percentages of variance explained by the first (x axis) and the second (y axis) factor.

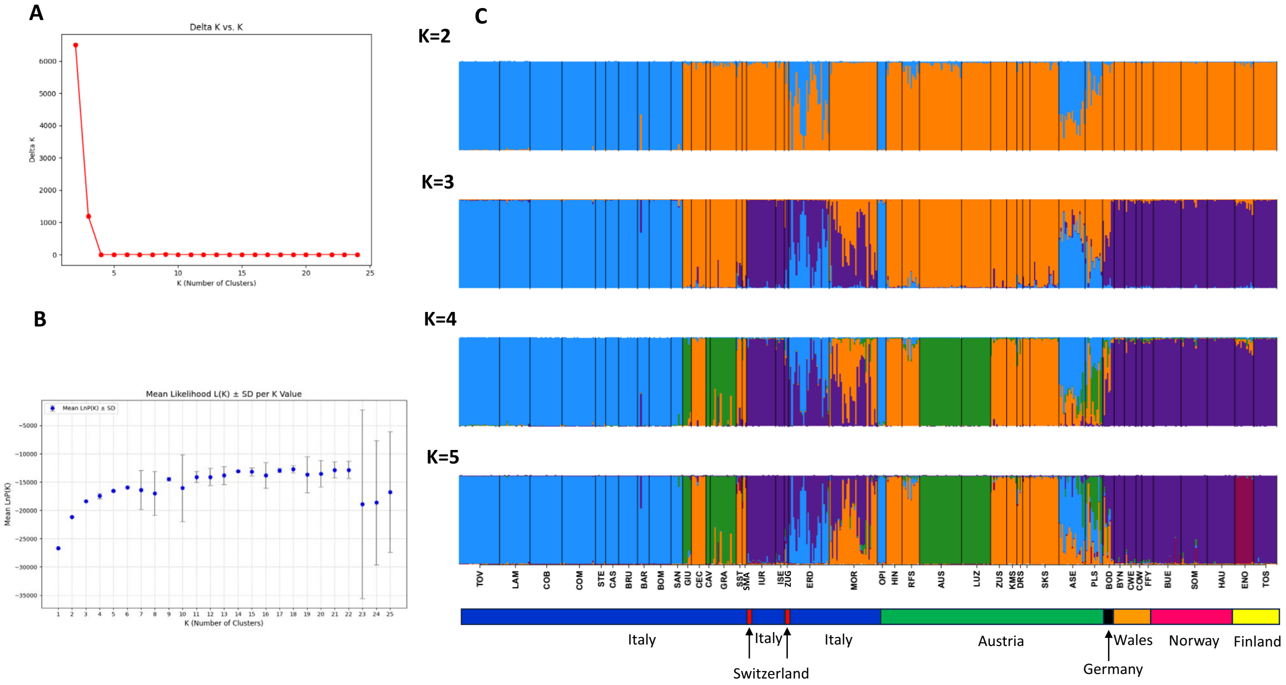

Fig. 3. STRUCTURE analysis results. (**A**) Plot of Evanno's method DeltaK; (**B**) scree-plot of the Log Likelihood values (vertical bars correspond to Standard Deviation); (**C**) Barplot of the posterior probability of the coefficient of membership. Each vertical line represents an individual and colours represent the inferred ancestry from K ancestral populations. Results for K = 2–5 are shown.

as seen from the FCA (Fig. 2). The cross-entropy plot detected an inflection in the slope at K = 4 and the barplots for K = 3–5 confirmed the STRUCTURE admixture pattern, with the addition of a further independent ancestral gene pool including all populations from Wales at K = 5 (Fig. S12, C). The continuous decrease of the entropy suggested further sub-structure particularly with changes in slope at K = 12–20 (Fig. S12B). The corresponding barplots (Fig. S13) showed a complex structure with the Italian samples divided in 2 and 3 clusters as the number of K increases, but always included some degree of admixture with the Austrian samples. The only exception was two samples (TOV and LAM) that clustered separately for K > 15. Most of the Central and Northern European populations were assigned to individual clusters. In addition the Welsh, Bodlyn (BYN) population was assigned to a cluster different from other Welsh populations confirming previous findings[14,15].

Discussion

The demographic history of *S. alpinus* in Southern Europe is very complex. In the Tyrol region (the eastern Alps under both Austrian and Italian jurisdiction) *S. alpinus* has been affected by introduction and stocking/restocking activities started in the late 15th-early sixteenth century[16] (the times of the Holy Roman Empire) and continued afterwards. To correctly manage and potentially conserve the current populations of *S. alpinus* in Italy, their native status should be accurately defined. Native populations dating back to ancient natural colonisation events, or even expansions predating the post-glacial period, should be restored and protected. Conversely, all Italian Arctic charr populations resulting from recent or historical stocking activities should be managed as exotic species, and restocking or protection activities either stopped or appropriately and legally regulated. Our molecular results confirm the extensive gene flow among *S. alpinus* populations due to introduction and translocation as well as *S. fontinalis* introgression at rates varying between 2.7% and 6%, which is consistent with previous findings[11,12].

The mitochondrial CR DNA data clearly showed extensive mixing between the Austrian and Italian populations (Fig. 1, Hap_2), which is supported by historical sources dating back to the sixteenth-seventeenth centuries that show several Italian Tyrol lakes were stocked with *S. alpinus* originating from Austrian water bodies[6,13,17–19]. In the mitochondrial CR network, which included publicly available sequences (Fig. 1, Table S1), the Alpine *S. alpinus* haplotypes clustered within the Atlantic haplogroup, distinct from the Northernmost samples collected in eastern Russia, Siberia, Canada and Arctic area, which belong to the Siberian, Arctic and Acadian haplogroups[9]. The star-like shape of the Atlantic haplogroup sub-network suggested that *S. alpinus* populations experienced a sudden demographic growth, probably through a post-glacial expansion. The CR network also supports a connection between the European *S. alpinus* and those found in Russia. One of the major haplotypes, indeed, includes Russian sequences alongside those of Italy and Austria (Fig. 5). This analysis corroborates previous findings[9,10] that found that *S. alpinus* populations are geographically structured and well differentiated, on a wide spatial scale, with some connections between Scandinavian and Russian populations. Although there is no historical documentation reporting restocking of Italian Valbona (VAL) lake with Scandinavian samples, this

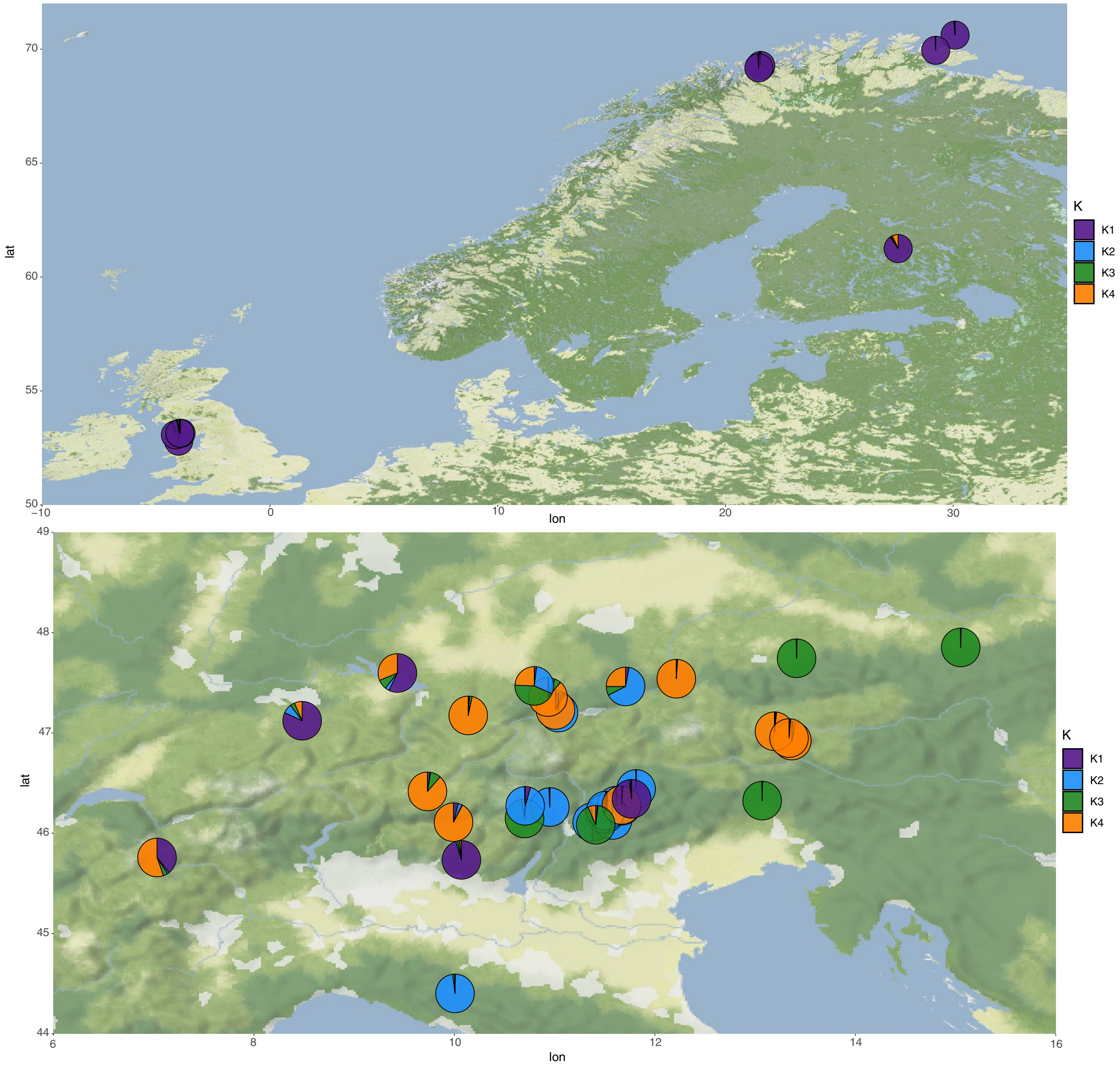

Fig. 4. Coefficients of membership map for K = 4. Map showing the membership coefficients calculated at K = 4 averaged per population and plotted as pie charts at the geographical coordinates of the sampling locations. The map has been constructed using *ggmap* (Kahle 2013) and *scatterpie* packages (Guangchuang Yu; https://CRAN.R-project.org/package=scatterpie) in R (R Core team 2023) with a Stadia Maps API key obtained for free after registration (https://stadiamaps.com/). At the top the Northern sampled populations (Wales, Norway and Finland), at the bottom the Alpine sampled populations (Italy, Switzerland, Austria and Germany).

population share two haplotypes with a Norvegian (Hap_3, BUE) and a Finnish population (Hap_5, ENO), and shows a private haplotype (Fig. S3, Hap_7) clustering with these two Northern haplotypes (Fig. S3). However, this should be evaluated with caution, considering the clear signals of introgression from *S. fontinalis* detected in this lake thus pointing to anthropogenic activities possibly originating this population (Fig. S8).

The mitochondrial COI locus showed lower diversity than CR, which was expected considering its lower mutation rate. In general, a predominant haplotype with a few private haplotypes were identified beyond the presence of a predominant haplotype (Fig. S5) which includes *S. alpinus* COI sequences from all the sampled countries. Unexpectedly, Austria and Wales shared a rare haplotype, despite no records exist of the possible introduction of Austrian charr to Wales or vice versa.

The expected heterozygosity values obtained from AFLPs data were highly variable, with particularly low values in populations collected in the Italian perialpine and Alpine area, resulting from the low number of polymorphic loci found in these samples (Table 1). The AMOVA performed on mitochondrial and nuclear loci

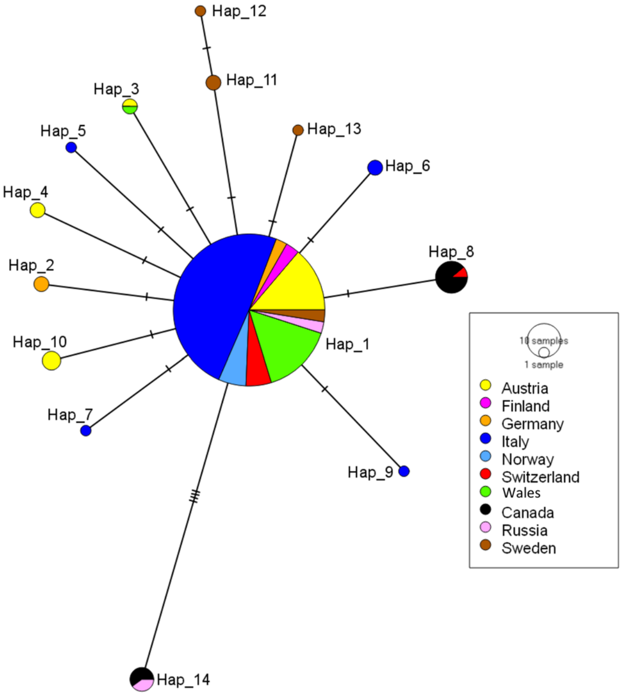

Fig. 5. TCS network constructed using the COI sequences of *S. alpinus* and reference sequences obtained from NCBI database (Canada, Russia, Sweden).

showed partially contrasting results: the two mitochondrial loci detected a significant partitioning of variance among populations within countries, whereas AFLPs identified significant differentiation among countries. This difference may be partly due to the particularly low level of mtDNA polymorphism displayed by the species, and partly from the uni- vs. bi-parental mode of inheritance of mitochondrial vs AFLP markers. Factorial Correspondence Analysis (Fig. 2) clearly distinguished Northern from Southern European populations and showed the different origin of several Italian samples. This signal of admixture can be ascribed both to the Pleistocene events, which shaped the original distribution of *S. alpinus* and promoted the isolation of some populations, and to anthropogenic activities of introduction and restocking from diverse areas. Worth noting in this context is the admixed nature of the individuals reared at the hatchery facility of Morgex (MOR) which showed signals of an admixed Central and Northern European ancestry, thus raising concerns regarding the use of these individuals for restocking.

The same admixture pattern was seen in the membership coefficient plot obtained with STRUCTURE. Considering K = 4 as a probable scenario (Fig. 3), which is suggested by the Neighbor-net analysis performed on the same data, three core groups of populations account for the Northern (Norway, Finland and Wales) Central (Austria) and southern European (Italy) gene pools, accompanied by populations displaying varying degrees of admixture. There were two well defined clusters in Austria, as well as a signal of admixture between Austrian and some Italian populations supporting the TCS network of mitochondrial DNA CR sequences, FCA analysis and Neighbor-net analysis of AFLPs (Figs S3, 4 and S11).

The genetic signature of past admixture events was also confirmed by sNMF software results when considering K = 4 and further refined for K = 5 (Fig. S12), with a well differentiated cluster for the Welsh populations. Considering K = 12–20 (Fig. S13), a very complex scenario emerged which suggested various potential admixture episodes. Several Italian and Austrian populations shared the same ancestry. Conversely, the Northern European populations (Norway, Finland and Wales) had diverse and well differentiated clustering patterns: i) the ENO population was differentiated from the other Finnish populations and confirmed previous finding based on simple tandem repeat (STR)[20]; ii) TOS and SOM populations clustered together, probably due to the close geographical proximity of the two lakes; iii) within the Welsh (UK) group, the BYN population, which is native to North-Western Wales, was assigned to a separate cluster from the CWE (native), FFY and COW populations (non-native) confirming the interconnection between FFY and COW[21]. Notably, Morgex samples (MOR, Italian hatchery), showed an admixed origin whose ancestry can be traced back to the Austrian and Northern European gene pools. This pattern was shared with the Lake Iseo (ISE, Italy) population, suggesting a possible stocking from the Morgex hatchery. Lastly, two Italian populations (TOV and LAM) showed some degree of distinctiveness and formed a sub-cluster starting from K = 14 (Fig. S13), even though this behaviour may be partially explained by the low number of polymorphic markers displayed by these populations (Table 1).

This work describes a very complex scenario of genetic differentiation and admixture of *S. alpinus* populations inhabiting the European area. The samples analysed in this study date back to the early twenty-first century, i.e., about five generations ago (assuming a generation time of 3–5 years for *S. alpinus*[22–24]), therefore providing a thorough description of the current genetic variation of the species over the study area. The distribution

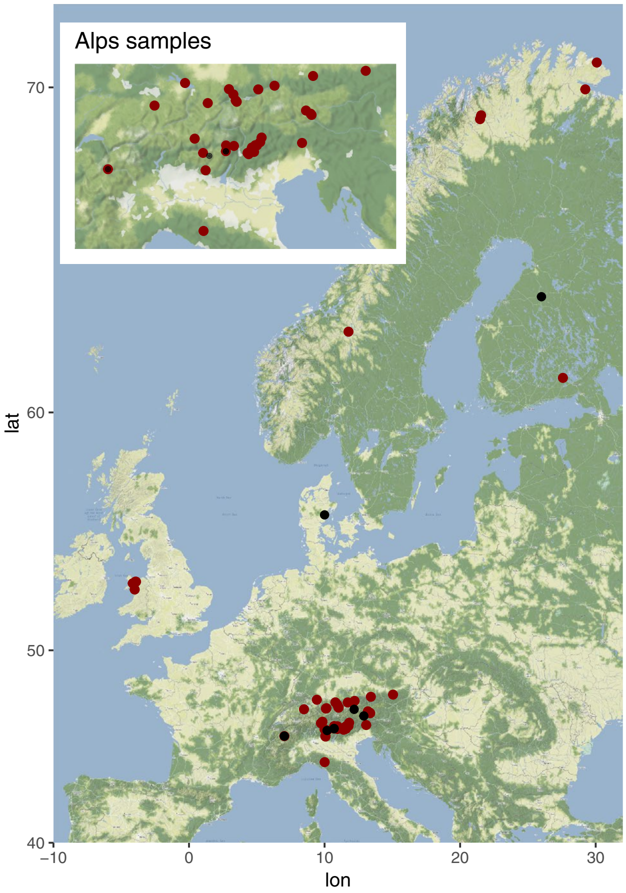

Fig. 6. Map of the sampling locations. Further details can be found in Table 1 and Table S1. The inset shows a close-up of the Alpine and perialpine region. The red dots indicate sampling sites for *S. alpinus*, while the black dots show the sites for *S. fontinalis*. The map has been constructed using *ggmap* package (Kahle 2013) in R (R Core team 2023) with a Stadia Maps API key obtained for free after registration (https://stadiamaps.com/).

of molecular variation we observed may account for introduction and translocation events dating back to different time points over the last 500 years or even earlier. Several populations from different countries showed signals of extensive admixture suggesting that different source populations were probably used for stocking/restocking over time. Unfortunately, the lack of detailed historical information makes it difficult to disentangle the postglacial history of the species and to determine how much of the current diversity pattern can be ascribed to the consequences of Pleistocene events or anthropogenic activities. However, interesting results emerged regarding the occurrence of well differentiated gene pools with a clear geographical connotation, i.e., Northern, Central and Southern Europe. Regarding the Italian populations inhabiting the Southern Alps, the extensive sampling and the use of both mitochondrial and nuclear markers allowed us to highlight the potential source of restocking, especially when inspecting AFLPs admixture analysis. Our results, in fact, revealed the presence of clear Northern and Central European ancestries in the Italian territory which poses several important questions about the status of *S. alpinus* in Southern Europe and especially in Italian lakes, and raises concerns on the management of the hatcheries' stocks and on how to address the conservation of Italian Arctic charr nuclei. In fact, among the Italian populations that belonged to the Southern European cluster those of Lake Tovel and Lagorai Maggiore (TOV and LAM) were found to be genetically distinct from the others, an evidence which seems to sustain the view of some authors who considered the population from Lake Tovel as native[5,6].

Material and methods

A total of 693 *S. alpinus* (Table 1), 57 *S. fontinalis* and 30 hybrid individuals (Table S1) were collected between 1999 and 2009 from 51 populations sampled in 41 alpine and 10 Northern European lakes from eight countries (Austria, Italy, Germany, Norway, Finland, United Kingdom (Wales), Switzerland and Denmark, Table 1, Table S1 and Fig. 6). DNA was extracted from fin clips using the AquaPure Genomic DNA tissue kit (BioRad – Hercules, CA, USA) following the manufacturer instructions.

A fragment of 552 bp of the mitochondrial Control Region (CR) was amplified using primers HN20 (5'-GTGTTATGCTTTAGTTAAGC-3') and Tpro2 (5'-ACCCTTAACTCCCAAAGC-3') and amplification conditions described in Brunner et al.[9]. For the Cytochrome Oxidase I (COI) a fragment of 707 bp was amplified by using primers FISH-COF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FISH-COR1 (5'-TAGACTTCTG

GGTGGCCAAAGAATCA-3’) following the amplification conditions described in Ward et al.[25]. PCR products were purified with Wizard® SV Gel and PCR Clean-up System (Promega) and sequenced in the forward direction in outsourcing partly at BMR Genomics (Padova, Italy) and partly at Macrogen (Seoul, Korea). The electropherograms were visually inspected and edited by using the Sequence Alignment Editor v7.0.9.0[26]. Text format sequences were aligned with Clustal-W as implemented in MEGA version v5[27]. The software DNAsp v5.00.07[28] was used to identify the haplotypes and to compute haplotype and nucleotide diversity. Expected heterozygosity He[29] and haplotype frequencies were computed using Arlequin v3.5.2.2 software[30]. The same package was used to run an Analysis of MOlecular VAriance (AMOVA[31]) to test for the hierarchical partitioning of mitochondrial genetic variation between populations and countries. TCS[32] haplotype networks were constructed from the aligned sequences using the PopArt software[33]. The same analyses were performed using the concatenated sequences of the CR and COI (41 sequences of *S. alpinus*). Moreover, a haplotype network was constructed including 193 mitochondrial CR reference sequences collected from the NCBI database, considering only sequences whose sampling site was recorded (Table S2).

AFLP (Amplified Fragment Length Polymorphism) profiles were obtained by digestion with EcoRI and TaqI restriction enzymes and P[33] radioactive labelling following the procedure described by Ajmone-Marsan et al.[34]. Four primer combinations were used: Eco32(AAC)/Taq32(AAC), Eco35(ACA)/Taq32(AAC), Eco45(ATG)/Taq32(AAC), Eco33(AAG)/Taq33(AAG). To assess the occurrence of *S. fontinalis* introgression, monomorphic bands present exclusively in the Brook charr samples were identified and their occurrence was evaluated both in the hybrid individuals and in all samples that were described as *S. alpinus* from a phenotypic point of view. The markers were binary scored in a dominant manner as 1 = presence of the band, 0 = absence of the band or 2 = missing data. To estimate the occurrence of Brook charr introgression, an assignment test was performed with the Bayesian approach implemented in the software STRUCTURE ver. 2.1[35,36]. The analysis was run for K = 2 in a supervised fashion by flagging the *S. fontinalis* genotypes as reference samples (USEPOPINFO = 1). The run was replicated 10 times with 100,000 MCMC iterations with a burn-in period of 50,000. We used the no-admixture model with uncorrelated allele frequencies. Arctic charr bearing signs of Brook charr introgression above 2% were then excluded from subsequent analyses.

Polymorphic loci specific to *S. alpinus* were detected and scored in the same way, and subsequently analysed to assess the genetic variation of Arctic charr populations. A Bayesian approach with a uniform distribution was used to estimate allelic frequencies with the software AFLP-surv ver. 1.0[37]. Further analyses were performed at the level of single individuals by a Factorial Correspondence Analysis (FCA) using the software Genetix[38]. Expected heterozygosity was calculated following Nei[29] and an AMOVA analysis was performed to estimate the partitioning of diversity between populations and countries using Arlequin v3.5.2.2 software[30]. A neighbor-network based on a Reynolds[39] genetic distance matrix computed with Arlequin v3.5.2.2[30] was then constructed with Splitstree software[40] with default settings.

Population structure was investigated through a model-based Bayesian clustering approach implemented inthe software STRUCTURE ver. 2.1[35,36]. The analysis was run for K (i.e., the number of ancestral populations that gave rise to the current pattern of admixture) values between 1 and 25, with 10 replicates per run, 800,000 MCMC iterations with a burn-in period of 80,000. We used the admixture model with uncorrelated allele frequencies[35,36]. The most likely number of clusters was inferred using both the standard method (plotting ln Pr (X|K) vs K) and the ΔK statistic based on the rate of change in the log probability of the data[41]. The results were analysed with the CLUMPAK (Cluster Markov Packager Across K) website program[42] (https://clumpak.tau.ac.il/index.html) and displayed using R[43] and Rstudio[44] programs. To assess the consistency of population structure estimation with an approach different from STRUCTURE, both a Principal Component Analysis (PCA) and a non-negative matrix factorization algorithm[45] (sNMF) analyses were carried out using the R[43] package LEA[46]. This method is based on sparse non-negative matrix factorisation (NMF) and least-squares optimisation[47–49]. Like principal component analysis (PCA), NMF algorithms are flexible approaches that are robust to departures from the assumptions of the traditional population genetic model. Furthermore, NMF algorithms provide ancestry proportion estimates in much shorter runtimes than STRUCTURE. The analysis was performed with K = 1–20, 200 iterations, entropy = T, 100 repetitions and alpha = 100. All the runs reached convergence and the cross-entropy criterion was used to identify the best K. For graphical purposes, the best run with the minimum cross-entropy out of 100 was used.

Data availability

CR and COI DNA sequences obtained during the current study were deposited in the European Nucleotide Archive (ENA, http://www.ebi.ac.uk/ena) under the project number PRJEB89968 (http://www.ebi.ac.uk/ena/data/; accession numbers OZ271921-OZ272152 for dloop, accession numbers OZ272153-OZ272378 for COI sequences). A text file of AFLPs data is available as Supporting Information.

Received: 5 June 2025; Accepted: 17 October 2025

References

1. Klemetsen, A. The charr problem revisited: exceptional phenotypic plasticity promotes ecological speciation in postglacial lakes. *Freshw. Rev.* **3**, 49–74 (2010).
2. Elliott, J. M. & Elliott, J. A. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. *J. Fish Biol.* **77**, 1793–1817 (2010).
3. Kottelat, M. & Freyhof, J. *Handbook of European Freshwater Fishes* Vol. 13 (Publications Kottelat Cornol, 2007).
4. Nordeng, H. Solution to the “char problem” based on Arctic char (*Salvelinus alpinus*) in Norway. *Can. J. Fish. Aquat. Sci.* **40**, 1372–1387 (1983).

5. Ford, M. 2024. *Salvelinus alpinus*. The IUCN Red List of Threatened Species 2024: e.T242222668A242224493.(Accessed on 23 October 2025); https://dx.doi.org/10.2305/IUCN.UK.2024-2.RLTS.T242222668A242224493.en.
6. Machino, Y. History and status of Arctic charr introductions in southern Europe. *ISACF Inf. Ser.* **7**, 33–39 (1999).
7. Bianco, P. G. *et al.* Salvelinus alpinus. The Italian IUCN red list of threatened species. http://www.iucn.it/scheda.php?id=972302088.
8. Piccinini, A., Nonnis Marzano, F. & Gandolfi, G. I. Salmerino alpino (*Salvelinus alpinus*): prove storiche alla sua introduzione sul territorio italiano. *Atti* **9**, 259–264 (2004).
9. Brunner, P. C., Douglas, M. R., Osinov, A., Wilson, C. C. & Bernatchez, L. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. *Evolution (N. Y.).* **55**, 573–586 (2001).
10. Colli, L. et al. Molecular characterization of Alpine and Northern European populations of Arctic charr *Salvelinus alpinus* (Linnaeus, 1758) by means of nuclear and mitochondrial markers. *Stud. Trentini di Sci. Nat.* **87**, 61–65 (2010).
11. Gross, R., Gum, B., Reiter, R. & Kühn, R. Genetic Introgression Between Arctic Charr (*Salvelinus alpinus*) and Brook Trout (*Salvelinus fontinalis*) in Bavarian Hatchery Stocks Inferred from Nuclear and Mitochondrial DNA Markers. *Aquac. Int.* **12**, 19–32 (2004).
12. Faulks, L. & Östman, Ö. Genetic Diversity and Hybridisation between Native and Introduced Salmonidae Fishes in a Swedish Alpine Lake. *PLoS ONE* **11**, e0152732 (2016).
13. Stolz, O. *Geschichtskunde Der Gewässer Tirols* (Wagner, 1936).
14. Child, A. R. Biochemical polymorphism in char (*Salvelinus alpinus* L.) from LLYNNAU Peris, Padarn. *Cwellyn and Bodlyn. Heredity (Edinb)* **38**, 359–365 (1977).
15. Beck, S. V. et al. Genetic insights into the management and conservation of Arctic charr in North Wales. *bioRxiv* https://doi.org/10.1101/2021.12.03.470319 (2021).
16. Pechlaner, R. Historical evidence for the introduction of Arctic charr into high-mountain lakes of the Alps by man. in *Proceedings of the International Symposium on Arctic Charr* 449–557 (University of Manitoba Press, 1984).
17. von Wolkenstein, M. S. *Landesbeschreibung von Südtirol, Verfasst Um 1600* Vol. 34 (Universitäts-verlag Wagner, 1936).
18. Bernardi, C. L. ricostruzione del patrimonio ittico del lago di Molveno. *Stud. Trentini di Sci. Nat.* **3**, 3–19 (1953).
19. Bernardi, C. *Considerazioni Sulla Paleodiffusione Nelle Nostre Acque Alpine Di Alcune Rare Specie Di Salmo e Sulle Ragioni Che Impongono l'adozione Di Misure Protettive Idonee Ad Impedirne Il Prevedibile Prossimo Annientamento* (Istituto poligrafico dello Stato, 1956).
20. Primmer, C. R. et al. Microsatellite analysis of hatchery stocks and natural populations of Arctic charr, *Salvelinus alpinus*, from the Nordic region: implications for conservation. *Hereditas* **130**, 277–289 (1999).
21. McCarthy, I. D. The Welsh Torgoch (*Salvelinus alpinus*): a short review of its distribution and ecology. *Ecol. Freshw. Fish* **16**, 34–40 (2007).
22. Jonsson, B. & Hindar, K. Reproductive Strategy of dwarf and normal Arctic Charr (*Salvelinus alpinus*) from Vangsvatnet lake, Western Norway. *Can. J. Fish. Aquat. Sci.* **39**, 1404–1413 (1982).
23. Jonsson, B. et al. Life history variation of polymorphic Arctic Charr (*Salvelinus alpinus*) in Thingvallavatn, Iceland. *Can. J. Fish. Aquat. Sci.* **45**, 1537–1547 (1988).
24. Bradbury, C., Roberge, M. H. M. & Minns, C. K. *Life History Characteristics of Freshwater Fishes Occurring in Newfoundland and Labrador, with Major Emphasis on Lake Habitat Requirements* (Minister of Supply and Services Canada, 1999).
25. Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R. & Hebert, P. D. N. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B Biol. Sci.* **360**, 1847–1857 (2005).
26. Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* vol. 41 95–98 ([London]: Information Retrieval Ltd., c1979-c2000., 1999).
27. Tamura, K. et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739 (2011).
28. Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X. & Rozas, R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496–2497 (2003).
29. Nei, M. DNA polymorphism within and between populations. In *Molecular Evolutionary Genetics* (ed. Nei, M.) (Columbia University Press, 1987).
30. Excoffier, L. & Lischer, H. E. L. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**, 564–567 (2010).
31. Excoffier, L., Smouse, P. E. & Quattro, J. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491 (1992).
32. Templeton, A. R., Crandall, K. A. & Sing, C. F. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data III. Cladogram estimation. *Genetics* **132**, 619–633 (1992).
33. Leigh, J. W., Bryant, D. & Nakagawa, S. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, (2015).
34. Ajmone-Marsan, P. et al. AFLP™ markers for DNA fingerprinting in cattle. *Anim. Genet.* **28**, 418–426 (1997).
35. Pritchard, J. K., Stephens, M. & Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959 (2000).
36. Falush, D., Stephens, M. & Pritchard, J. K. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587 (2003).
37. Vekemans, X. AFLP-SURV 1.0: A program for genetic diversity analysis with AFLP (and RAPD) population data. http://www.ulb.ac.be/sciences/lagev/aflp-surv.html (2002).
38. Belkhir, K. 1996–2004 GENETIX 4.05, logiciel sous Windows TM pour la genetique des populations. http://www.univmontp2.fr/~Genet.html (2004).
39. Reynolds, J., Weir, B. S. & Cockerham, C. C. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics* **105**, 767–779 (1983).
40. Huson, D. H. & Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254–267 (2006).
41. Evanno, G., Regnaut, S. & Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620 (2005).
42. Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A. & Mayrose, I. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.* **15**, 1179–1191 (2015).
43. R Core Team. R: A language and environment for statistical computing. https://www.r-project.org/ (2023).
44. Racine, J. S. RStudio: a platform-independent IDE for R and Sweave. (2012).
45. Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G. & François, O. Fast and efficient estimation of individual ancestry coefficients. *Genetics* **196**, 973–983 (2014).
46. Frichot, E. & François, O. LEA: An R package for landscape and ecological association studies. *Methods Ecol. Evol.* **6**, 925–929 (2015).
47. Lee, D. D. & Seung, H. S. Learning the parts of objects by non-negative matrix factorization. *Nature* **401**, 788–791 (1999).
48. Kim, H. & Park, H. Sparse non-negative matrix factorizations via alternating non-negativity-constrained least squares for microarray data analysis. *Bioinformatics* **23**, 1495–1502 (2007).
49. Kim, J. & Park, H. Fast nonnegative matrix factorization: An active-set-like method and comparisons. *SIAM J. Sci. Comput.* **33**, 3261–3281 (2011).

Acknowledgements

We thank Elisabetta Milanesi and Marco Pellecchia for technical support in laboratory procedures. Jorma Piironen, Heikki Hirvonen and Peder Jansen are acknowledged for providing samples from Finnish and/or Norwegian populations.

Author contributions

G.R. analysed the data, wrote the main manuscript text and prepared all the figures and tables. M.P. performed lab analysis to produce dloop and COXI sequences. E.S. performed preliminary data analysis on AFLPs data. R.N. provided helpful insights into data interpretation. E.E. performed preliminary lab analysis. A.G. provided research funds and *S. alpinus* samples. F.C. provided helpful insights into data interpretation. E.V. performed preliminary data analysis on AFLPs data. I.M. provided *S. alpinus* samples from Wales. M.S.G. provided research funds and *S. alpinus* samples. F.N.M. provided *S. alpinus* samples. C.P. provided *S. alpinus* samples from Finland. J.L.W. contributed to structuring the paper. P.A.M. provided research funds and contributed to structuring the paper. L.C. Performed preliminary lab analysis on AFLPs data, provided research funds and contributed to structuring the paper. All authors reviewed the manuscript.

Funding

This work was supported by the project POPSAL "Genetic diversity and aquaculture potential of natural salmonid populations from Trentino" Funded by the Autonomous Province of Trento. Financial support to the publication of this manuscript was provided by the Linea D.3.1 (years 2020 and 2022) of the Università Cattolica del S. Cuore within its activities of promotion and dissemination of scientific research.

Declarations

Competing interests

The authors declare no competing interests.

Ethics declarations

Both *Salvelinus alpinus* and *Salvelinus fontinalis* are commercial species therefore neither special permits nor ethics approval were required for their sampling, all methods were carried out in accordance with relevant guidelines and regulations.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-25095-0.

Correspondence and requests for materials should be addressed to G.R.