Book Chapter

Brown trout (*Salmo trutta*) Phylogentics

Edo D'Agaro[1]*, PierPaolo Gibertoni[2] and Stefano Esposito[2]

[1]Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Italy
[2]Mediterraneam Trout Research Group, Italy

***Corresponding Author:** Edo D'Agaro, Department of Agricultural, Food, Environmental and Animal Sciences (Di4A), University of Udine, Via delle Scienze 206, 33100, Udine, Italy

Published **July 18, 2022**

This Book Chapter is a republication of an article published by Edo D'Agaro, et al. at Applied Sciences in March 2022. (D'Agaro, E.; Gibertoni, P.; Marroni, F.; Messina, M.; Tibaldi, E.; Esposito, S. Genetic and Phenotypic Characteristics of the *Salmo trutta* Complex in Italy. Appl. Sci. 2022, 12, 3219. https://doi.org/10.3390/app12073219)

How to cite this book chapter: Edo D'Agaro, PierPaolo Gibertoni, Stefano Esposito. Brown trout (*Salmo trutta*) Phylogentics. In: Prime Archives in Applied Sciences. Hyderabad, India: Vide Leaf. 2022.

Abstract

Salmonid fish have become ecological and research models of study in the field of conservation genetics and genomics. Over the last decade, brown trout have received a high level of interest in research and publications. The term *Salmo trutta* complex is used to indicate the large number of geographic forms present in the species *Salmo trutta*. In Europe, the *S. trutta* complex consists (based on mitochondrial DNA control region analysis) of seven major evolutionary lineages: Atlantic (AT),

Mediterranean (ME), Adriatic (AD), Danubian (DA), Marmoratus (MA), Duero (DU) and Tigris (TI). In several nations, the difficulty of identifying some lineages derives from their wide phenotypic and geographic plasticity and the presence of mixed lineages (due to introgressive hybridization with domestic AT populations). In Italy, the *S. trutta* complex populations living in the Tyrrhenian area and on the main islands (Sicily, Sardinia and Corsica) showed high genetic diversity. Currently, on the Italian Red List, the protected (near threatened) populations are the AD and ME lineages. Recent studies based on traditional (mitochondrial and nuclear markers) and NGS (next-generation sequencing) analyses have clarified some genetic differences between the populations of the Tyrrhenian region, Sicily, Sardinia and Corsica. Native populations in Sardinia belong to the AD lineage, while those living in Corsica are mainly characterized by the AD, MA and ME haplotypes. In Sicily, in the area of the Iblei mountains, an AT lineage (North African) exists. According to some authors, the term *S. macrostigma* should only be used for populations in North Africa. The use of genotyping methods based on mtDNA and nuclear markers and the latest generation sequencing techniques can improve the study of populations and evolutionary lineages in areas where there are overlaps and hybridization phenomena.

Keywords

Salmo trutta Complex; Phylogenetic Analysis; Genomic Analysis

Phylogenetic analysis of Brown trout *Salmo trutta*

The family *Salmonidae* includes three subfamilies: whitefish (*Coregoninae*), grayling (T*hymallinae*) and salmonids (*Salmonidae*). The genus Salmo and six other genera, *Brachymystax*, *Salmothymus*, A*cantholinqua*, *Hucho*, *Salvelinus* and *Oncorhynchu*s, form the subfamily *Salmonidae* [1].

The genus *Salmo* Linnaeus, 1758 is characterized by a wide distribution range and species and local ecotype subdivisions. The genus Salmo includes only two species (*Salmo salar* and *Salmo trutta*). All brown trout in Europe are referred to as the

Salmo trutta complex, which includes most of the European taxa.

The ancestor of the family *Salmonidae* appeared early in the Cretaceous period (between 63 and 135 MYA) in freshwater [2]. Genomic duplication of *Salmonidae* became evident approximately 60 MYA [3,4]. The subfamily *Salmonidae* appeared after the Tertiary period, more precisely, during the Miocene (between 13 and 25 MYA) [5]. The separation between the ancestors *S. salar* (Atlantic salmon) and *S. trutta* occurred approximately 9-15 MYA [4], and that between *S. marmoratus* and *S. trutta* occurred approximately 4 MYA. The nucleotide divergence between *S. salar* and the *S. trutta* complex is less than 2% [6]. However, chromosomal rearrangements [6], chromosome number (*S. trutta*: 80 vs. *S. salar*: 54-58) and the degrees of residual tetrasomy [4] differ significantly between the two species.

The *S. trutta* complex consists of more than 60 species, including several ecotypes and evolutionary lineages. *S. trutta* originated from an ancestral form in the Palearctic region. Bernatchez [7] analysed the mitochondrial DNA control region (mtDNA CR) and found that the *S. trutta* complex consists of five main evolutionary lines, Atlantic (AT), Mediterranean (ME), Adriatic (AD), Danubian (DA) and Marmoratus (MA), which evolved independently following allopatric fragmentation. Additional evolutionary lineages have been identified in the Duero River (Duero, DU) in Spain [8] and the Dades River (DA) [9] and Tigris River (TI) [10] in Turkey. Recently, the *S. obtusirostris* and *S. ohridanus* forms have been included in the genus *Salmo* as distinct species [11]. The lineage AT is distributed throughout the Atlantic basin, from Morocco to Scandinavia. The ME lineage has a native range in the Mediterranean rivers of the Iberian Peninsula, southern France, Corsica, Italy and Greece. Currently, the ME lineage is present in the middle–lower reaches of many watercourses in the Alps, from the Aosta valley to Slovenia. The ME lineage was the first to separate from the AT lineage. The AD lineage is distributed along Mediterranean rivers from the southern Iberian Peninsula to Turkey and the Apennine area in Italy and Corsica. The MA lineage originates from the rivers of the Northern Adriatic basin.

The DA lineage is present in rivers flowing into the Black, Caspian, D'Aral and Persian Gulf Seas. In some areas, the AD and ME lineages show overlaps. For example, in Corsica, both forms have been reported [12], while in Sardinia, native populations are of the AD lineage. In the Iberian Peninsula, populations comprising both AD and ME and pure ME or AD populations have been reported in the Ebro basin [7].

The results of genetic analysis indicate that the different evolutionary lineages of the *S. trutta* complex were modified by glaciation processes and specific colonization histories [13]. The diversification of the *S. trutta* complex occurred mainly during the Pliocene and Pleistocene and was associated with the climate cooling that affected the Northern Hemisphere [14]. The oldest fossil of European trout was found in the Caucasus region and dates back to the Upper Pliocene [15]. During glacial periods, European fauna were confined to ice-free refuges in Spain, Italy and the Balkans and spread northwards during the interglacial warm periods. Populations living in these territories are the result of complex evolutionary processes involving older evolutionary lineages and local adaptations. The occupation of the territory by glaciers was a limiting factor for the distribution of the species even though brown trout can survive in near-zero temperatures. In addition, individuals could move across seas and oceans when the sea water was cool enough. It is probable, therefore, that glacial periods may have caused the isolation of some populations and, at the same time, favoured the spread of others who had free access to the sea. In southern Europe, during interglacial phases, warming favoured the spread and fragmentation of populations living in single catchment areas (Figure 1). Based on the current southern limit of the anadromous populations of *S. trutta*, which approximately corresponds with the northern Atlantic coast of the Iberian Peninsula, we can deduce that S. trutta could form stable anadromous populations until the surface temperature of the seas reached approximately 16-18 °C [14]. During glacial periods, most of the Mediterranean Sea was suitable for salmonid life, providing the possibility of colonizing all accessible watercourses (Figure 2).

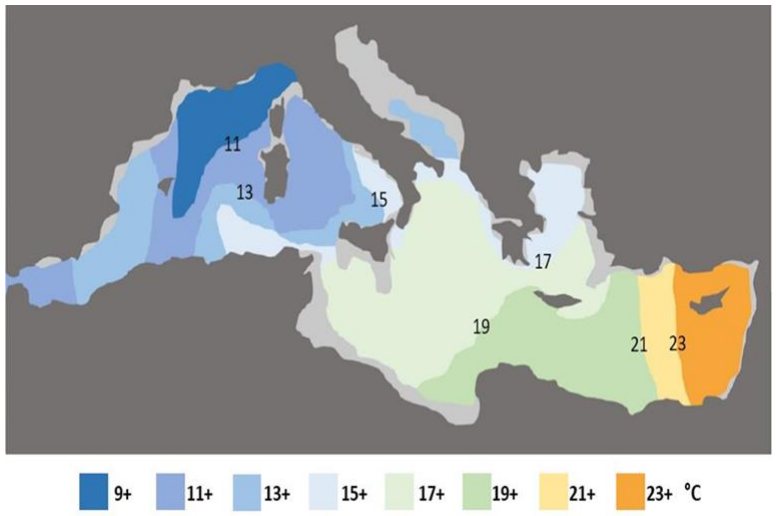

Figure 1: Summer surface temperature (° C) of the Mediterranean sea during the last glacial maximum (LGM). Maps modified from Hayes *et al.* [16]. The light gray coasts are the emerged lands.

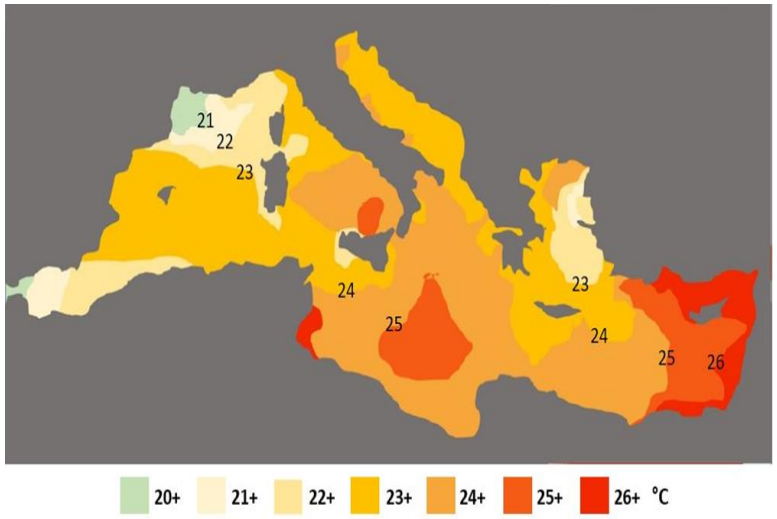

Figure 2: Current surface temperatures (°C) of the Mediterranean Sea.

The spatial genetic differentiation of the *S. trutta* complex is confirmed by the biogeographic hypothesis. The evolutionary lineages in the Mediterranean area (ME–AD–MA) originated from a common ancestor [7, 17]. Within anadromous migratory

populations, only a portion of progeny return to the sea, while some individuals remain as residents and grow to sexual maturity in freshwater. During the Pleistocene, the chemical and physical conditions of the Mediterranean Sea were not always favourable to the survival of anadromous populations, causing alternating situations characterized by phases of isolation and redistribution within the entire Mediterranean basin [14]. The three Mediterranean lineages separated from an anadromous ancestral population approximately 150,000 years ago [15]. This date corresponds with the date of the glacial maximum of the penultimate glaciation (Riss glaciation). Refuge areas have been identified to the west in the major catchment areas of the Rhone and Ebro rivers and to the east in the Anatolian Peninsula, which was scarcely affected by glaciation [7]. The earliest separation was probably followed by a subsequent fragmentation within the Mediterranean basin, leading to the divergence of the ME, MA and AD lineages. Compared to the AT line, ME retains more ancestral features in its genetic sequence [18]. The presence of these lineages confirms the hypothesis that the Mediterranean area was a refuge during glaciations. The richness and diversity found in Southern Europe is probably the result of the persistence of refugia and the accumulation of variations during glaciations, while the rapid postglacial colonization was the result of the less complex evolutionary lineages in northern Europe.

Mitochondrial (mtDNA) and nuclear DNA analysis

Genetic markers based on mtDNA analysis are simple to use and inexpensive. Mitochondria contain most of the genes that code for cellular energy production and electron transfer (the NADH dehydrogenase subunit, the cytochrome oxidase subunit, ATPases 6 and 8, cytochrome b, rRNAr, RNA, 12S and 16S) [19-21]. Compared to nuclear DNA, mtDNA markers show a higher rate of nucleotide substitution (by 5 to 10 times), no (or little) recombination, maternal inheritance and neutral (or nearly neutral) evolution [7]. The choice of the sequence used for genetic analysis depends on the phylogenetic hypothesis to be tested—the D-loop is used in the case of rapid evolution; cytochrome b is used in the case of moderate evolution; and 12S

or 16S rRNA and cytochrome oxidase I are used in the case of slow evolution. mtDNA is used to study the direction of hybridization and the incidence of introgression [22, 23]. Note that in the case of hybridization, the use of mitochondrial markers alone is not sufficient and may generate erroneous inferences. For this reason, other analyses (e.g., based on nuclear genes) are also considered in phylogenetic and phylogeographic studies [24]. Recently, Ravinet *et al.* [25] suggested a Bayesian coalescence-based method for mitochondrial DNA analysis.

Traditionally, the nuclear genes most often used in phylogenetic analyses are histone (H3) and ribosomal (18S and 28S) genes. At the population level, introns or noncoding regions are usually used, as their evolutionary rate is higher and they are not affected by selection [26]. To resolve the simplest phylogeny cases, ITS (internal transcribed spacer) regions offer sufficient variability, although in some cases, paralogy effects and recombination events can complicate analyses [18].

Electrophoretic analyses of proteins and the LDH-C1 allozyme (lactate dehydrogenase-C1 locus) were used in early studies of brown trout [12]. Genetic differences between the AT and ME evolutionary lineages were confirmed using mtDNA and restriction fragment length polymorphism (RFLP) analyses. This method facilitated the distinction between the AT and ME lineages by amplifying the mitochondrial 16S rDNA and control region (D-loop) genes using the restriction enzyme RsaI and amplifying the nuclear LDH-C1* gene using the restriction enzyme BslI [**27]**. The LDH-C1* 90 allele is specific to the AT line. Several authors have used mtDNA markers for genetic analysis of brown trout [14, 28, 29]. The control region sequence is the marker most widely used to identify main evolutionary lineages [**14**]. This gene is characterized by the absence of introns and recombination. *S. marmoratus* is characterized by the mtDNA MA and LDH-C1*(120) alleles [30, 31]. Meraner *et al.* **[**32**]** using the complete mtDNA control region in *S. trutta* identified the MA, AT and DA lineages. In a recent study, ancient DNA was used for phylogeographic analysis of the *S. trutta* complex in Italy **[31],** highlighting that the AD and ME evolutionary lines are also present in museum-preserved samples.

Microsatellite Analysis

Microsatellites are also known as simple sequence repeats (SSRs) or short tandem repeats (STRs). Microsatellites are distributed throughout the genome, with a greater presence in noncoding regions. Microsatellites are mainly neutral and unaffected by selection [33]. Regions flanking repeated sequences tend to be highly conserved. The advantages of using microsatellites are their high reproducibility and high resolution. The most common repeats are di-, tri- and tetra-nucleotides, and the maximum length is approximately 200 bp. Microsatellite loci with a dinucleotide motif are the most widely used due to their higher density (on average, one dinucleotide per 30-50 kb) [34]. Microsatellite markers are very useful in analyses of population structures and gene flow.

The main limitations of the use of SSRs derive from possible errors during PCR amplification [35] and the occurrence of homoplasy products (equal types and numbers of different alleles) [36]. These phenomena lead to underestimations of the true level of divergence between two populations. Genotyping errors can also occur due to the preferential amplification of small alleles (while larger alleles are not amplified) [37] or replication slippage, which can produce additional elements (in the form of allelic prepeaks) [38]. Most of these problems can be solved by using markers from different sources, e.g., nuclear and mitochondrial genes. Microsatellites can be easily identified in new species by de novo techniques [39]. Numerous authors have used microsatellite markers for genetic analysis of brown trout [32, 40, 41].

Combined Analysis of different Genetic Markers

Some authors have used different combinations of mitochondrial and nuclear genetic markers in genetic analyses of the *S. trutta* complex [32]. Berrebi *et al.* [41] used a set of 6-12 microsatellites in combination with 16S rDNA (or D-loop) and LDH-C1* markers. Spendiani *et al.* [42] used two genes, mtDNA (control region) and LDH-C1*, in their first experiment

and three genes, mtDNA (control region), 11 microsatellites and LDH-C1*, in their second work [43]. Fabiani *et al.* [44] used an mtDNA gene and microsatellite markers, while Chiesa *et al.* [45] used the mtDNA 16SrDNA gene and microsatellite markers in a study of *S. marmoratus*.

Next Generation Sequencing (NGS)

Since the 2000s, the development of single nucleotide polymorphism (SNP) analysis has brought ecological genetics into a new era. The development of new sequencing techniques has allowed for the simultaneous analysis of thousands of markers [46]. The first sequencing technology was proposed by Sanger [47] and produced a sequence for each fragment (> 700 bp) with a very low error (<0.01%). This technique has been used to sequence various fish, and it is still used in some laboratories to sequence single genes or microsatellites. New second- and third-generation sequencing techniques (such as single molecule real time (SMRT) sequencing) have improved genomic assembly by the parallel sequencing of millions of fragments [48] and have been used for Atlantic salmon [4], rainbow trout (*Oncorhynchus mykiss*) [49] and brown trout [50]. Currently, several high-density genomic maps are available for brown trout [6], Atlantic salmon [51] and Artic char [52]. Several research projects are underway to study the genomes of Atlantic salmon and rainbow trout (including cGRASP, Consortium for the Genomic Research of all Salmonids, and ICSASG, International Collaboration for the Study of the Sequence of the Genome) [53], perform comparative genome analyses of similar species, and conduct genome resequencing and de novo transcriptome analyses of Atlantic salmon, rainbow trout, brown trout and Arctic charr [54, 55]. These initiatives have enabled the creation of an expressed sequence tag (EST) database and the development and commercialization of a number of microarray SNPs for rainbow trout, Atlantic salmon and other salmonid species. The creation of the PhyloFish database (which published the transcriptomes of 15 fish species) provided another important contribution to the development of the sector [56].

The availability of reference genomes for the main salmonid species is essential for analysing their complex traits [48]. The discovery and annotation of genomic variants allow the production of new microarray SNPs and, accordingly, the implementation of specific genomic programs. Recently, the availability of microarray SNPs for salmonids has allowed the simultaneous analysis of thousands of SNPs at low cost. In the aquaculture sector, recent studies have shown that the genomic selection method can be used to improve several production traits and disease resistance in rainbow trout and Atlantic salmon [57, 58]. Population genomic data also provide valuable information for studying aspects of the eco-evolutionary history of different salmonid species, such as population structures, local adaptations and genetic diversity between populations. In several fish species, genome-wide distributed sets of SNP markers have been successfully used for phylogenetic analyses. The use of SNPs has also contributed to the development of new conservation strategies [59]. Hand *et al.* [60] used 10267 SNPs in the USA to study the introgression of O. mykiss into wild populations of *Oncorhynchus clarkii lewisi* and to identify chromosomal regions affected by selection. To date, these methods have rarely been used for *S. trutta*, and currently, only a limited number of SNP markers for the species have been developed. Recently, for the first time, a commercial SNP microarray derived from a 57K SNP chip originally developed for rainbow trout was used in a *S. trutta* complex selection program [61]. The identification of approximately 900 polymorphic sites facilitated studying the genetic structure of wild Mediterranean brown trout populations in Italy and to begin the selection of native broodstocks. Zhang *et al.* [62] used a 57K DNA chip developed for rainbow trout in different salmonid species, and Drywa *et al.* [63] used a SNP array developed for Atlantic salmon to analyse a population of lake brown trout (*S. trutta trutta*).

In fish phylogenetic and phylogeographic studies, the most popular NGS methods are restriction site-associated DNA sequencing (RAD-seq), transcriptome sequencing (RNA-seq), targeted amplicon sequencing (TAS) and hybrid enrichment using probes. In salmonid population genetic studies, the RAD-seq technique has been widely used [64]. RAD-seq technologies

facilitate overcoming some limitations of traditional methods by increasing the number of genes analysed [6]. The main applications of the RAD-seq method are the identification and annotation of novel SNPs and the genotyping of thousands of SNPs in population genetic studies. The RAD-seq method proposed by Baird [65] showed some limitations, and therefore, subsequent modifications were proposed (ddRAD [66] and 2b-RAD [67]. In Baird's method [65], sequences between known restriction sites and random sites are obtained, while with the ddRAD method, sequences between two known restriction sites are obtained. The results of RAD-seq analysis can be analysed using several software programs, such as STACKs [68], IQ-TREE [69], UNEAK (TASSEL) [70], PyRAD [71], dDOCENT [72] and AftrRAD [73] [57]. The most popular software is the STACKs program. The results obtained in the first analytical step using this software are then transferred to the GENEPOP or STRUCTURE programs for further analysis. The ddRAD method was used by Letwein *et al.* [6] to obtain a dense association map for *S. trutta* by crossing parents of the AT and ME lineages. Lecaudey *et al.* [74] used a RAD-seq method to study the different genera of the subfamily *Salmonidae.* Leitwein *et al.* [75] analysed the introgression of the AT lineage into local ME populations in southern France using the ddRAD technique, and Magris *et al.* [76] used the same method for different populations of the *S. trutta* complex in Italy (northern Italy, the Tyrrenian area, Sardinia and Corsica). Vendrami *et al.* [77] and Lemopoulos *et al.* [33] reported improved genetic analyses using RAD-seq methods compared to microsatellite markers, particularly in resolving population structures. Saint-Pé *et al.* [78] used a RAD-seq method to identify 12,204 SNPs throughout the genome of *S. trutta* and a low-density SNP array (192 SNPs).

Transcriptome sequencing (RNA-seq) is used to analyse the sequences of coding regions, which usually account for approximately 1.5% of the total genome. Carrauthers *et al.* [54] obtained 35,736 protein-coding transcripts for the AT lineage.

Description of the Broun trout *Salmo trutta* complex in Europe

The *S. trutta* complex, with all its variations, is present throughout Europe. The northern limit of its range extends from Iceland to Russia (territories north of the Volga) and the northern part of Scandinavia. The southern limit of its territory is the Atlas Mountains (between Algeria and Morocco) and includes both Italian islands of Sicily and Sardinia. From west to east, brown trout are present from territories along the Atlantic European coast to the Caspian Sea and Aral Sea. The anadromous marine form is present in the rivers of the Cheshkaya Gulf, Baltic Sea, North Sea, Irish Sea, English Channel, Black Sea, Caspian Sea and Aral Sea. Sea trout are absent from the Mediterranean area. Lake trout occur in lakes in the Alps, Scandinavia, Great Britain and northern central Europe [79]. The distribution within this range varies according to the following specific characteristics:

- A water temperature within a very narrow range (averaging less than 20 °C in summer);
- Fast currents;
- Good water quality with pH values close to neutral;
- Easily accessible spawning areas (clean bottoms with coarse gravel and pebbles).
  Examples of mixed evolutionary lineages [7] have been reported in several European countries [41].

Description of the Brown trout *Salmo trutta* complex in Italy

In its wide distribution range, the *S. trutta* complex shows extreme phenotypic diversity and considerable variation in biological characteristics. Italy, due to its latitude, central position in the Mediterranean area and abundant mountains, is a very important hotspot of the *S. trutta* complex. In Italy, the *S. trutta* complex is widely distributed in all suitable waters. From north to south, country ranges from the rivers, streams and lakes of the Alps, which are characterized by seasonality, to the

streams of the southern Apennines, which are characterized by summer heat waves at the survival limits of salmonids. In Italy, available habitats range from the cold springs of the great rivers of the central Apennines to the streams that flow through the middle of the Mediterranean scrub on the main Italian islands.

In recent decades, knowledge of the state and distribution of native populations of the *S. trutta* complex in Italy has received a considerable boost, especially for conservation purposes. This attention has led to the publication of numerous scientific papers that have revealed a certain degree of introgression of domestic populations of northern European origin (AT lineage) into native strains [80, 81]. Clearly, this characteristic makes the systematic classification of the *S. trutta* complex even more complicated.

In recent decades, several taxonomic classification methods have been proposed. Bianco in 1995 [82] identified three species, *S. trutta*, *S. marmoratus* and *S. carpio*, considering *S. marmoratus* and *S. carpio* to be the only Italian endemic species. More recently, Bianco [83] identified six species, *S. fibreni*, *S. carpio*, *S. cettii*, *S. marmoratus*, *S. cenerinus* and *S. rhodanensis*, the latter of which is endemic to the Roja River on the Italian–French border. In 2004, Zerunian [84] identified two species (*S. fibreni* and *S. carpio*) and a superspecies consisting of three semispecies: *S. trutta* semi species, *S. trutta macrostigma*, and *S. trutta marmoratus*. In this case, the endemic species of the Garda and Fibreno lakes were identified as species, and the other forms present in Italy were combined into a single superspecies (*S. trutta*). It is worth noting that this approach was based on the hypothesis existing at the time, which was that the native trout of central–northern Italy were similar to the trout in northern Europe and that in the Tyrrhenian area, some populations similar to *S. macrostigma* occurred, as suggested by Vinciguerra in the early 1900s [84]. Later, according to the first studies based on molecular markers, it became clear that the brown trout of Italy were phylogenetically separated from northern European trout [14]. During this period, Mediterranean Alpine and northern–central Apennine trout were referred to as "Mediterranean brown trout", distinct from *S. macrostigma [42]*.

The taxonomic classification of the S. trutta complex in Italy was revised by Kottelat and Freyhof in 2007 [85]. According to their study, the term *S. macrostigma* should be used only for the populations of Morocco, and the correct name for the "Tyrrhenian and Apennine trout" was *S. cettii* Rafinesque, Schmaltz 1810, which was originally used by the Franco–German zoologist to describe trout living in the rivers of eastern Sicily. Additionally, these authors proposed the name *S. cenerinus* Chiereghini, 1847 for the populations of the *S. trutta* complex living along the Adriatic side of the Apennines (up to the Vomano River in Abruzzo) and in the high portions of Alpine streams in the Po basin. Using several mitochondrial markers (the mtDNA control region) and nuclear markers (11 microsatellites and 8 intronic genes), Gratton *et al.* [86] identified two main native evolutionary lineages: a "peninsular lineage", comprising samples of *S. cenerinus*, *S. cettii and S. fibreni*, and the lineage of *S. carpio* and *S. marmoratus*. *S. marmoratus* is the only native brown trout present in the middle and lower river courses of the Po River and the basins of the upper Adriatic area [87]. According to Tortonese [88], the MA lineage represents a subspecies within the *S. trutta* complex. *S. carpio* was also considered a subspecies by Tortonese [88].

In 2014, the Italian Association of Fresh Water Ichthyologists (AIIAD) [89] proposed a classification system based on evolutionarily significant units (ESU, populations that are partially genetically differentiated as a result of significant evolutionary separation). According to this classification, the 5 evolutionary lineages present in Italy (*S. trutta* complex) are as follows: 1. *S. marmoratus* distributed in the Po valley, Croatia and Slovenia; *S. cettii* (former name *S. macrostigma*) distributed in Sardinia and Sicily, Lakes Posta Fibreno and Ninfa and some Tyrrhenian waterways; *S. ghigii* distributed in the Apennine and Tyrrhenian basins; *S. Fibreni* distributed in the Posta and Fibreno Lakes; and *S. carpio* distributed in Lake Garda. Lake trout are not considered a separate entity but an ecotype of *S. trutta* and *S. marmoratus*. In the literature, Tyrrhenian and Adriatic trout are often associated with the ME and AD mitochondrial haplotypes. In fact, this classification is inaccurate because the ME haplotype is present in some Adriatic

populations, and the AD haplotype is common in some populations living in central Italy and on the main islands [86].

Native *S. trutta* are present in the Western Alps (20%), the Apennines (2.8%), Sardinia (50%) and Sicily (33.3%) [42]. Watercourses with nonnative brown trout populations are common in the Eastern Alps (94.3%), Central Alps (100%) and Western Alps (60.0%) and less common in the Apennines (59.2%) and Sardinia (50.0%). For *S. marmoratus*, all populations in the Central Alps are introduced, while original populations include 75% of those in the Western Alps and 100% of those in the Eastern Alps [43]. Although the mechanisms behind the variation in the rate of introduction remain poorly documented, geological and climatic conditions seem important in the distribution of the *S. trutta* complex. Native trout tend to be absent or rare in rivers with modified flow regimes. Other threats to the *S. trutta* complex include poaching, habitat fragmentation, pollution, water diversion, and competition with invasive nonnative species.

The IUCN Red List of Threatened Species (Version 2016) considers *S. cettii* to be almost threatened, and the populations themselves are listed (as *S. macrostigma*) in Annex II to the Habitats Directive (92/43/EEC). For these populations, the main threats are sport fishing and restocking with nonnative fish. The marbled trout was included on the IUCN Red List as critically endangered [90]. *S. carpio* and *S. fibreni* are considered to be in a critical state due to illegal fishing, the introduction of nonnative species (e.g., *Coregonus* sp.), pollution and habitat degradation.

Description of the morphological characteristics of the Mediterranean brown trout of Italy

All *S. trutta* complex populations living on the Italian mainland and islands (Sicilia, Sardegna and Corsica), except the Sicilian populations in the Iblei mountains, are included in this group [91]. Certain morphological features can help identify the native

forms, even though most of these characteristics are not universal and vary relative to the environment [92] (Figure 3).
In general, the Mediterranean brown trout is described by the following characteristics:

- Parr spots (dark, rounded spots on the flanks typical of juvenile salmonids) also present in adults;
- An evident preopercular dark spot;
- Fine black and red spots with no or a slight white halo.
  These general characteristics are valid for many of the populations of the central and northern Apennines and the Alpine area (91]. These features have recently been updated to include additional frequent characteristics:
- A dark or bluish preopercular spot, with other retro-mandibular and suborbital spots of the same colour in some populations;
- Parr spots inconsistently present in adults (parr spots are always present in numbers greater than nine and have a narrow oval shape compared to the round shape of trout of northern European origin), with multiple rows of parr spots often occurring;
- Black and red spotting present in varying proportions, with some individuals displaying only red or black spotting;
- Primary spotting often consisting of irregularly shaped patches and never including perfectly oval spots with white halos;
- Populations displaying four vertical dark bands alternating with three light bands, which may be more or less evident in different populations;
- Extensive spraying is absent in the dorsal zone, except in some rare hyperpigmented individuals who also have spots on the upper and lower jaws;
- 2-4 dark ocular spots frequently symmetrically arranged around the pupil;
- Complete absence of the black margin, with white bands occurring on the first rays of the anal and dorsal fins (in adults), although this reference characteristic is only valid for peninsular populations; and

- The adipose fin frequently occurring without points, with the exception of a few peninsular populations and many island populations.

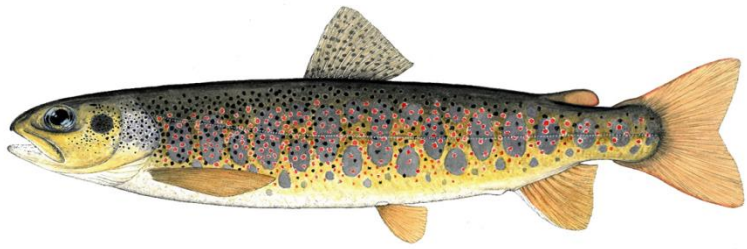

Figure 3: The Mediterranean brown trout of the Northern Apennines in Italy.

Mediterranean brown trout may have two "extreme" eco-phenotypes: one phenotype with black dots and another with predominantly red dots. These two eco-phenotypes show adaptations to different environmental conditions. The phenotype with black dots is usually present in areas with medium–low slopes that are rich in macrophytes. The phenotype with red dots lives in areas steeper slopes and/or waters that are always very cold and oxygenated. The Atlantic trout of livestock origin is described in Figure 4. This general scheme is simplified and does not take into account all environmental variables and possible Mediterranean trout habitats [85, 93, 94] (Figure 5).

Figure 4: The Atlantic brown trout of livestock origin living in the wild.

Figure 5: Some phenotypes of the brown trout living in Italy: 1) Alpine Mediterranean brown trout (Ripa stream basin (TO)); (2) Alpine Mediterranean brown trout (Rio Freddo (CN)); (3) Mediterranean brown trout (Serchio River (LU)); (4) Apennine Mediterranean brown trout (Fegana Stream (LU)); (5) Mediterranean brown trout (Fibreno River (FR)); (6) Apennine Mediterranean brown trout (Secchia River (RE)); (7) Mediterranean brown trout (Santa Croce River (LT)); 8) Mediterranean brown trout (Volturno River (IS)); 9) Mediterranean brown trout (Fibreno River); 10) Mediterranean brown trout (Calore Irpino River (BN)); 11) Mediterranean brown trout (Tanagro River (SA)); Sardinian brown trout (Rio Camboni Basin (CA)); Sardinian brown trout (Flumineddu River Basin (NU)); 14) Corsican brown trout.

Native Mediterranean brown trout in the Alps

The presence of Mediterranean trout in Alpine areas has long been debated. Some authors have reported that the brown trout is an indigenous species of the Alps. Zerunian [84] reported that the brown trout originated in Alpine areas and the northern part of the Apennines, stating, however, that most current populations are totally or partially composed of material of alien origin (AT). Forneris *et al.* [95] reported that the ME lineage cannot be considered native to the left tributaries of the Po River or the tributary waterways of the Adriatic Sea. The only exceptions are some watercourses in the western Alpine area. It should be noted that in the southwestern Alpine area (Liguria and Piedmont), the presence of ME and AD lineage has been reported by several authors, and recently, Splendiani *et al.* [96] (using the mtDNA control region and LDH-C1* genes) confirmed the native origin in this territory. According to these authors, in the southwestern section of the Alps, during the last glaciation, a unidirectional corridor of dispersion was active from the Adriatic side to the French side. For this reason, the French origin hypothesis for the Mediterranean trout populations in the western sector of the Alps must be definitively rejected. Gibertoni *et al.* [97] pointed out that the Po basin (west of the Garda lake) can be considered a potential region of spreading by native mediterranean trout. This hypothesis was recently reinforced by studies conducted by Splendiani *et al.* [31], which analysed museum DNA samples collected from the western Alpine sector, and the results indicated the presence of haplotypes characteristic of Mediterranean brown torut. For example, an ME haplotype was found in a museum sample (dated 1876) from Lake Moncenisio (Dora Riparia basin), and AD haplotypes were isolated in samples from Lake Maggiore (dated 1879) and Lake Garda (dated 1877). Giuffra (1994) reported the presence of the ME lineage in the Dora Riparia basin (MtDNA: cytochrome b and D-loop), and this presence was also confirmed by reports from Cuvier and Valenciennes in approximately the mid-19th century [28]. Genetic studies have been conducted on the *S. trutta* complex in Switzerland using microsatellites and AFLP (Amplified Fragment Length Polymorphism) markers. In these studies, eight populations in

various river basins, including Torrente Poschiavino (Adda basin), Allaine (Rhône basin), the Rhine basin and the Ticino basin, were analysed. Although the aim of the research was to highlight possible neutral and adaptive genetic divergences, the authors found a clear separation between populations in the Allaine, Poschiavino and Rhine Rivers. The authors reported that although alien *S. trutta* have been introduced on a large scale, it was still possible to distinguish three distinct genetic groups, which attests to the residual original native genetic pool. In particular, the authors describe that AD and MA genetic variants were observed in the Poschiavino Stream [99].

Brown Trout of Sicily

In 1810, in a catalogue of Sicilian fish, Rafinesque included the new species *S. cetti*, dedicating it to the zoologist Francesco Cetti, who published the book "Amphibians and Fish of Sardinia" in 1778. Rafinesque described a generic trout with mixed black and red spotting present in the waterways of the Noto Valley and the Demon Valley. In 1896, Vinciguerra identified the Algerian *S. macrostigma* trout described by Dumeril in 1858, which has rounded black spots that are larger than normal and almost no red spots, in the Ragusa area. The similarity between the two fish is evident upon comparing the specimen collected Sicily with the original description. Indeed, oval-shaped spots with clear white halos and a coarse morphology in large and well contracted patches and the presence of black margins on the anal and dorsal fins are distinctive features of Sicilian trout compared to the ME lineage [99].

Trout in the Iblei Mountains of Sicily have a small number of points (on average, 21) and a lower number of spots compared to the ME lineage. The shape of their parrs is also different, presenting as more rounded [99]. These morphological differences have also been confirmed by molecular genetic analyses [100]. The first studies focused on the analysis of mitochondrial DNA, also identifying the AT haplotype in North Africa and the Atlantic regions of the Iberian Peninsula. The presence of the same haplotype in Palaeolithic fossils found in

the caves of Praia a Mare in Calabria [43] suggests that colonization was not limited to Sicily. No other populations belonging to the AT lineage have been reported in other areas of southern Italy. Therefore, the Sicilian populations of the Iblei Mountains are the only AT *S. trutta* complex of South Atlantic origin [30]. Tougard *et al.* [30] analysed the complete mtDNA sequences and the cytochrome b gene of four musem samples (attributed to *S. macrostigma*) and concluded that they belonged to the AT lineage. In the same study, samples from Corsica and Sardinia were attributed to the AD, ME and AT lines [41]. Zaccara *et al.* [94] (based on mtDNA and LDH-C1*) and Sabatini *et al.* [101] reported similar results for *S. trutta* complex populations in Sardinia. The results of a recent study definitively clarified the genetic differences between the Sicilian, Sardinian and peninsular populations [102]. The term *S. cettii* originally used by Rafinesque (1810) to identify the trout in southeastern Sicily should reasonably be used only for *S. trutta* populations in the Iblei Mountains.

Figure 6: The Sicilian brown trout from the Iblei Mountains.

Brown trout of Sardinia and Corsica

Brown trout from Corsica and Sardinia, along with several other Mediterranean trout, are often referred to as *S. macrostigma* [103], which is a species native to Algeria. The name *S. macrostigma* refers to parr signs preserved in adults (Duméril, 1858). Recently, Berrebi *et al.* [41, 104] analysed morphological and genetic characteristics of populations in Sardinia and Corsica. According to the study, *S. trutta* populations living in Sardinia show larger head sizes than those of the AT lineage. Conversely, other characters, such as the body spotting, black

and white edges of fins, body depth, and number of epurals in the caudal skeleton, are polymorphic. The morphological data available for populations living in Corsica are limited to the number of pyloric caves and colour descriptions among populations [105]. Autochthonous *S. trutta* populations in Sardinia (without introgression) are characterized by the AD haplotype and the LDH-C1 100/100 allele [101], while hybrid populations in Sardinia and Corsica are characterized by the AD, MA and ME haplotypes [41, 93].

Conclusions

Genetic studies carried out over the last decade have shown considerable genetic heterogeneity within the *S. trutta* complex in Europe and Italy. *S. trutta* evolutionary lineages living in Italy, particularly in the Tyrrenian area and on the main islands (Sicily, Sardinia and Corsica), have complex genetic structures that are still not fully known in some areas. Results of numerous researches have provided a clear evidence of the introgression of AT lineages into others populations, especially in central Italy. The combined use of traditional genotyping techniques (based on mtDNA and nuclear genetic analyses) and latest-generation sequencing techniques (e.g., the SNP microarray and ddRAD methods) facilitates the more accurate identification of native evolutionary lineages and the measurement of their levels of hybridization with alien lineages. In particular, RAD-seq techniques can be used to identify different lineages and the level of inbreeding within populations. In-depth knowledge of the genetic characteristics of *S. trutta* complex populations present in Italy is important for defining the objectives and priorities of conservation activities and for improving native fish resource restocking and management methods.

References

1. Nelson JS. Fishes of the world, 3rd ed. New York: John Wiley. 1994.
2. Tchernavin V. The origin of Salmon. Salmon Trout Mag. 1939; 95: 120-140.

3. Macqueen DJ, Johnston IA. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proc. Biol. Sci. 2014; 281: 20132881.
4. Lien S, Koop BF, Sandve SR, Miller JR, Kent MP, et al. The Atlantic salmon genome provides insights into rediploidization. Nature. 2016; 533: 200–205.
5. Legendre V. Les ager géologiques et quelques uns de leurs vivants d'apres les fossils. M.L.C.P. Montreal: Service de l'Aménagement et de l'exploitation de la Faune. 1980.
6. Leitwein M, Garza JC, Pearse DE. Ancestry and adaptive evolution of anadromous, resident and adfluvial rainbow trout (Oncorhynchus mykiss) in the San Francisco Bay Area: Application of adaptive genomic variation to conservation in a highly impacted landscape. Evol. Appl. 2017; 10: 56–67.
7. Bernatchez L. The evolutionary history of brown trout (Salmo trutta L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. Evolution 2001; 55: 351–379.
8. Vera M, Cortey M, Sanz N, García-Marín JL. Maintenance of an endemic lineage of brown trout (Salmo trutta) within the Duero river basin. J. Zool. Syst. Evol. Res. 2010; 48: 181–187.
9. Snoj A, Marić S, Sušnik S, Bajec S, Berrebi P, et al. Phylogeographic structure and demographic patterns of brown trout in North-West Africa. Mol. Phylogenetics Evol. 2011; 61: 203–211.
10. Bardakci F, Degerli N, Ozdemir O, Basibuyuk HH. Phylogeography of the Turkish brown trout Salmo trutta L.: mitochondrial DNA PCR-RFLP variation. J. Fish Biol. 2006; 68: 36–55.
11. Snoj A, Marić S, Berrebi P, Crivelli A, Shumka S, et al. Genetic architecture of trout from Albania as revealed by mtDNA control region variation. Genet. Sel. Evol. 2009; 41: 22.
12. Berrebi P. Three brown trout Salmo trutta lineages in Corsica described through allozyme variation. J. Fish Biol. 2015; 86: 60–73.

13. Hewitt HGM. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 1996; 58: 247–276.
14. Cortey M, Pla C, García-Marín JL. Historical biogeography of Mediterranean trout. Mol. Phylogenet Evol. 2004; 33: 831–44.
15. Cortey M, Vera M, Pla C, Garcìa-Marìn J. Northern and Southern expansions of Atlantic brown trout (Salmo trutta) populations during the Pleistocene. Biol. J. Linn. Soc. 2009; 97: 904–917.
16. Hayes A, Kucera M, Kallel N, Sbaffi L, Rohling EJ. Glacial Mediterranean sea surface temperatures based on planktonic foraminiferal assemblages. Quat. Sci. Rev. 2005; 24: 999-1016.
17. Hamilton KE, Fergusson A, Taggart JB, Tómasson T. Post-glacial colonization of brown trout, Salmo trutta L.: Ldh-5 as a phylogeographic marker locus. J. Fish Biol. 1989; 35: 651–664.
18. Snoj A, Melkiè E, Sušnik S, Muhamedagic S, Dovè P. DNA phylogeny supports revised classification of Salmothymus obtusirostris. Biol. J. Linn. Soc. 2002; 77: 399–411.
19. Avise JC, Tatarenkov A. Population genetics and evolution of the mangrove rivulus Kryptolebias marmoratus, the world's only self-fertilizing hermaphroditic vertebrate. J. Fish. Biol. 2015; 87: 519-538.
20. Patarnello T, Bargelloni L, Caldara F, Colombo L. Cytochrome b and 16S rRNA sequence variation in the Salmo trutta (Salmonidae, Teleostei) species complex. Mol. Phylogenet Evol. 1994; 3: 69-74.
21. Splendian A, Giovannotti M, Nisi Cerioni P, Caniglia ML, Caputo V. Phylogeographic inferences on the native brown trout mtDNA variation in central Italy. Ital. J. Zool. 2006; 73: 179-189.
22. Rubinoff D, Holland BS. Between Two Extremes: Mitochondrial DNA is neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference. Syst. Biol. 2005; 54: 952–961.
23. Tobe SS, Kitchener AC, Linacre AMT. Reconstructing Mammalian Phylogenies: A Detailed Comparison of the

Cytochrome b and Cytochrome Oxidase Subunit I Mitochondrial Genes. PLoS ONE. 2010; 5: e14156.
24. Brito PH, Edwards SV. Multilocus phylogeography and phylogenetics using sequence-based markers. Genetica. 2009; 135: 439–455.
25. Ravinet M, Yoshida K, Shigenobu S, Toyoda A, Fujiyama A, et al. The genomic landscape at a late stage of stickleback speciation: High genomic divergence interspersed by small localized regions of introgression. PLoS Genet. 2018; 14: e1007358.
26. Berrebi P, Caputo Barucchi V, Splendiani A, Muracciole S, Sabatini A, et al. Brown trout (Salmo trutta L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear And mitochondrial markers. Hydrobiologia. 2019; 826: 209–231.
27. McMeel OM, Hoey EM, Ferguson A. Partial nucleotide sequences, and routine typing by polymerase chain reactionrestriction fragment length polymorphism, of the brown trout (Salmo trutta) lactate dehydrogenase, LDH - C1*90 and *100 alleles. Mol. Ecol. 2001; 10: 29–34.
28. Giuffra E, Bernatchez L, Guyomard R. Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of brown trout Salmo trutta from northern Italy. Mol. Ecol. 1994; 3: 161–171.
29. Cortey M, Garcìa-Marìn JL. Evidence for phylogeographically informative sequence variation in the mitochondrial control region of Atlantic brown trout. J. Fish Biol. 2002; 60: 1058–1063.
30. Tougard C, Justy F, Guinand B, Douzery EJ, Berrebi P. Salmo macrostigma (Teleostei, Salmonidae): Nothing more than a brown trout (S. trutta) lineage? J. Fish Biol. 2018; 93: 302–310.
31. Splendiani A, Fioravanti T, Giovannotti M, Olivieri L, Ruggeri P, et al. Museum samples could help to reconstruct the original distribution of Salmo trutta complex in Italy. J. Fish Biol. 2017; 90: 2443–2451.
32. Meraner A, Gratton P, Baraldi F, Gandolfi A. (2013). Nothing but a trace left? Autochthony and conservation status of Northern Adriatic Salmo trutta inferred from PCR

multiplexing, mtDNA control region sequencing and microsatellite analysis. Hydrobiologia 2013; 702: 201-213.
33. Lemopoulos A, Prokkola JM, Uusi-Heikkilä S, Anti Vasemägi A, Ari Huusko A, et al. Comparing RADseq and microsatellites for estimating genetic diversity and relatedness — Implications for brown trout conservation. Ecol. Evol. 2019; 9: 2106– 2120.
34. Jarne P, Lagoda PJL. 1996 Microsatellites, from molecules to populations and back, Trends Ecol. Evol. 1996; 11: 424-429.
35. Dieringer D, Schlötterer C. Two distinct modes of microsatellite mutation processes: evidence from the complete genomic sequences of nine species. Genome Res. 2003; 13: 2242-2251.
36. Estoup A, Jarne P, Cornuet JM. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Mol. Ecol. 2002; 11: 1591-1604.
37. Wattier R, Engel CR, Saumitou-Laprade P, Valeo M. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in Gracilaria gracilis (Rhodophyta). Mol. Ecol. 1998; 7: 1569-1573.
38. Shinde D, Lai Y, Sun F, Arnheim N. Taq DNA polymerase slippage mutation rates measured by PCR and quasi-likelihood analysis: (CA/GT)n and (A/T)n microsatellites, Nucleic Acids Res. 2003; 31: 974–980.
39. Grohme MA, Soler RF, Wink M, Frohme M. Microsatellite marker discovery using single molecule real-time circular consensus sequencing on the Pacific Biosciences RS. Biotechniques. 2013; 55: 253-256.
40. Araguas RM, Vera M, Aparicio E, Sanz N, Fernández-Cebrián R, et al. Current status of the brown trout (Salmo trutta) populations within eastern Pyrenees genetic refuges. Ecol. Freshw. Fish. 2017; 26: 120-132.
41. Berrebi P, Caputo Barucchi V, Splendiani A, Muracciole S, Sabatini A, et al. Brown trout (Salmo trutta L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. Hydrobiologia. 2019; 826: 209–231.

42. Splendiani A, Ruggeri P, Giovannotti M, Pesaresi S, Occhipinti G, et al. Alien brown trout invasion of the Italian peninsula: the role of geological, climate and anthropogenic factors. Biol. Invasions. 2016; 18: 2029–2044.
43. Splendiani A, Giovannotti M, Righi T, Fioravanti T, Cerioni PN, et al. Introgression despite protection: the case of native brown trout in Natura 2000 network in Italy. Conserv. Genet. 2019; 65: 460–473.
44. Fabiani A, Gratton P, Zappes IA. Investigating the genetic structure of trout from the Garden of Ninfa (central Italy): Suggestions for conservation and management. Fish. Manag. Ecol. 2018; 25: 1–11.
45. Chiesa S, Filonzi L, Ferrari C, Vaghi M, Bilò F, et al. Combinations of distinct molecular markers allow to genetically characterize marble trout (Salmo marmoratus) breeders and stocks suitable for reintroduction plans, Fish. Res. 2016; 176: 55-64.
46. McKelvey KS, Young MK, Knotek WL, Carim KJ, Wilcox TM. Padgett-Stewart, T.M. and Schwartz, M.K. Sampling large geographic areas for rare species using environmental DNA: a study of bull trout Salvelinus confluentus occupancy in western Montana. J. Fish Biol. 2016; 88: 1215-1222.
47. Sanger F. "The Croonian Lecture, 1975: Nucleotide Sequences in DNA. Proceedings of the Royal Society of London. Series B, Biological Sciences. 1975; 191: 317–33.
48. Gonzalez-Pena D, Gao G, Baranski M, Moen T, Cleveland BM, et al. Genome-Wide Association Study for Identifying Loci that Affect Fillet Yield, Carcass, and Body Weight Traits in Rainbow Trout (Oncorhynchus mykiss). Front. Genet. 2016; 7: 203.
49. Berthelot C, Brunet F, Chalopin D, Juanchich A, Bernard M, et al. The rainbow trout genomeprovides novel insights into evolution after whole-genome duplication invertebrates. Nat. Commun. 2014; 5: 3657.
50. Hansen T, Fjelldal PG, Lien S. The genome sequence of the brown trout, Salmo trutta Linnaeus 1758. Wellcome Open Res. 2021; 6: 108.
51. Tsai HY, Matika O, Edwards SMK, Antolín–Sànchez R, Hamilton A, et al. Genotype Imputation To Improve the

Cost-Efficiency of Genomic Selection in Farmed Atlantic Salmon. G3-genes genom. Genet. 2017; 7: 1377–1383.
52. Sutherland BJG, Gosselin T, Normandeau E, Lamothe M, Isabel N, et al. Salmonid Chromosome Evolution as Revealed by a Novel Method for Comparing RADseq Linkage Maps, Genome Biol. Evol. 2016; 8: 3600–3617.
53. Houston RD, Bean TP, Macqueen DJ, Gundappa MK, Jin YH, et al. Harnessing genomics to fast-track genetic improvement in aquaculture. Nat. Rev. Genet. 2020; 21: 389–409.
54. Carruthers M, Yurchenko AA, Augley JJ, Adams CE, Herzyk P, et al. De novo transcriptome assembly, annotation and comparison of four ecological and evolutionary model salmonid fish species. BMC Genomics. 2018; 19: 32.
55. Gao G, Magadan S, Waldbieser GC, Youngblood RC, Wheeler PA, et al. A long reads-based de-novo assembly of the genomeof the Arlee homozygous line reveals chromosomal rearrangements in rainbow trout. G3-genes genom genet. 2021; 15: jkab052.
56. Froese R, Pauly D. FishBase, The Global Database of Fishes. 2019. available online at: http://www.fishbase.org.
57. D'Agaro E, Favaro A, Matiussi S, Gibertoni PP, Esposito S. Genomic selection in salmonids: new discoveries and future perspectives. Aquac. Int. 2021; 29: 2259–2289.
58. Yoshida GM, Carvalheiro R, Lhorente JP, Correa K, Figueroa R, et al. Accuracy of genotype imputation and genomic predictions in a two-generation farmed Atlantic salmon population using high-density and lowdensity SNP panels. Aquaculture. 2018; 491: 147–154.
59. Shafer ABA, Gattepaille LM, Stewart REA, Wolf JBW. Demographic inferences using short-read genomic data in an approximate Bayesian computation framework: in silico evaluation of power, biases and proof of concept in Atlantic walrus. Mol. Ecol. 2015; 24: 328-345.
60. Hand BK, Hether TD, Kovach RP, Muhlfeld CC, Amish SJ, et al. Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing, Curr. Zool. 2015; 61: 146–154.
61. Palombo V, De Zio E, Salvatore G, Esposito S, Iaffaldano N, et al. Genotyping of two Mediterranean trout populations in

Central-Southern Italy for conservation purposes using a rainbow-trout-derived SNP array. Animals. 2021; 11: 1803.
62. Zhang HY, Zhao ZX, Xu J, Xu P, Bai QL, et al. Population genetic analysis of aquaculture salmonid populations in China using a 57K rainbow trout SNP array. PLoS ONE. 2018; 13: e0202582.
63. Drywa A, Pocwierz-Kotus A, Wąs A, Dobosz S, Kent MP, et al. Genotyping of two populations of southern Baltic Sea trout Salmo trutta m. trutta using an Atlantic salmon derived SNParray. Mar. Genomics. 2013; 9: 25–32.
64. Robledo D, Palaiokostas C, Bargelloni L, Martinez P, Houston R. Applications of genotyping by sequencing in aquaculture breeding and genetics. Rev. Aquacult. 2018; 10: 670-682.
65. Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, et al. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE. 2008; 3: e3376.
66. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE. 2012; 7: e37135.
67. Wang N, Fang L, Xin H, Wang L, Li S. Construction of a high-density genetic map for grape using next generation restriction-site associated DNA sequencing. BMC Plant Biol. 2012; 12: 148.
68. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. Stacks: building and genotyping Loci de novo from short-read sequences. G3 (Bethesda). 2011; 1: 171–182.
69. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol. Biol.Evol. 2020; 37: 1530–1534.
70. Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, et al. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics. 2007; 23: 2633-2635.
71. Eaton DAR. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses , Bioinformatics. 2014; 13: 1844–1849.

72. Puritz JB, Hollenbeck CM, Gold JR. dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ. 2014; 2: e431.
73. Sovic MG, Fries AC, Gibbs HL. AftrRAD: a pipeline for accurate and efficient de novo assembly of RADseq data. Mol. Ecol. Resour. 2015; 15: 1163-1171.
74. Lecaudey LA, Schliewen UK, Osinov AG, Taylor EB, Bernatchez L, et al. Inferring phylogenetic structure, hybridization and divergence times within Salmoninae (Teleostei: Salmonidae) using RAD-sequencing, Mol. Phylogenetics Evol. 2018; 124: 82-99.
75. Leitwein M, Gagnaire PA, Desmarais E, Berrebi P, Guinand B. Genomic consequences of a recent three-way admixture in supplemented wild brown trout populations revealed by local ancestry tracts. Mol. Ecol. 2018; 27: 3466–3483.
76. Magris G, Marroni F, D'Agaro E, Vischi M, Chiabà C, et al. ddRAD-seq reveals the genetic structure and detects signals of selection in Italian brown trout. Genet. Sel. Evol. 2022; 1: 8.
77. Vendrami DLJ, De Noia M, Telesca L. RAD sequencing sheds new light on the genetic structure and local adaptation of European scallops and resolves their demographic histories. Sci. Rep. 2019; 9: 7455.
78. Saint-Pé K, Leitwein M, Tissot L. Development of a large SNPs resource and a low-density SNP array for brown trout (Salmo trutta) population genetics. BMC Genomics. 2019; 20: 582.
79. Melhaoui M. Elements d'ècologie de la truite de lac (Salmo trutta) du Leman dans le système lac-affluent. Fac. Sci. Paris: Univ. Paris. 1985.
80. Caputo V, Giovannotti M, Nisi Cerioni P, Caniglia ML, Splendiani A. Genetic diversity of brown trout in central Italy. J. Fish Biol. 2004; 65: 403-418.
81. Marzano FN, Corradi N, Papa R, Tagliavini J, Gandolfi G. Molecular Evidence for Introgression and Loss of Genetic Variability in Salmo (trutta) macrostigma as a Result of Massive Restocking of Apennine Populations (Northern and Central Italy). Environ. Biol. Fishes 2003; 68: 349–356.
82. Bianco PG. Mediterranean endemic freshwater fishes of Italy. Biol. Conserv. 1995; 72: 159–170.

83. Bianco PG. An update on the status of native and exotic freshwater fishes of Italy. J. Appl. Ichthyol. 2014; 30: 62–77.
84. Zerunian S. Pesci delle acque interne d'Italia. Modena: Quaderni di Conservazione della Natura. Rome: Ministero Ambiente – Istituto Nazionale Fauna Selvatica. 2004.
85. Kottelat M, Freyhof JR. Handbook of European Freshwater Fishes. Cornol: Kottelat Publications. 2007.
86. Gratton P, Allegrucci G, Sbordoni V, Gandolfi A. The evolutionary jigsaw puzzle of the surviving trout (Salmo trutta L. complex) diversity in the Italian region. A multilocus Bayesian approach. Mol. Phylogen. Evol. 2014; 79: 292–304.
87. Sommani E. 1960. Il Salmo marmoratus Cuv.: Sua origine e distribuzione nell'Italia settentrionale. Bollettino della Pesca, Piscicoltura e Idrobiologia. 1960; 15: 41–47.
88. Tortonese E. Osteichthyes, parte I. Fauna d'Italia. Vol. X. Bologna: Calderini. 1970.
89. Zanetti M. La gestione della fauna salmonicola in italia - prime indicazioni dal gruppo salmonidi dell'A.I.I.A.D.. Ital. J. Fresh Ichthyol. 2022. Available online at: www.aiiad.it/ijfi/index.php/ijfi/article/view/15
90. Rondinini C, Battistoni A, Peronace V, Teofili C. Lista Rossa IUCN dei Vertebrati Italiani. Roma: Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare. 2013.
91. Lorenzoni M, Carosi A, Giovannotti M, La Porta G, Splendiani A. Caputo Barucchi V. Morphological survey as powerful detection tool of pure and local phenotypes in Salmo trutta complex. Knowl. Manag. Aquat. Ecosyst. 2019; 420: 48.
92. Aparicio E, García-Berthou E, Araguas RM, Martínez P, García-Marin JL. Body pigmentation pattern to assess introgression by hatchery stocks in native Salmo trutta from Mediterranean streams. J. Fish Biol. 2005; 67: 931–949.
93. Delling B, Sabatini A, Muracciole S, Tougard C, Berrebi P. Morphologic and genetic characterisation of Corsican and Sardinian trout with comments on Salmo taxonomy. Knowl. Manag. Aquat. Ecosyst. 2020; 421: 21.
94. Zaccara S, Trasforini S, Antognazza CM, Puzzi C, Britton JR, et al. Morphological and genetic characterization of

Sardinian trout Salmo cettii Rafinesque, 1810 and their conservation implications. Hydrobiologia. 2015; 760: 205–223.
95. Forneris G, Merati F, Pascale M, Perosino GC. Proposta di indice ittico (I.I.) per il bacino occidentale del Po e prime applicazioni in Piemonte. Rivista Piemontese di Storia Naturale. 2005; 26: 3-39.
96. Splendiani A, Berrebi P, Tougard C, Righi T, Reynaud N, et al. The role of the south-western Alps as a unidirectional corridor for Mediterranean brown trout (Salmo trutta complex) lineages, Biol. J. Linn. Soc. 2020; 131: 909–926.
97. Gibertoni P, Penserini M, Esposito S, Foglia A, Dagani D, et al. Presence of a migratory lacustrine life-history strategy in the marble trout (Salmo marmoratus): The case of the native trout population of Lake Maggiore spawning in the Toce River (Italy). Ital. J. Freshw. Ichthyol. 2014; 1: 25–37.
98. Keller I, Taverna A, Seehausen O. Evidence of neutral and adaptive genetic divergence between European trout populations sampled along altitudinal gradients. Mol. Ecol. 2011; 20: 1888–1904.
99. Duchi A. Flank spot number and its significance for systematics, taxonomy and conservation of the near-threatened Mediterranean trout Salmo cettii: Evidence from a genetically pure population. J. Fish Biol. 2018; 92: 254–260.
100. Fruciano C, Pappalardo AM, Tigano C, Ferrito V. Phylogeographical relationships of Sicilian brown trout and the effects of genetic introgression on morphospace occupation, Biol. J. Linn. Soc. 2014; 112: 387–398.
101. Sabatini A, C Podda, G, Frau MV, Cani A, Musu M, et al. Palmas Restoration of native Mediterranean trout Salmo cetti Rafinesque, 1810 (Actinopterygii, Salmonidae) populations using an electric barrier as mitigation tool. Eur. Zool. J. 2018; 85: 137-149.
102. Segherloo IH, Freyhof J, Berrebi P, Ferchaud AL, Geiger M, et al. A genomic perspective on an old question: Salmo trouts or Salmo trutta (Teleostei: Salmonidae)?, Mol. Phylogenetics Evol. 2021; 162: 107204.

103. Duméril A. Note sur une truite d'Algérie (Salar macrostigma, A. Dum.). C R Acad. Sci. Paris. 1858; 47: 160–162.
104. Berrebi P, Horvath Á, Splendiani A, Palm S, Bernaś R. Genetic diversity of domestic brown trout stocks in Europe, Aquaculture. 2021; 544: 737043.
105. Guyomard R. Diversite´ gènètique de la truite commune. Bull. Fr. Pêche Piscic. 1989; 314: 118–135.