Brown trout (Salmo trutta L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers

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Abstract

The brown trout (*Salmo trutta* L.) is widely distributed all around Europe but its natural diversity is threatened by massive stocking with Atlantic domestic strains. Describing the remaining natural genetic diversity and the proportion of domestic hatchery strains in rivers is a prerequisite for smart conservation. The high genetic diversity of brown trout populations around the Tyrrhenian Sea is well known. Use of twelve microsatellites has allowed description of the natural genetic structure of populations and detection of the consequences of stocking. Mitochondrial DNA control region sequences and the *LDH-C1** gene enabled placement of each population into one of the six mitochondrial and two allozymic known

evolutionary lineages. The Corsican populations showed low intra-population genetic diversity but an exceptionally high level of inter-population differentiation. More southern Tyrrhenian regions exhibited opposite pattern of diversity, partly due to the Atlantic domestic introgression. Globally, the natural structure outlines two north-south clines: high inter-population differentiation and predominance of the Adriatic lineage in the north, but lower inter-population differentiation and presence of the natural Atlantic lineage in the south. In addition, the Tyrrhenian region is the contact zone between the widespread Adriatic lineage and a local natural Atlantic lineage probably coming from North Africa through the Strait of Gibraltar.

Keywords - Microsatellites - mtDNA control region - *LDH-C1** - Tyrrhenian brown trout - Conservation

Running title: Tyrrhenian trout genetic structure

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Introduction

The brown trout is widespread all around Europe, western Asia and North Africa at medium and high elevation (Behnke, 1972, 1986). It is one of the most extensively managed freshwater fish species worldwide because of its high economical value, mainly for sport fishing. As a consequence, brown trout are regularly stocked in most part of its distribution from multiple hatcheries that breed various strains in many countries. This species has also been introduced and acclimatized in numerous countries around the world where sometimes it is considered as an invader (De Moor & Bruton, 1988; Olsson et al., 2006; Miró & Ventura, 2013).

The brown trout is a complex taxon, first investigated through morphology, then with molecular markers. The term of "*Salmo trutta* complex" (Patarnello et al., 1994; Bernatchez & Osinov, 1995; Giuffra et al., 1996; Bernatchez, 2001) is used to indicate that the complex is composed of numerous differentiated geographic forms (without consensus on the taxonomy) that can easily hybridize with each other (Largiadèr & Scholl, 1996; Meldgaard et al., 2007). Its ecological diversity is also remarkable with sedentary populations, sometimes in the cold upstream of rivers, and anadromous ones that migrate to seas or lakes. Based on phenotypes, many species have been described. Kottelat & Freyhof (2007) reviewed the published nomenclature and reached the number of 28 nominal species. This has been augmented by at least fifteen new species described during the last twelve years (Delling & Doadrio, 2005; Turan et al., 2009, 2010, 2011, 2012, 2014a, 2014b; Delling, 2010; Doadrio et al., 2015).

Phylogenetic studies based on mitochondrial DNA (mtDNA) provided a first clear and testable way of classification, dividing *S. trutta* into five main evolutionary lineages, considered as geographic variants but not as species. Thus, Atlantic (AT), Mediterranean (ME), Marble (MA), Adriatic (AD) and Danubian (DA) lineages have been described (Bernatchez et al., 1992) and confirmed in many publications. Other lineages, i.e. monophyletic regional variants at the base of large phylogenetic lineages, such as Duero (DU, Suárez et al., 2001), Tigris (TI, Bardakci et al., 2006), the Balkan cluster (Snoj et al., 2009) and Dades (Snoj et al., 2011) have also been proposed.

The Western Mediterranean basin is considered to extend from the Siculo-Tunisian to the Gibraltar Straits. Its eastern part consisted of more or less isolated zones constituting the Tyrrhenian and Ligurian seas. The Adriatic and Ionian seas belong to the Eastern Mediterranean basin and are at the contact with the Western basin. Several publications analyzed the genetic diversity of trout in limited parts of its distribution around the Tyrrhenian Sea using allozymes, RFLP and sequences of a portion of the mitochondrial control region (mtDNA CR) and microsatellites (Nonnis Marzano et al. 2003; Lucentini et al. 2006; Gratton et al. 2014; Fabiani et al. 2018), but the genetic diversity of brown trout in the Tyrrhenian Sea region has not yet been examined in detail.

According to the large literature based on morphology and molecular genetics proposing taxonomic organization of the trout populations inhabiting the Tyrrhenian periphery, a rather confusing picture has emerged. Nera River (Roma region) was considered to be an indigenous pool, home of the Mediterranean *S. trutta* lineage (Lucentini et al., 2006). Corsican rivers were proposed to host *S. macrostigma* (Roule, 1933; Guyomard, 1989; Bernatchez et al., 1992) or *S. trutta* (Berrebi, 2015), while Sardinian rivers were inhabited by *S. macrostigma* (Boulenger, 1901; Mola, 1928; Pomini, 1940; Gandolfi et al., 1991; Patarnello et al., 1994; Massidda, 1995; Sabatini et al., 2006; Orrú et al., 2010) or *S. trutta* (Sabatini et al., 2011) or *S. cettii* (Zaccara et al., 2015). South Sicilian trout are of Atlantic *S. trutta* lineage (Schöffmann et al., 2007) or *S. cettii* (Kottelat, 1997; Kottelat & Freyhof, 2007; Schöffmann et al., 2007; Duchi, 2011 & 2018; Bianco, 2014; Sabatini et al., 2018).

In the present study, the whole area around the Tyrrhenian Sea was sampled in order to describe the overall structure of trout populations. For this, microsatellite genotypes (12 loci), mtDNA CR sequences and *LDH-C1** genotypes were analyzed. In addition, the impact of stocking was measured. In light of this new knowledge, the principles of conservation are discussed.

Materials and methods

Sampling distribution

The present survey concentrates sampling to the freshwater basins distributed all around the Tyrrhenian Sea with some Ionian Sea tributaries (Fig. 1). In order to introduce comparative samples, a river entering the Ligurian Sea at the French-Italian boundary and three commercial Atlantic domestic samples from northern France and central Italy have been included (Table 1). The French hatchery is representative of the international commercial Atlantic strain and the Italian ones of local domestic strains, both derived from genitors from several countries (Bohling et al. 2016).

Electrofishing was conducted between 2004 and 2014. A total of 365 river trout (sub-adults and adults) was anaesthetized and a small fin clip taken (preserved in 96% ethanol) before each fish was returned to its river. In the three hatcheries sampled, the method was similar but the fish were caught with dip nets.

Some of the samples contained few fish (Table 1): lower Tiber in Lazio region (7 to 9 specimens), Calabria (3 to 7) and Sicily (6 to 11), except for the Anapo River. These samples were very difficult to constitute because of the low trout density. In Sicily and Calabria, the species is protected and special permits are needed with a given maximum number of trout to be caught. Fortunately, most often, this concerned nearby small rivers which could be concatenated for population parameter calculations.

DNA extraction

DNA was extracted using the Chelex/proteinase K based method described by Walsh et al. (1991) and Estoup et al. (1996) then improved by Yue & Orban (2005). A small piece of fin was incubated overnight at 56°C in 195 µL of 5% Chelex 100 Resin (Biorad) solution containing 50 mM of Tris-HCL (pH 7) and 500 µg/mL of proteinase K. Samples were then incubated at 95°C for 10 min before centrifugation at 3500 g for 5 min. Supernatants were recovered and frozen at -20°C until required for use.

119

120 Mitochondrial marker

121

122 The mtDNA CR was amplified by PCR using the PST and FST primers (Cortey & García-Marín, 2002).
123 Each 50 µL reaction included 0.4 µM of each primer (Eurofins MWG Operon), dNTP (2 mM each), 2 mM
124 of MgCl2, 10 µL of 5× PCR buffer, 1 U of *Taq* polymerase (GoTaq® Promega) and about 50 ng of
125 genomic DNA. The PCR conditions included initial denaturation (95°C, 5 min) followed by 30 strand
126 denaturation (94°C, 1 min), primer annealing (52°C, 1 min) and DNA extension (72°C, 1 min) cycles,
127 followed by a final extension (72°C, 5 min). All PCR amplifications were performed in Eppendorf
128 Mastercycler thermocyclers. The amplified DNA fragments were run on a 0.8% agarose gel to verify the
129 amplification efficiency. The amplified products were purified and sequenced in both directions to
130 confirm the polymorphic sites in an ABIPRISM 3130/xl/ sequencer (Applied Biosystems).
131 The mtDNA CR sequences were aligned using the computer program Clustal X (Thompson et al.,
132 1997) implemented in MEGA version 6 (Tamura et al., 2013). In order to assign Tyrrhenian trout to a
133 lineage, data were aligned and compared with reference *S. trutta* CR sequences from GenBank (AT, DU,
134 ME, MA, AD and DA) (see Table S1 for haplotype details). The genealogical relation of haplotypes was
135 depicted using a 95% statistical parsimony network constructed using TCS 1.3 (Clement et al., 2000).

136

137 *LDH-C1** genotypes

138

139 Some of the sampled specimens were genotyped at the *LDH-C1** gene coding the LDH enzyme (Table 1).
140 This marker, largely used in the past through allozyme electrophoreses (Hamilton et al., 1989; Almodóvar
141 et al., 2006; Berrebi, 2015) was analyzed by the PCR-RFLP method of McMeel et al. (2001). This popular
142 marker separates the ancestral allele *100 found in Atlantic salmon and southern brown trout lineages and
143 Atlantic domestic strains carrying the derived *90 allele (Hamilton et al., 1989; García-Marín et al., 1999;
144 Berrebi, 2015). Native Mediterranean trout populations belong to the mitochondrial trout lineages ME,

AD, MA and AT. This last lineage, is represented by the so-called southern Atlantic clade, *sensu* Cortey et al. (2004) and also exhibits the LDH-C1*100 allele (Aurelle & Berrebi, 2002). Therefore, this marker is used, here, mainly to distinguish between the AT haplotypes belonging to the southern Atlantic clade and the AT haplotypes of northern origin (marked with LDH-C1*90) that invaded the Mediterranean region subsequent to stocking activities. According to Hamilton et al. (1989), all Mediterranean trout populations are characterized by the allele **100*, except the marble trout (allele *LDH-C1*120*). On the contrary, most north Atlantic populations carry the allele **90* with several exceptions (García-Marín et al., 1999). This marker can be used to distinguish natural trout, including natural Atlantic populations in Sicily carrying the **100* allele (Schöffmann et al., 2007) from introduced hatchery Atlantic trout.

Microsatellite loci

The primers of the twelve microsatellite markers used in this study were obtained from the literature. Repeated sequences are all dinucleotide except Ssa197 which is a tetranucleotide microsatellite (O'Reilly et al., 1996). Details about each locus and multiplexes are given in Table 2.

For each marker, one of the 5' ends of the two primers was end-labelled with a fluorescent dye, either 6-FAM, HEX or NED. Polymerase chain reactions (PCR) were performed using the Qiagen multiplex PCR kit in a final volume of 10 µL, containing 3 µL of genomic DNA diluted at 10 ng/µL, 5 µL of Qiagen PCR Master Mix, 1 µL of Qiagen Q-solution, and 1 µL of primer mix at 2 µM each (Eurofins MWG Operon). Amplifications were carried out in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems), according to the supplier's instructions (Qiagen multiplex PCR kit): initial denaturation step (95°C, 15 min;) followed by 35 cycles of denaturation (94°C, 30 s), annealing (55°C for the three multiplexes, 90 s) and extension (72°C, 60 s); with a final extension step (60°C, 30 min). Amplified PCR fragments were then diluted and separated on an ABIPRISM 3130/xl/ sequencer (Applied Biosystems) with GeneScan 500 Rox dye size standards. Allele sizes were determined using the GeneMapper v4.1 software system (Applied Biosystems, Life Technologies).

171 A genotype matrix was then constructed and used as a basis for all the following statistical analyses
172 mainly carried out using GENETIX 4.05 (Belkhir et al., 2004).
173
174 Microsatellites statistics
175
176 For estimation of genetic polymorphism, expected heterozygosity (He), unbiased expected
177 heterozygosity (Hnb: Nei, 1978) and observed heterozygosity (Ho) were calculated for each sample or
178 river system. The mean number of alleles by locus (A) is another way to estimate the diversity in a
179 population. These calculations were limited to samples of ten or more specimens. For this reason, nearby
180 locations characterized by very low fish number were grouped in order to increase the sample size.
181 Inter-sample or inter-river system differentiations (Fst) and the intra-river panmixia (Fis) were
182 estimated (ɵ and *f* estimators of Weir & Cockerham, 1984, respectively). The significance of the Fst and
183 Fis values was tested by random permutation procedures: 5000 individual permutations between samples
184 for Fst and 5000 allele permutations within samples for Fis, processed using GENETIX.
185 The sequential Bonferroni correction was applied for multiple tests (Rice, 1989). Micro-Checker
186 software (van Oosterhout et al., 2004) was run in order to detect null alleles, drop-out or stuttering
187 perturbations.
188 A general picture of the trout genetic diversity was first obtained through multidimensional analyses.
189 Here Factorial Correspondence Analyses (FCA: Benzécri, 1973), allowing the overall structure of the
190 sampling to be explored, were carried out as implemented in GENETIX. Three focuses were chosen: all
191 the samples, the Sicilian two lineages and the Atlantic diversity of the sampling (Fig. 3a, b and c). The
192 clusters (or clouds) observed in the diagrams correspond to nuclear genetic homogeneous lineages. The
193 mathematical method is clearly detailed in She et al. (1987).
194 In order to detect differentiated subgroups, hierarchical STRUCTURE assignment analyses were
195 performed for the whole sample set (Vähä et al., 2007; Marić et al., 2017). The program STRUCTURE
196 2.3.2 (Pritchard et al. 2000) runs Markov chain Monte Carlo simulations to partition individuals into K

clusters (here, K was run between 1 and 24). Basic assignment criteria are the minimization of Hardy–Weinberg and of linkage disequilibria (Pritchard et al., 2000). Different run lengths were used at each step (from 20,000 to 100,000 burn-in and 100,000 to 500,000 total lengths, repeated seven times for each K) depending on convergence (proportion of identical structure among the seven repeated runs at each step). The admixture ancestry model and correlated allele frequency options were chosen. Sampling location was not used as prior information.

The ΔK method (Evanno et al., 2005) was applied to estimate the most probable K at each step (see Table S2). The first step determines the first hierarchical level, the whole sample set being split into K subgroups. Then each subgroup was analyzed separately, allowing for more precise clustering of individuals without eliminating admixed individuals. This hierarchical method was applied until no further substructure was observed. A comparison with the simple assignment test with the best K (here K=19) is provided in Fig. S5 and S6).

Finally, the parentage software COLONY 2.0.6.4. (Jones & Wang, 2010) was applied especially on Corsican and Sardinian samples. We have chosen the Pair-Likelihood-Score (PLS)/Full-Likelihood (FL) combined (=FPLS) algorithm in order to establish only full-sibs listing. The objective is to understand the family structure of these very low polymorphic and very differentiated/isolated populations.

Results

Mitochondrial sequences

A total of 144 mtDNA CR sequences were obtained in this survey corresponding to sixteen different haplotypes, seven of which are undescribed so far (AD-Tyrrh1 to 6 and AT-Tyrrh1: Tables 3 and S1 for distribution and accession numbers). The alignment length was 987 bp providing 34 parsimony

222 informative sites. Haplotype classification allows the relationships between Tyrrhenian populations and
223 the five main known lineages widespread around Europe plus the DU junior lineage to be understood. The
224 network (Fig. 2) represents the haplotype organization within the Tyrrhenian region, with GenBank
225 published haplotypes used as references. Tyrrhenian haplotypes all belong to AD or AT lineages
226 exclusively.

227 The new haplotype AD-Tyrrh1 is the most common, widespread in all regions except Sicily, and in a
228 central position in the AD haplogroup (Fig. 2). AD-Tyrrh5 and 6 are exclusive to Nera River (i.e. upper
229 Tiber). With nine different haplotypes detected, the AD haplogroup was the main non-domestic mtDNA
230 haplogroup observed in the Tyrrhenian region (56%, Table 3). Note that the sequence of the AD-Tyrrh4
231 haplotype, newly discovered in the present study, contains an 82 bp repeat towards the 3′-end of the CR.
232 As the elongation model of this repeat is generally thought to be the result of intra-molecular processes
233 (Buroker et al., 1990), only the first copy was kept in the analysis. The native AD lineage was present in
234 96% of the Corsican Tyrrhenian trout, 100% of the upper Tiber River (Nera), 67% of the lower Tiber
235 River (Simbrivio and Aniene), 50% of the Sardinian trout, 46% of Calabrian trout and 0% of Sicilian
236 ones, representing a north-south cline partly linked to stocking activities. In the present survey, six AD
237 haplotypes are new, suggesting their endemism (Table S1).

238 Corsica was mainly characterized by the new haplotype AD-Tyrrh1, except for the Acqua d'Acelli
239 population that had only the ADcs15 haplotype. AD-Tyrrh1 accounted for 88% of the Corsican AD
240 haplotypes, 31% of the Sardinian ones, 8% in the Tiber basin in central Italy, 50% of the Calabrian
241 diversity (but with a sample size of 6) and was not present in Sicily. Here again, a cline affected the
242 distribution of this variant, exhibiting a decrease from north to south. The other AD haplotypes were
243 distributed without clear structure in Sardinia, the Tiber River and Calabria (Table 3).

244 Among the AT lineage, the AT-s6 haplotype should represent a natural migration of Atlantic trout into
245 the Mediterranean (Schöffmann et al., 2007). This haplotype of 380 bp is included in the 985 bp haplotype
246 ATSic detected here. This haplotype was the only one present in the Anapo River (Sicily). "Clone JE1",
247 the other Sicilian AT haplotype, was the only one found in the Manghisi River, a tributary of the River

Cassibile. On the contrary, haplotypes 1 to 4 (Table 3) are well known to characterize north Atlantic populations and the domestic commercial Atlantic hatchery strain (Cortey & García-Marín, 2002; Cortey et al., 2004). These haplotypes, synonymous with ATcs1 to 4 (Cortey et al., 2004), are well represented in both Italian and French hatcheries, and in rivers where they had been introduced by stocking. They were observed in Corsica (globally 4%), Sardinia (50%), Calabria (54%) and in the lower Tiber basin (33%). The new and rare haplotype AT-Tyrrh1 was found only in the Cantiano hatchery (sample 15) but not in any river. In Sicily, only the Atlantic haplotype was observed. Their natural/domestic origin is discussed below.

*LDH-C1** genotypes

Genotyping this gene is especially useful to distinguish between natural and domestic origins of the AT haplotypes of Sicily. Some of the samples were tested, especially those with AT mtDNA haplotypes (Table 1). Hatchery samples were nearly all characterized by the *LDH-C1*90* allele except for one **90/100* heterozygote genotype among the 20 Cantiano hatchery trouts (sample 15). This hatchery **90* allele was also found in 8/8 in the Allaro River and 7/10 in the Diga Giulia River, both in Calabria, and just 1/10 in the Anapo River in Sicily. The domestic allele *LDH-C1*90* was not found in Corsica (but only four fishes analyzed) nor in the Manghisi River in Sicily (five trouts).

Microsatellite genotypes

For samples numbering over ten specimens (20 of the 30 considered), the Micro-Checker software made no drop-out detection, two tests among 240 had suspect stuttering and 17 among 240 suggested the occurrence of null alleles. The 17 possible null allele detections were spread among 8 of the 12 loci, with 0 to 3 cases each, showing that there was no systematic presence of null alleles in a given marker, probably not disturbing the calculations.

The first representation of overall microsatellite genetic diversity is given by multidimensional analyses. Fig. 3a first isolates Corsican populations at the negative part of the first axis, with very low intra-population and very high inter-population diversity, together with absence of Atlantic influence. The southern samples of Sardinian trout (numbers 12 to 14) clustered in one group. Note that because this is a 2D representation of a 3D simplification of the 273D hyperspace (274 x 324 matrix), Sardinian autochthonous trout (green envelope) are clearly separated from E Maghjine Corsican trout (red circles) along axis 3 despite the apparent overlapping in the diagram (see Fig. S4). The same is true between Lataga (grey circles) and Acqua d'Acelli (dark green circles) fishes (Fig. S4).

In contrast, several trout populations seemed to be introgressed or hybridized with the commercial international Atlantic strain represented here by the Italian and French hatcheries (Cantiano, Visso and Isère hatcheries, samples 15, 16 and 30). They are positioned at the very end of the positive part of axis one. In order to understand this part of the diagram, Atlantic-like samples were reanalyzed alone. The second analysis (Fig. 3b) showed clear separation of the Anapo population (Sicily, sample 27) from the remaining populations. Finally, the third analysis (Fig. 3c) that considered the Atlantic-like samples without the Anapo one, gathered at the left the domestic Atlantic strains and the Ermolinus and Sadali samples of central-eastern Sardinia (numbers 10 and 11 in Table 1) and those from Allaro and Precariti in Calabria (23 and 26), probably of domestic origin. The remaining samples collected around two clusters: first, numbers 18, 19, 28 and 29 (respectively from the Tiber River in Lazio province and the southern samples in Sicily), and second, numbers 20 to 25 corresponding to most samples from Calabria (except for 23, the Allaro River, probably of full domestic origin).

Summarizing, the multidimensional analyses highlighted several types of populations:

- insular/endemic differentiated populations: all Corsican (samples 2 to 9) and southern Sardinian samples (12 to 14); the Nera population in continental Italy (17) (Fig. 3a);
- the Anapo River population (27) clearly isolated from the remaining Atlantic populations (Fig. 3b);

- southern populations, close to the domestic cluster: downstream Tiber River samples (numbers 18 and 19); two Sicilian samples (28 and 29); most Calabrian samples (numbers 20 to 24, except for sample 23 from the Allaro River that clustered with domestic trout);

- domestic Atlantic samples: Italian and French hatchery strains (numbers 15, 16 and 30); central-eastern Sardinian samples (10 and 11); the Simbrivio River, a Tiber River tributary (18); the Allaro River in Calabria (23) (Fig. 3c).

The hierarchical STRUCTURE analysis gave very similar results (Fig. 4, Table S2). In the first hierarchical step, trouts were separated into two groups. Group A mostly consisted of natural samples from Corsica and Sardinia, while group B was mainly composed of samples containing the AT lineage. In group B (subgroup B2) some are domestic hatchery samples of AT lineage (Cantiano, Visso and Isere). Others are an assemblage of domestic AT individuals in different proportions with native ME (Roya) and AD lineage (U Furcone, Ermolinus, Sadali, lower Calabria and lower Tiber), which is evident from the second to the fifth step, depending on the sample (subgroups B2 and B3). Finally, several are native samples of AT (Anapo and south Sicily) and AD (Ancinale and Nera) lineages (subgroup B1). However, in southern Sicilian rivers, there is a small proportion of domestic AT genotypes visible in the second step of the hierarchical assignment. This heterogeneity (especially in group B) explains that the hierarchical analysis reached up to 22 clusters. Some clusters showed substructures (lower Tiber, lower Calabria, Isere hatchery, Cantiano hatchery, Ermolinus, U Furcone and Marroccu) which are marks of migrations or introductions. The Nera sample is probably not sub-structured since the program cuts each individual into two lineages but do not partition them.

Unlike the hierarchical Structure analysis and ΔK method (Evanno et al., 2005) which estimated 22 groups, method based on maximizing the mean estimated ln probability of data (ln P(D); Pritchard et al., 2000) show that the most probable K = 19 (Fig. S5 & S6). This last analysis method is less informative than hierarchical Structure, and, unlike hierarchical method, some of populations are not detected as distinct (Pozzi, Val d'Esse and Marroccu), which is very important for delimitation of MU.

323 Parentage analyses performed with COLONY software detected one to three families in each
324 autochthonous Corsican or Sardinian population. However, in north-east Sardinian populations, the
325 Ermolinus and Sadali populations are composed of 7 to 9 families of generally 1 or 2 individuals (except
326 two families of 3 and 5 trouts, see Table S3).

327

328 Comparing different marker systems

329

330 Comparing data provided by the three categories of markers allowed the general genetic structure of trout
331 around the Tyrrhenian Sea to be described. The *LDH-C1** marker is monomorphic for the considered
332 natural populations and was used as a diagnostic marker between natural and domestic trout.
333 The Roya trout population belongs to the ME lineage, according to mtDNA CR sequences
334 (unpublished data) constituting the ME lineage reference for microsatellite diversity and assignment
335 (Table 4, Fig. 4). In the sequence data constituting the present study, this ME lineage was not observed all
336 around the Tyrrhenian Sea.
337 Corsican and Sardinian natural populations were mostly characterized by the AD-Tyrrh1 haplotype
338 (with a few AD-Tyrrh2 and AD-Tyrrh4). Among this mitochondrial homogeneity, there were two
339 exceptions: the Corsican population of Acqua d'Acelli had only the ADsc15 haplotype and the Sardinian
340 population Is Abius was characterized by the A_2 Adriatic haplotype. The microsatellite markers divided
341 Corsican and Sardinian natural populations into nine clusters in Fig. 3a and at the third step of the
342 hierarchical assignment analysis (Fig. 4).
343 Along the Tyrrhenian-Ionian Italian Peninsula the upper Tiber River trout (Nera River, sample 17)
344 appear as a separate cluster in the third step of the hierarchical analysis, which is congruent with the
345 mtDNA haplotype composition. Here, two private haplotypes were observed (AD-Tyrr5 and 6) that
346 probably have a trans-Apennine origin due to their genetic similarity with haplotype ADcs11 (see Fig. 2),
347 this latter being very common in the Adriatic region (Sušnik et al., 2007; Berrebi et al., 2013).

On the contrary, the lower Tiber River trout (samples 18 and 19) showed wide and distinct haplotype diversity of more western origin (AD-Tyrrh1, 2 and 4).

Further south, the Calabrian samples were separated into two lineages at the second step of the hierarchical analysis (B1 and B2): the Ancinale River system (samples 20 to 22) and lower Calabria (samples 23 to 26). The Ancinale cluster was characterized by the common haplotype AD-Tyrrh1 and the lower Calabrian by two private haplotypes: AD-Tyrrh3 and ADcs1.

Sicilian trout populations exhibited two AT haplotypes (ATSic and "clone JE1"). The clone JE1 haplotype, fixed in the Manghisi River population, can be considered to be of native origin on the basis of the associated fixation of the *LDH-C1*(100)* allele (Table 3). Microsatellite Sicilian clusters separated lately (at the third step) the Manghisi and San Marco populations (south Sicily - samples 28 and 29) from the Anapo population (27) in correlation with the two haplotypes (Table 3). With only one exception, all analyzed Sicilian trouts showed the *LDH-C1*100* wild genotype.

All around the Tyrrhenian Sea, AT haplotypes AT-Tyrrh1 and "haplotypes 1 to 4" marked the domestic lineage assigned to the B2 cluster (Fig. 4) based on microsatellites and recognizable with the *LDH-C1*90* allele, while clusters B1 and B3 dominantly represented composite natural lineages.

Population parameters

Population parameters add some biological information. Heterozygosity, as measured with various H parameters and with the mean number of alleles A (Table 4), was very low in Corsica (Hnb=0.17), but very high in Sardinia (0.58). Parameter A, the mean number of alleles by locus, was also very distinctive (respectively 1.9 and 4.7). Hatchery populations are considered to be highly polymorphic (Berrebi et al., 2000; Bohling et al., 2016) and displayed large values: $0.67<Hnb<0.78$ and $5.7<A<8.3$.

Inter-sample differentiation can be determined using the estimations of Fst (Table 5). Most sample pairs was highly significantly differentiated except for a few cases which became moderately significant after Bonferroni correction. Considering only feral trout, in Corsica the mean inter-sample Fst was 0.74,

374 which is a very high value among neighboring rivers (0.47>Fst>0.90). The mean Fst was lower in Sicily
375 (0.34) and among the Tiber tributaries (0.29). In Sardinia, the Ermolinus and Sadali trout populations were
376 deeply introgressed by domestic forms. Only Marroccu, Is Abius and Camboni populations are native,
377 with very low differentiation (0.03<Fst<0.08). In Calabria, the small sample sizes limited the estimations
378 between groupings to an opposition between the Ancinale and Alaro/Assi/Stilaro/Precariti basins (Fst was
379 0.17).
380

381 **Discussion**

382

383 Combining nuclear and mitochondrial markers provided a rather clear structure of the Tyrrhenian (and
384 Ionian) trout populations. Very high inter-population diversity was observed in Corsica. A double north-
385 south cline was detected: (i) high inter-population differentiation in the north (mainly Corsica) and lower
386 in the south (ii) predominance of the Adriatic lineage in the north, which decreased towards the south due
387 to stocking in Sardinia, the Tiber basin and Calabria and because of the natural Atlantic lineage settlement
388 in Sicily.
389

390 Origin of Tyrrhenian trout diversity

391

392 Corsican trout can be considered as the best conserved natural stock around the Tyrrhenian Sea, since
393 all samples (except number 2) were in the A cluster, which characterizes mostly AD individuals (Fig. 4).
394 As a possible explanation, the riverine ecosystems have been relatively well preserved in the island and
395 the upstream part of the watersheds is generally free of pollution. Hydrogeological characteristics are also
396 favorable to salmonids. According to Gauthier & Berrebi (2007), the ancestral lineage (AD) has been
397 isolated upstream by impassable waterfalls since the last glacial maximum. Below these waterfalls,
398 postglacial Mediterranean invader trout (ME, not sampled here) hybridized with resident AD populations.

399 The numerous upstream isolated ancestral populations are free of ME invader or of domestic introduction
400 (Fig. 4), confirming the Berrebi (2015) survey of 38 samples, some of which were re-sampled for this
401 study (stations 2, 6 and 7). Traces of stocking (AT) are rare there (imperceptible with nuclear markers and
402 4% according to the mitochondrial sequences). This favorable situation explains the preserved high
403 differentiation between populations (0.47>Fst>0.90; mean value 0.74, the highest value among all the
404 samples). Moreover, the small size of streams together with possible bottlenecks could explain the very
405 low intra-population diversity (0.08<Hnb<0.26, the lowest values among all the samples) compared to
406 native grouped Tiber populations (0.51<Hnb<0.75), native Sardinian trout (0.45<Hnb<0.51), and even the
407 grouped Calabrian (0.62<Hnb<0.78) and Sicilian populations (0.49<Hnb<0.59). Table 3 shows that these
408 populations exhibit only one haplotype each (except for sample 9 from the Lataga River), mostly the
409 newly described AD-Tyrrh1.
410 In order to better describe these island isolated small populations, COLONY software (Jones & Wang
411 2010) was used to research family structure in Corsican populations (not shown). In Corsica, each
412 population shows only one to three families (Table S3). In fact, the polymorphism is so low
413 (0.08<Hnb<0.26) that we can deduce that these small populations, totally isolated, have suffered several
414 drastic recent bottlenecks. We can also suppose that one or two pairs of parents have recently re-funded
415 each population making naturally each sample 1 to 3 families. The other interpretation is that such low
416 polymorphism does not allow families detection. In Sardinia, the landscape is totally different: (i) in north-
417 east populations (Flumendosa basin), mostly composed of Atlantic domestic lineage, the Ermolinus and
418 Sadali populations are composed of 7 to 9 families of generally 1 or 2 individuals. These highly
419 polymorphic populations (here 0.74<Hnb<0.79) are composed of various origins. (ii) In the south, the
420 same local lineage has been observed in three samples of the same watershed (Cixerri basin) with possible
421 exchanges. The medium level of polymorphism (0.41<Hnb<0.51) corresponds to rather large populations
422 and possibly exchanges between them. The whole Cixerri sampling forms 12 families of full-sibs
423 frequently composed of individuals of the three sampled locations (Table S3).

If we compare these values with populations analyzed in the literature with similar markers (9 to 11 microsatellite loci), the first difficulty is the diversity of cases mainly in terms of population size. However, with the filter of case similarity, we observed very high diversity in northern populations (generally of AT mtDNA lineage) as in Switzerland (Stelkens et al, 2012: 0.73<He<0.81) or in Romania (Popa et al. 2016: 0.79<He<0.82), but lower values in Catalonia/Spain, as around the Tyrrhenian Sea (Araguas et al., 2017: 0.36<He<0.66).

The Sardinian populations seem to be far more degraded than the Corsican ones. Currently trout populations can be found in a few basins in Sardinia, where they are confined to areas of medium elevation. In this island, known pure natural populations are limited to one southern basin located within the Regional Natural Reserve (Foresta di Monte Arcosu): the Cixerri basins (Sabatini et al., 2006; 2011; Zaccara et al., 2015; Sabatini et al., 2018). Cixerri basin samples (numbers 12 to 14) were grouped at the second step of hierarchical assignment (Fig. 4). Flumendosa basin (samples 10 and 11), located east-central of the island, is inhabited by hybrid trout with domestic dominance, assigned to the B2 cluster gathering the domestic Atlantic lineage (Fig. 4), with a majority (76%) of hatchery haplotypes (Table 3).

Tiber River trout are probably influenced by the numerous upstream hatcheries settled in the Marche (samples 15 and 16) and Umbria regions. However, this influence seems limited in the wild according to the mitochondrial marker (no AT haplotype upstream in the Nera River and 33% downstream). Nuclear markers clearly isolate a first natural group, the upstream Nera River sample alone, visible outside the central part of the multidimensional diagram (Fig. 3a) in the B1 lineage (Fig. 4) and with haplotypes AD-Tyrrh5 and 6, not observed elsewhere (Table 3). According to microsatellites (Fig. 3c and the B2 cluster in Fig 4.), another lower Tiber group is genetically closer to the domestic samples (haplotypes AD-Tyrrh 1, 2 and 4, all three not endemic to the Tiber River).

Calabria is a dry area cut at the south by small parallel coastal rivers flowing into the Ionian Sea. Domestic Atlantic strains were introduced into this region, and totally replaced the Allaro population (Table 3, Fig. 3c and B2 cluster in Fig. 4). The Diga Giulia population is hybridized and shows 70% of

449 domestic LDH alleles (**90*) and 30% of Atlantic haplotypes (Table 3). Natural Calabrian trout are limited
450 to Ancinale and partly in the Diga Giulia Rivers with haplotypes AD-Tyrrh1 and 3, and ADcs1.
451 According to Schöffmann et al. (2007) and the present study, Sicily is inhabited by trout
452 belonging only to the AT mitochondrial lineage. Schöffmann et al. (2007) described haplotype AT-s6 in a
453 sample of 26 specimens from three rivers including the River Anapo. The AT-s6 haplotype is a short
454 synonym (380 bp reported by Bernatchez, 2001) of three published longer sequences: the ATSic
455 haplotype (endemic to Sicily, Snoj et al., 2011), and the ATM1 and ATM6 haplotypes, known in Morocco
456 (Snoj et al., 2011). These natural AT haplotypes are phylogenetically far from "haplotypes 1 to 4" marking
457 domestic AT trout (Snoj et al., 2011). Using one enzymatic marker, Schöffmann et al. (2007) indicated
458 that the *LDH-C1*100/100* genotype is dominant in Sicily (24/26). This last genotype is absent from north
459 Atlantic domestic strains (Berrebi et al., 2000; Cortey et al., 2004). It was thus deduced that the Sicilian
460 lineage should be a natural immigration of the south Atlantic lineage, characterized by the *LDH-C1*100*
461 allele. Schöffmann et al. (2007) also indicated that the AT-s6 haplotype sequence is close to those found
462 in Atlantic basins in the Iberian Peninsula (Weiss et al., 2000; Suárez et al., 2001) and southern France
463 (Aurelle & Berrebi, 2001). The other haplotype found in Sicily in the Manghisi River (tributary of the
464 River Cassibile), "clone JE1", was first described in the Jerte River (tributary of the River Tajo) in Spain
465 (Suárez et al., 2001). This haplotype is phylogenetically close to the ATSic natural haplotype but also to
466 several domestic haplotypes like "haplotype 3" (Fig. 2). Therefore, the presence of the sole allele *LDH-*
467 *C1*100* in a sub-sample from the Manghisi River (Table 3) demonstrates the natural origin of the
468 haplotype clone JE1. Taking these new results into account confirm the delimitations of two Atlantic
469 sublineages: the natural one mainly in Sicily and the domestic ones mainly in hatchery samples but
470 dispatched over the whole zone. This is justified by (i) the two exclusive groups of haplotypes, haplotypes
471 1 to 4 *vs* ATsic and clone JE1 for domestic and natural AT and (ii) by Fig. 4, where the Atlantic lineage is
472 in black at step 1 and in green and black (B1 and B2) at step 2 for natural and domestic Atlantic
473 sublineages.

Recently, Splendiani et al. (2016) detected the ATsc33 haplotype in sub-fossil remains of brown trout from a Tyrrhenian locality of northern Calabria dating about 13,000-8,000 years BP. This haplotype is considered to belong to the southern Atlantic haplogroup together with ATSic and clone JE1 haplotypes, and was already detected in Sicily (Fruciano et al., 2014), Spain (Suárez et al., 2001; Cortey et al., 2009) and Morocco (Snoj et al., 2011). This observation is coherent with the recent expansion of this haplogroup related to the last glacial maximum as proposed by Snoj et al. (2011).

In the samples from Calabria analyzed in the present study, no native Atlantic haplotypes were found. However, due to the very low size of the Calabrian samples analyzed here (13 sequenced specimens), we cannot make any assumptions about a possible natural presence of the Atlantic lineage in current brown trout populations of the southern Italian peninsula.

Conservation purposes

The brown trout is generally accepted as a diversified and complex assemblage of salmonids of the genus *Salmo*. It has been investigated for a long time by several methods, but without a clear description of its taxonomy. According to Sanz (2018), who reviewed the entire *S. trutta* complex data, the origin of this difficulty is due to the multiple episodes of colonization and secondary contacts. This high phylogenetic complexity with large genetic differences within lineages is accompanied by morphological and life-history diversity that has led to the description of dozens of nominal species (Kottelat & Freyhof, 2007).

The increase in the number of new trout species, sometimes elevating known subspecies to the species rank (Isaac et al., 2004; Zachos et al., 2013), sometimes accepting ancient morphological species as true species (Ninua et al., 2018), is a strategy to be considered as a threatened taxon in the IUCN Red List criteria. This should be a help for conservation (Garnet & Christidis, 2007) but such a strategy can lead to mismatches with described molecular lineages (Phillimore & Owens, 2006). Considering the complex

499 evolutionary history of the brown trout, Sanz (2018) recommended defining conservation units based on
500 genetic assessments at the population level.
501 Thirty years ago, molecular investigations proposed a first global description of the *S. trutta* complex.
502 The most popular description of genetic structure is based on the mtDNA CR sequences, showing five
503 main clusters closely linked to geographic distribution (Bernatchez et al., 1992; Bernatchez, 2001). This
504 organization has been confirmed with rDNA ITS markers (Presa et al., 2002).
505 Numerous publications used the CR marker, increasing the number of sequences or the sequence
506 length (Weiss et al., 2000; Cortey & García-Marín, 2002) until sequencing the 1250 bp that cover the
507 whole CR in Giuffra et al. (1994) or 1013 bp in Cortey et al. (2004). This marker is perhaps not the best
508 for phylogenetic reconstruction, but it is the most used and so the most practical for general phylogenies
509 with numerous GenBank sequences involved.
510 Beside the *S. trutta* complex, few taxa have been demonstrated as being outside the species complex
511 according to mtDNA phylogenies. *S. ohridanus* and *S. obtusirostris* are rare *Salmo* taxa considered as
512 distinct species (Snoj et al., 2002; Sušnik et al., 2006; Snoj et al., 2009) as is *S. salar*. At the same time,
513 other morphological species have been returned to the *S. trutta* complex (*S. platycephalus*: Sušnik et al.,
514 2004; *S. dentex*: Snoj et al., 2010).
515 Most molecular publications apply an exclusivity criterion which is the reciprocal monophyly, the
516 basis of the phylogenetic species concept (Hebert et al., 2003; Sites & Marshall, 2004), so that taxonomy
517 is as close as possible to the phylogeny. However, discrepancies between morphology and genetics
518 (especially for recent species) should be explained by the expected discrepancy between gene history
519 (Avise 2000) and/or gene phylogeny and species phylogeny (Knowles & Carstens, 2007).
520 According to Moritz (1994) and to the arguments given here, conservation units should be management
521 units (MUs), i.e. conservation units based on molecular assessments at the population level, without
522 phylogenetic prerequisites, considering population assemblages forming units that deserve distinct
523 management for conservation.

524 In the Tyrrhenian region, several attempts at nomenclature have been made. The proposition of
525 Kottelat & Freyhof (2007) to retain the name *S. cettii* for the whole Tyrrhenian region ignores the local
526 high diversity of trout populations and numerous genetic sub-groups observed with two molecular markers
527 (Fig. 2, 3 and 4). Trout in the Tyrrhenian Sea region were rarely investigated with molecular methods and
528 never as a whole in order to distinguish the different taxa and conservation units, although these data are
529 necessary for intelligent management and prioritization of stock preservation. The present survey
530 demonstrated that several categories of molecular markers are necessary for this. Crossing the well visible
531 clusters in Fig. 2, 3a and 3b and the distinct lineages obtained by assignment (Fig. 4), the following
532 significantly differentiated MUs, can be proposed:
533 The eight Corsican samples (U Furcone, A Tassineta, Aqua d'Acelli, E Maghjine, Speloncellu, Pozzi,
534 Val d'Ese and Lataga Rivers) and the three Sardinian samples of the Cixerri basin divided into two MUs
535 (Marroccu and Is Abius-Camboni) constitute ten MUs, genetically clearly different, mostly grouped in
536 cluster A of Fig. 4. They constitute the island part of the Tyrrhenian trout diversity. The detection of these
537 MUs allows much more isolated and differentiated populations to be described in Corsica. These island
538 MUs are almost not subject to domestic admixture.
539 Secondly, continental Italy revealed only two MUs positioned in cluster B1 of Fig. 4, i.e. dominated by
540 natural trout: i) the Nera River (sample 17, central Italy) and ii) the Ancinale River (sample 22, Calabria),
541 which seems to be the only local population dominated by a natural lineage.
542 Third, the Sicilian trouts, confirmed in their natural AT lineage, are nevertheless separated into two
543 MUs: i) the well-known Anapo River (Schöffmann et al., 2007) characterized by haplotype ATSic and ii)
544 the pure wild populations of Manghisi and San Marco with the "clone JE1" haplotype, although the San
545 Marco River specimens were not haplotyped.
546
547 Conclusions
548

549 Analyzed as a whole for the Tyrrhenian Sea region, trout have revealed their geographical genetic
550 organization.
551 The northern islands host precious MUs (not seen elsewhere) with special mention of Corsica, where
552 the relative good health of very small populations suggests that dozens of MUs need protection. Sardinia
553 is similar except that very few trout populations remain for investigation with molecular markers: two
554 MUs could be defined in the Cixerri basin. Sicily is similar to Sardinia since only the south of the island is
555 populated by small populations, but at least two MUs were detected. We do not know if the limited trout
556 range in Sicily is natural or due to human activity.
557 Populations from continental Italy appear less structured, probably as a consequence of both fewer
558 sampling efforts and a strong influence of genetic introgression with the Atlantic genome of domestic
559 origin. Here, therefore, on one hand, two MUs are still recognizable (in central and south Tyrrhenian
560 Italy). On the other hand, caution should be taken for estimation of the number of MUs detected. In future,
561 more effort should be made to include samples from other parts of Tyrrhenian Italy where the historical
562 presence of native brown trout genetic diversity has been highlighted (e.g, Splendiani et al., 2017; Fabiani
563 et al., 2018).
564 The maintenance of these natural lineages, some without domestic introgression, allows optimism
565 regarding conservation of Tyrrhenian trout, which is indispensable for their role in freshwater biodiversity.
566 In view of the great genetic diversity that characterizes Mediterranean trout and the risks of undesirable
567 genetic homogenization by domestic forms, an intelligent conservation strategy should never be carried
568 out without preliminary genetic description of the populations involved in conservation actions.
569 Unfortunately, the number of conservation programs carried out without any kind of control is becoming
570 more and more frequent.
571
572
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876

Table captions

Table 1: Details about the trout samples. Italian hatcheries were linked to the host river because a possible exchange was expected. The French hatchery has a national dispatch and no link with a river. Map = see Fig. 1, N = number of analyzed individuals, MS = microsatellites, mtDNA = control region haplotype, *LDH-C1** = enzyme gene RFLP. FR=France, IT=Italy, AM=Alpes-Maritimes, a southern France region/area, W. Med=Western Mediterranean basin. Very small samples are due to low density of natural populations or limited capture authorization. In the calculations, they are grouped by region.

Table 2: List of the twelve microsatellites genotyped. Their grouping into three multiplexes and their original publication are indicated.

Table 3: Control region haplotypes and *LDH-C1** allele distribution. Map = see Fig. 1.

Table 4: Parameters estimating the genetic diversity and the panmixia. Map = see Fig. 1, He = calculated heterozygosity under the hypothesis of Hardy-Weinberg Equilibrium, Hnb = similar to He parameter but weighted depending on the sample size (Nei, 1978), Ho = observed heterozygosity, A = mean number of allele by locus. Values of Fis between parentheses have no real sense as several samples were grouped to obtain a sample size over 10.

Table 5: Double matrix indicating the Fst (Θ, Weir & Cockerham, 1984) values (upper triangle) and their significance (lower triangle). All Fst were very highly significant ***. After Bonferroni correction, only the level of significance was reduced, indicated between parentheses, for some comparisons simply significant (*) or highly significant (**). Med. = Roya River flowing to the Ligurian Sea, Dom. = domestic Atlantic commercial strain. Numbers refer to the first column of Table 1 and Fig. 1. A, B, C and D are samples grouped in order to reach sample sizes of 10 and more.

900 **Figure captions**

901

902 **Fig. 1:** Geographic position of 29 samples (the last one, number 30, positioned far in the north, is not
903 shown). The station numbers refer to the first column of Table 1.

904 **Fig. 2:** Network presenting the relationships between Tyrrhenian control region sequences (colored
905 circles) and published ones (white circles). This network allows classification of the Tyrrhenian
906 haplotypes within the five main known lineages (plus DU). The circle sizes are proportional to the
907 haplotype occurrence except the white ones.

908 **Fig. 3:** Sampling diversity analyzed through Factorial Correspondences Analysis. Circles correspond to
909 France and Corsica, diamonds to Sardinia, triangles to continental Italy, squares to Sicily and crosses to
910 domestic samples. The different colors distinguish different populations of the same region.

911 **a:**. General picture gathering all the samples. C. Italy = Central Italy (the Marche and Lazio); ellipses: red
912 = Corsica, green = part of Sardinia, blue = part of C. Italy, black = domestic Atlantic, dotted orange =
913 overlapping samples developed in Fig. 3b.

914 **b:** In order to focus on the samples included in the doted orange envelope of Fig. 3a, a new FCA was
915 performed with these samples only (except the Roya River ME sample), giving new insight into the
916 Anapo River differentiation.

917 **c:** Focus on the samples included in the dotted orange envelope of Fig. 3a, after withdrawal of the Roya
918 River ME and the Anapo River AT samples. Black ellipse = true domestic Atlantic trout (samples 10,
919 11, 15, 16, 18, 23 and 30). Red ellipse = Calabrian sample containing both wild and domestic trout
920 (samples 20 to 26). Lower Tiber = populations close to Rome. The upper Tiber gathers the two
921 domestic strains from the hatcheries Cantiano and Visso (samples 15 and 16). South Sicily samples are
922 Manghisi and San Marco (28 and 29).

923 **Fig. 4:** Population structure as inferred by hierarchical STRUCTURE analysis of microsatellite data.
924 White lines separate the sampling sites. Cluster names correspond to river names or to regions. In order
925 to link each cluster to the elementary sample(s) involved, the numbers from the first column in Table 1

926 are indicated at the left of the figure - first histogram. The most probable K for the analyzed samples
927 shown in the arrows is based on the ΔK method; no further structures were detected in subsequent
928 rounds (after the sixth step) and within the excluded clusters (K = 1). Arrows delineate the progress of
929 the hierarchical approach, where subsets of the data were subsequently analyzed. Corresponding
930 mtDNA (control region haplotypes) and *LDH-C1** alleles are indicated at the right of the figure.
931

Supporting information

Table S1: Accession numbers of all haplotypes positioned in Fig. 2 (* = new), completed with their geographic distribution according to twenty main publications.

Table S2: Hierarchical steps in estimating K (the number of genetic clusters) from STRUCTURE runs using the ΔK method. L(K) - posterior probability of K; stdev - standard deviation of L(K) from seven independent runs; ΔK - an ad hoc quantity, predictor of the real number of clusters (Evanno et al., 2005), best ΔK are highlighted.

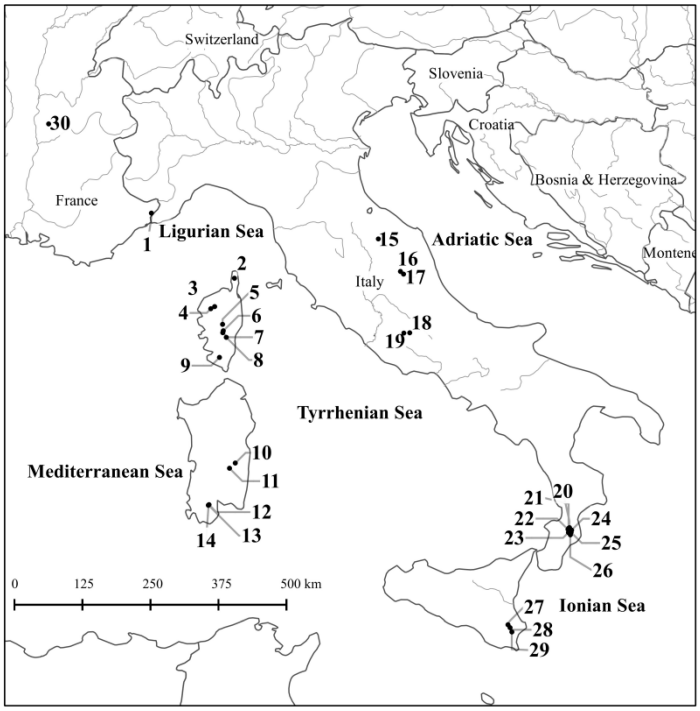
Table S3: Detection of fullsibs in Corsican and Sardinian samples.

Fig. S4: New projection of the FCA analysis presented in Fig. 3a according to axes 1 and 3. In this new perspective, Sardinian samples and E Maghine Corsican one, together with the Corsican samples Lataga and Aqua d'Acelli, which seemed similar in Fig. 3a, are in fact clearly different (red arrows) while Corsican samples Pozzi and Val d'Ese confirm their similarity.

Fig. S5: Estimation of the number of genetic clusters (K) for the first level, from STRUCTURE runs using the ΔK method.

Fig. S6. Estimated population structure as inferred by STRUCTURE analysis of microsatellite marker DNA data. White lines separate sampling sites, the most probable K = 19 is based on maximizing the mean estimated ln probability of data (Pritchard et al., 2000). Names and codes of sampling sites/clusters are reported in Table 1 and 4 and Figure 4.

951 **Figure 1**



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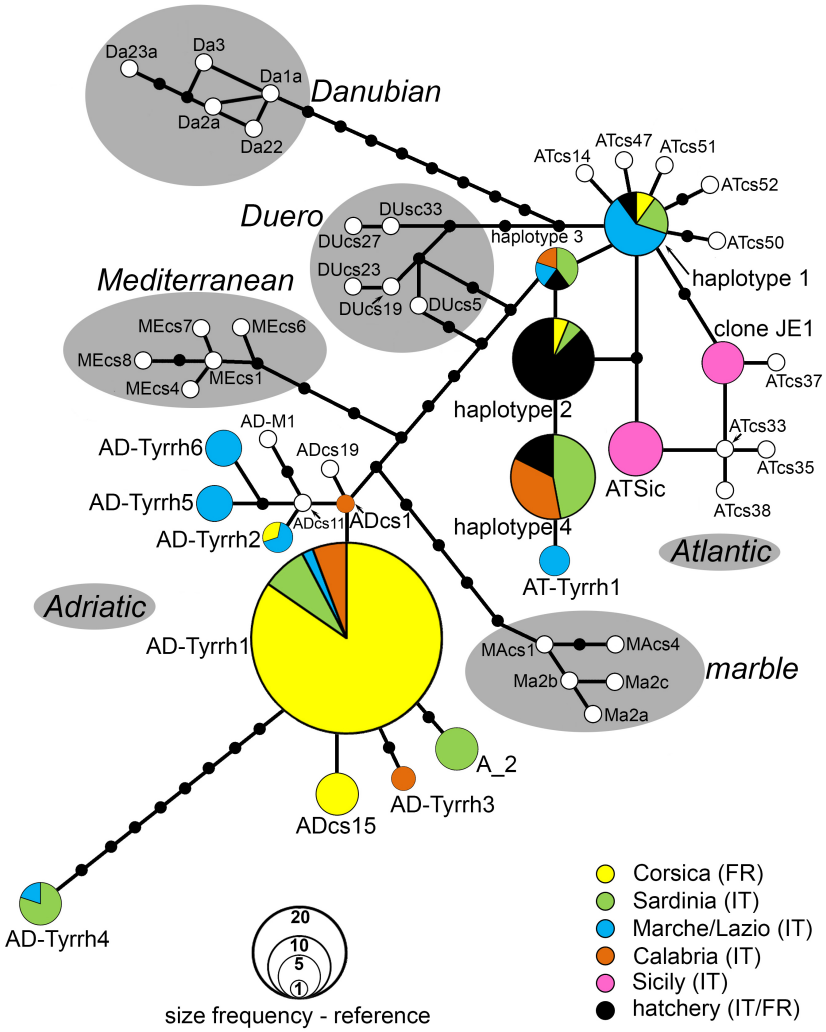
954 **Figure 2**



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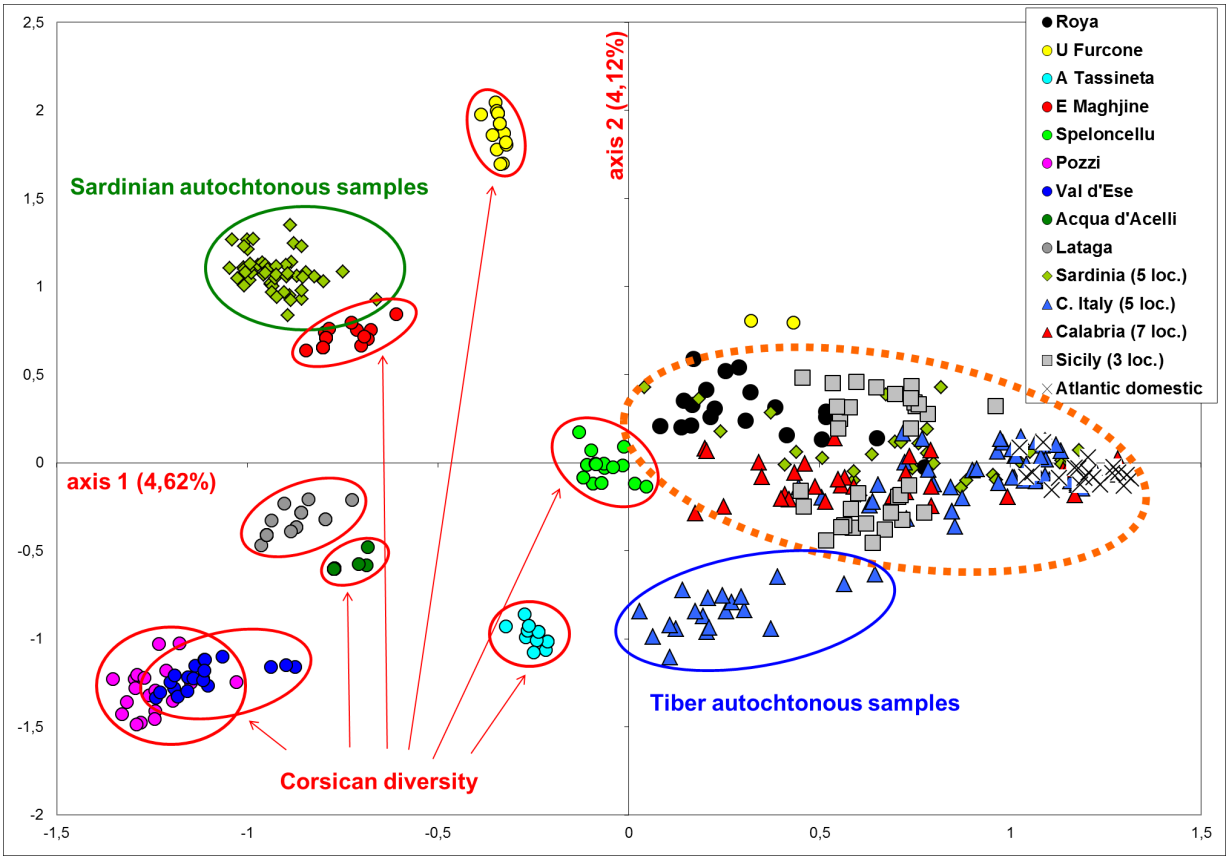
957 Figure 3a



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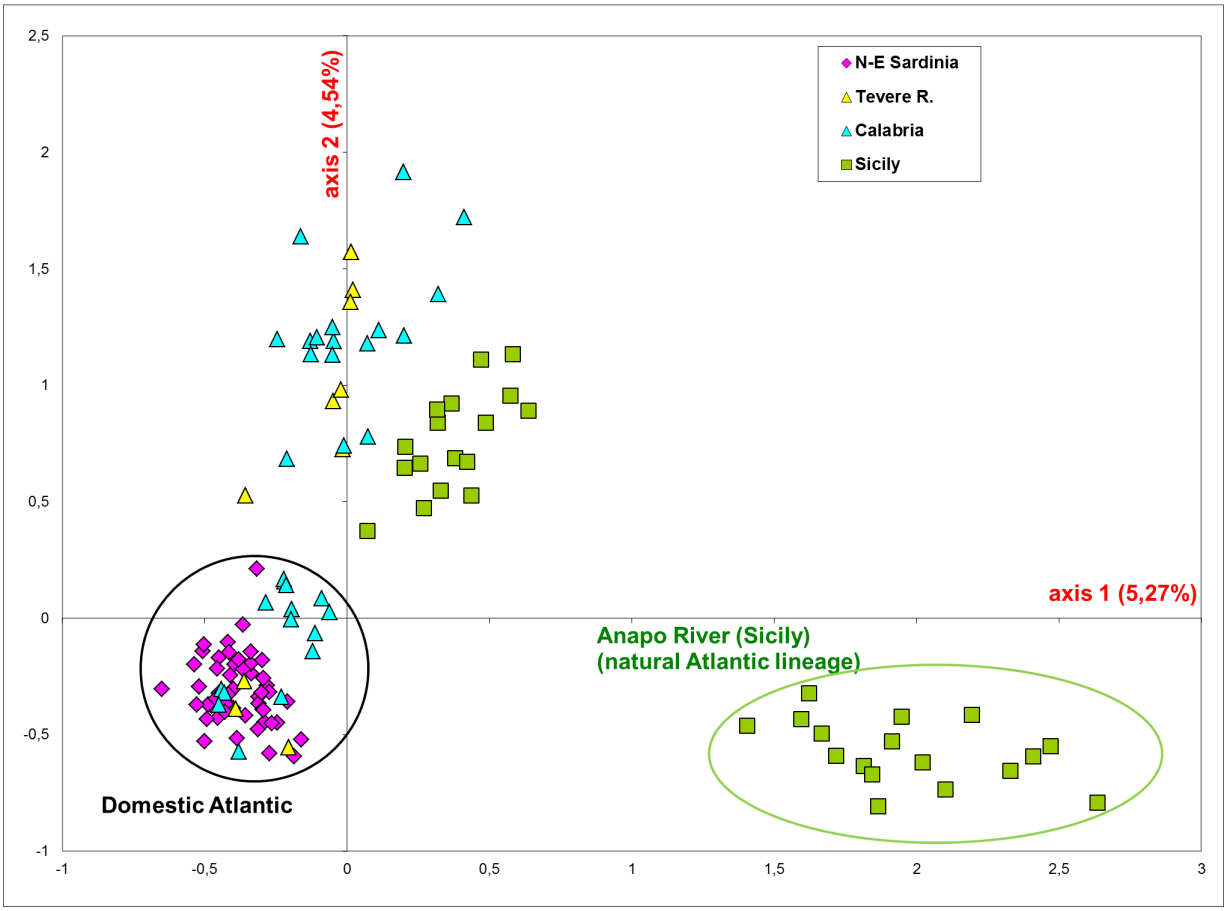
960 Figure 3b



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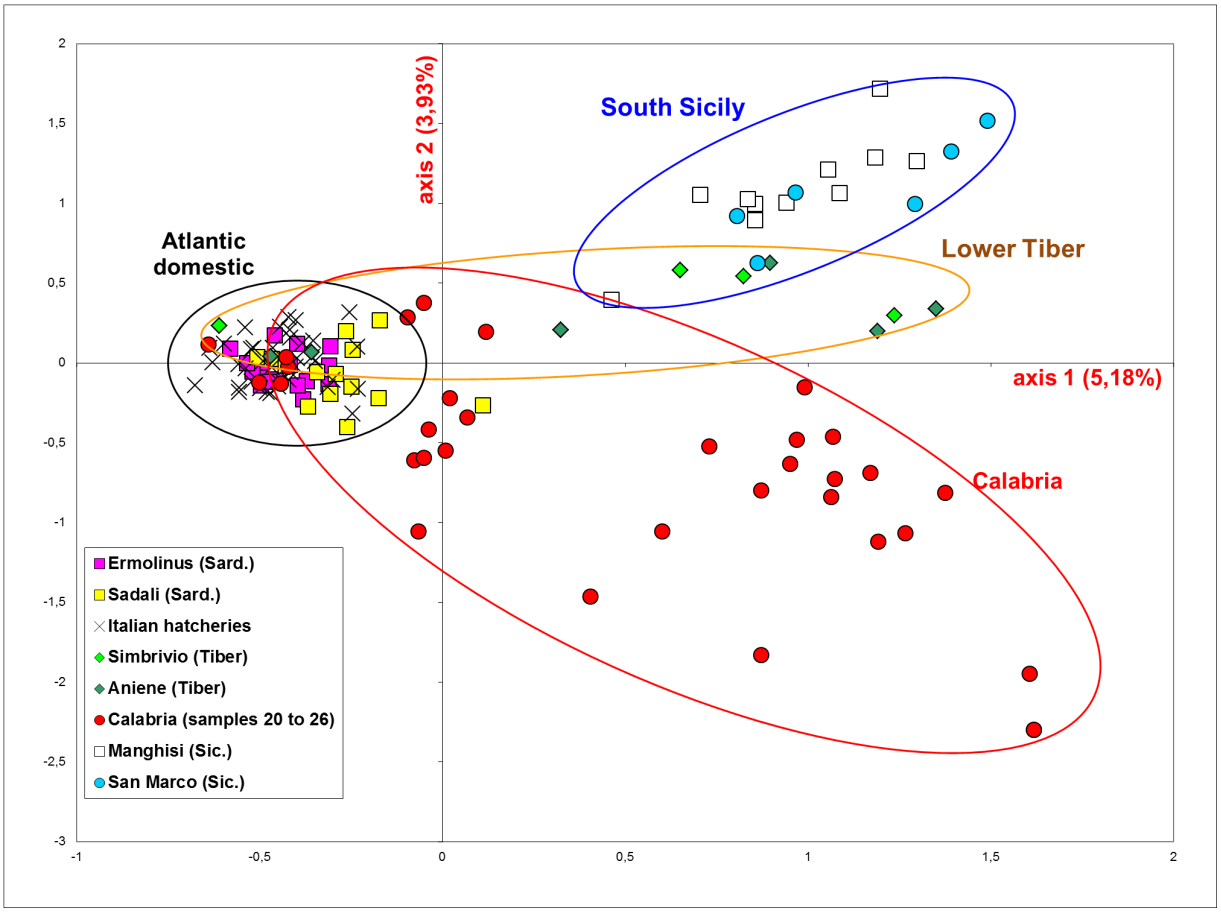
963 Figure 3c



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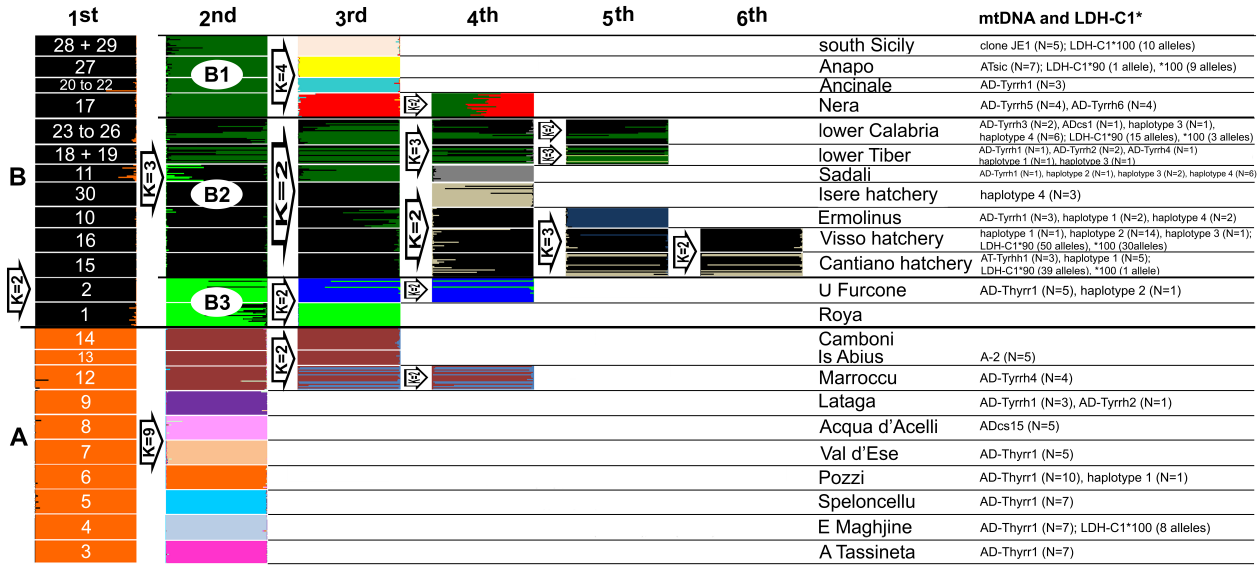
966 Figure 4



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969 Figure S4

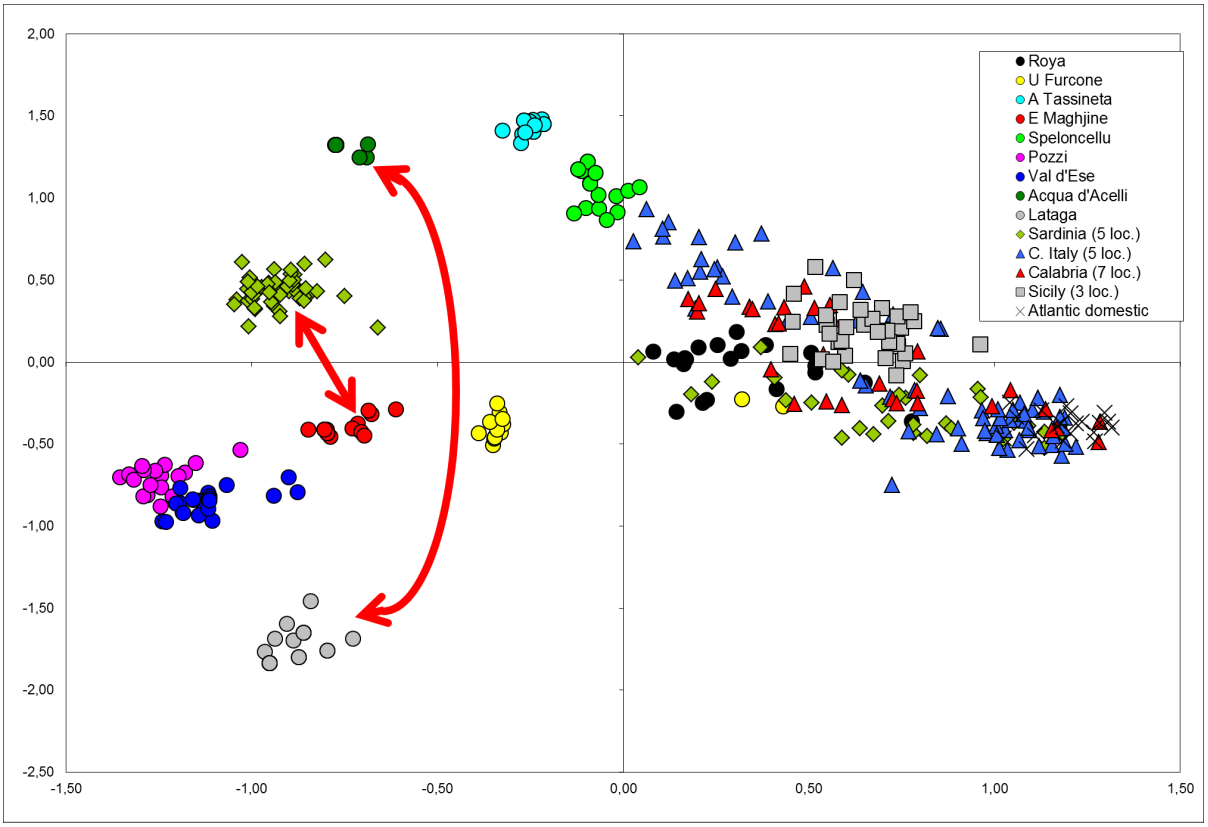

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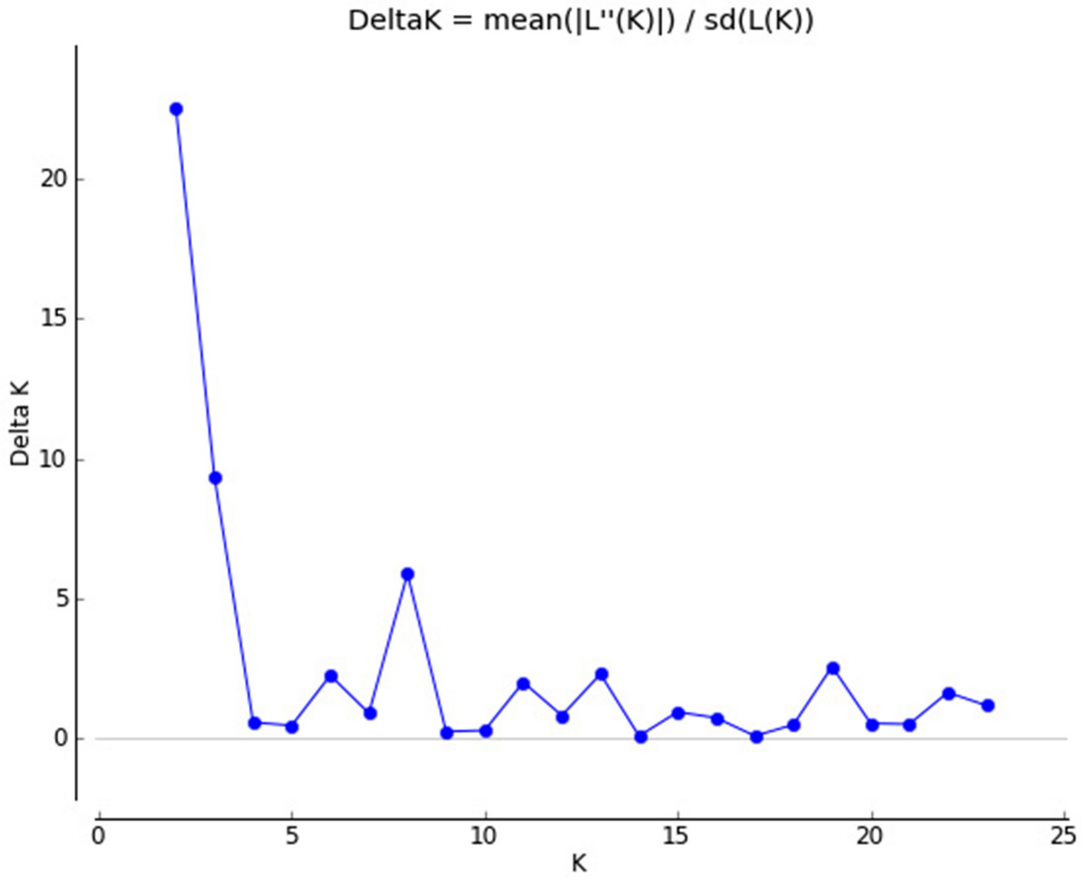
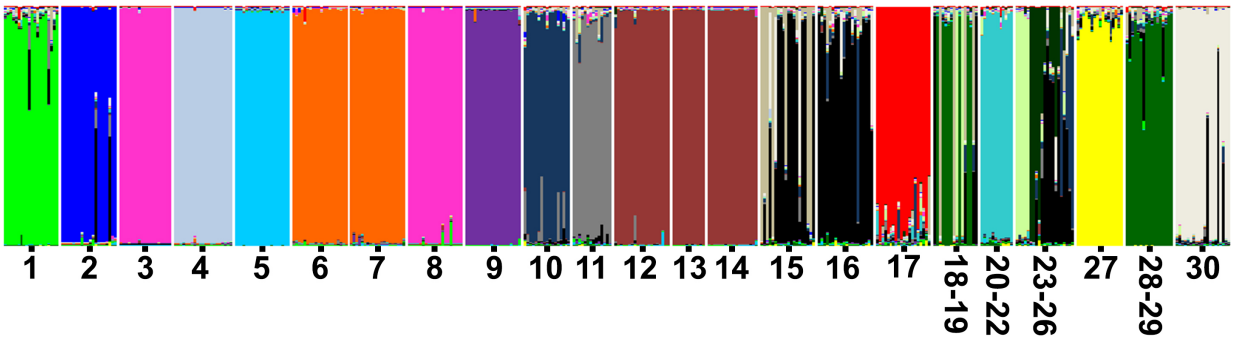
Figure S5



975 Figure S6



976

977

978 **Table 1:** Details on the trout samples. Italian hatcheries were linked to the host river because a possible exchange is
979 expected. The French hatchery has a national dispatch and no link with a river. Map = see Fig. 1, N = number of
980 analyzed individuals, MS = microsatellites, mtDNA = control region haplotype, LDH-C1* = Enzyme gene
981 RFLP. FR=France, IT=Italy, AM=Alpes-Maritimes, a southern France region/area, W. Med=Western
982 Mediterranean basin. Very small samples are due to low density of natural populations or limited capture
983 authorization. In the calculations, they are grouped by region.

Map	River or hatchery	Watershed	Sea	Region (country)	Date	N MS	N mtDNA	N *LDH-C1**
1	**Roya**	Roya	Ligurian	AM (FR)	2007	20	0	-
2	**U Furcone**	Luri	Tyrrenian	Corsica (FR)	2011	20	6	-
3	**A Tassineta**	Golo	Tyrrenian	Corsica (FR)	2012	19	7	-
4	**E Maghjine**	Fango	W. Med.	Corsica (FR)	2012	21	7	4
5	**Speloncellu**	Tavignanu	Tyrrenian	Corsica (FR)	2013	20	7	-
6	**Pozzi**	Fium'Orbu	Tyrrenian	Corsica (FR)	2004	20	11	-
7	**Val d'Ese**	Prunelli	W. Med.	Corsica (FR)	2004	20	5	-
8	**Acqua d'Acelli**	Travo	Tyrrenian	Corsica (FR)	2013	20	5	-
9	**Lataga**	Ortolo	W. Med.	Corsica (FR)	2005	20	4	-
10	**Ermolinus**	Flumendosa	Tyrrenian	Sardinia (IT)	2007	17	7	-
11	**Sadali**	Flumendosa	Tyrrenian	Sardinia (IT)	2009	14	10	-
12	**Marroccu**	Cixerri	Tyrrenian	Sardinia (IT)	2007	20	4	-
13	**Is Abius**	Cixerri	Tyrrenian	Sardinia (IT)	2009	12	5	-
14	**Camboni**	Cixerri	Tyrrenian	Sardinia (IT)	2009	19	0	-
15	**Cantiano hatchery**	Tiber	Tyrrenian	Marche (IT)	2006	19	8	20
16	**Visso hatchery**	Tiber	Tyrrenian	Marche (IT)	2000	20	16	40
17	**Nera**	Tiber	Tyrrenian	Marche (IT)	2006	20	8	-
18	**Simbrivio**	Tiber	Tyrrenian	Lazio (IT)	2014	9	3	-
19	**Aniene**	Tiber	Tyrrenian	Lazio (IT)	2014	7	3	-
20	**Rotta**	Ancinale	Ionian	Calabria (IT)	2013	3	0	-
21	**Carusi**	Ancinale	Ionian	Calabria (IT)	2013	5	0	-
22	**Ancinale**	Ancinale	Ionian	Calabria (IT)	2013	4	3	-
23	**Allaro**	Alaro	Ionian	Calabria (IT)	2013	5	3	4
24	**Mula**	Assi	Ionian	Calabria (IT)	2013	4	0	-
25	**Diga Giulia**	Stilaro	Ionian	Calabria (IT)	2013	7	5	5
26	**Precariti**	Precariti	Ionian	Calabria (IT)	2013	5	0	-
27	**Anapo**	Anapo	Ionian	Sicily (IT)	2013	17	7	5
28	**Manghisi**	Casibile	Ionian	Sicily (IT)	2013	11	5	5
29	**San Marco**	Asinaro	Ionian	Sicily (IT)	2013	6	0	-
30	**Isère hatchery**	-	-	(FR)	2008	20	3	-

984

985

986 **Table 2.** List of the 12 microsatellites genotyped, their grouping into three multiplexes and their original publication.

Locus	multiplex	Reference
MST543	**C**	Presa et al., 1994
MST85	**B**	Presa & Guyomard 1996
Omm1105	**A**	Rexroad et al., 2002
OMY21DIAS	**B**	Holm & Bendixen 2000
Oneμ9	**C**	Scribner et al., 1996
Sfo1	**A**	Angers et al., 1995
Ssa197	**A**	O'Reilly et al., 1996
SsoSL311	**C**	Slettan et al., 1995
SSOSL417	**A**	Slettan et al., 1995
SSOSL438	**C**	Slettan et al., 1996
STR591	**B**	Presa & Guyomard 1996
STRBS131	**C**	Charles et al., 2005

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Table 3. Control region haplotypes and *LDH-C1** allele distribution. Map = see Fig. 1.

				Haplotype frequency																
Region	**Map N°**	**River or hatchery**	**sequence numbers**	**AD-Tyrrh1**	**AD-Tyrrh2**	**AD-Tyrrh3**	**AD-Tyrrh4**	**AD-Tyrrh5**	**AD-Tyrrh6**	**ADcs1**	**ADcs15**	**A_2**	**AT-Tyrrh1**	**haplotype 1**	**haplotype 2**	**haplotype 3**	**haplotype 4**	**ATSic**	**clone JE1**	**LDH-C1*90**	**LDH-C1*100**
Corsica	**2**	**U Furcone**	6	5											1					-	-
	3	**A Tassineta**	7	7																-	-
	4	**E Maghjine**	7	7																0	8
	5	**Speloncellu**	7	7																-	-
	6	**Pozzi**	11	10										1						-	-
	7	**Val d'Ese**	5	5																-	-
	8	**Acqua d'Acelli**	5								5									-	-
	9	**Lataga**	4	3	1															-	-
Sardinia	**10**	**Ermolinus**	7	3										2			2			-	-
	11	**Sadali**	10	1											1	2	6			-	-
	12	**Marroccu**	4				4													-	-
	13	**Is Abius**	5									5								-	-
Tiber River	**15**	**Cantiano hatchery**	8										3	5						39	1
	16	**Visso hatchery1**	8											1	6	1				40	0
	-	**Visso hatchery2**	8												8					10	30
	17	**Nera**	8					4	4											-	-
	18	**Simbrivio**	3		1									1		1				-	-
	19	**Aniene**	3	1	1		1													-	-
Calabria	**22**	**Ancinale**	3	3																-	-
	23	**Allaro**	5														5			8	0
	25	**Diga Giulia**	5			2				1						1	1			7	3
Sicily	**27**	**Anapo**	7															7		1	9
	28	**Manghisi**	5																5	-	10
	30	**Isere hatchery**	3														3			-	-
	Total		144	52	3	2	5	4	4	1	5	5	3	10	16	5	17	7	5	105	61

Table 4. Parameters estimating the genetic diversity and the panmixia. Map = see Fig. 1, He = calculated heterozygosity under the hypothesis of Hardy-Weinberg Equilibrium, Hnb = similar to He parameter but weighted depending on the sample size (Nei, 1978), Ho = observed heterozygosity, A = mean number of allele by locus. Values of Fis between parentheses have no real sense since several samples have been grouped for a sample size over 10.

Map	River or hatchery	N	He	Hnb	Ho	A	Fis	Fis signif.
1	**Roya**	**20**	0.63	0.65	0.55	7.75	0.164	***
2	**U Furcone**	**20**	0.21	0.22	0.20	2.92	0.075	ns
3	**A Tassineta**	**19**	0.15	0.15	0.18	1.42	-0.148	ns
4	**E Maghjine**	**21**	0.01	0.10	0.09	1.50	0.144	ns
5	**Speloncellu**	**20**	0.20	0.20	0.18	1.75	0.113	ns
6	**Pozzi**	**20**	0.23	0.23	0.21	2.08	0.104	ns
7	**Val d'Ese**	**20**	0.25	0.26	0.28	2.17	-0.094	ns
8	**Acqua d'Acelli**	**20**	0.08	0.08	0.08	1.75	-0.041	ns
9	**Lataga**	**20**	0.08	0.08	0.07	1.58	0.083	ns
10	**Ermolinus**	**17**	0.72	0.74	0.67	6.25	0.102	ns
11	**Sadali**	**14**	0.76	0.79	0.78	7.17	0.008	ns
12	**Marroccu**	**20**	0.44	0.45	0.40	3.25	0.118	**
13	**Is Abius**	**12**	0.40	0.41	0.47	2.58	-0.133	ns
14	**Camboni**	**19**	0.49	0.51	0.51	4.42	-0.009	ns
15	**Cantiano hatchery**	**19**	0.73	0.75	0.73	7.42	0.031	ns
16	**Visso hatchery**	**20**	0.76	0.78	0.75	8.83	0.029	ns
17	**Nera**	**20**	0.49	0.51	0.47	5.83	0.063	ns
18&19	**lower Tiber**	**16**	0.72	0.75	0.62	6.42	(0.177)	ns
20to**22**	**Ancinale**	**12**	0.59	0.62	0.57	5.00	(0.082)	ns
23to**26**	**lower Calabria**	**21**	0.76	0.78	0.55	8.33	(0.310)	(***)
27	**Anapo**	**17**	0.48	0.49	0.47	4.67	0.046	ns
28&**29**	**south Sicily**	**17**	0.57	0.59	0.49	6.33	(0.182)	(***)
30	**Isere hatchery**	**20**	0.65	0.67	0.67	5.75	0.007	ns

Table 5. Double matrix indicating the Fst (Θ, Weir & Cockerham, 1984) values (upper triangle) and their significance (lower triangle). All Fst were very highly significant ***. After Bonferroni correction, only the level of significance was reduced, indicated between parentheses, for some comparisons simply significant (*) or highly significant (**). Med. = Roya River flowing to the Ligurian Sea, Dom. = domestic Atlantic commercial strain. Numbers refer to the first column of Table 1 and Fig. 1. A, B, C and D are grouped samples in order to reach sample sizes of 10 and more.

	Med.	Corsica								Sardinia					Central Italy				Calabria		Sicily		Dom.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	A	B	C	27	D	30
1	0	0.48	0.58	0.59	0.47	0.53	0.51	0.59	0.57	0.26	0.18	0.40	0.40	0.37	0.23	0.22	0.38	0.22	0.27	0.18	0.36	0.26	0.26
2	***	0	0.81	0.82	0.77	0.76	0.74	0.84	0.82	0.48	0.44	0.58	0.64	0.55	0.49	0.46	0.63	0.52	0.61	0.46	0.61	0.57	0.54
3	***	***	0	0.87	0.77	0.75	0.77	0.84	0.87	0.55	0.51	0.68	0.73	0.65	0.54	0.50	0.54	0.52	0.61	0.50	0.66	0.57	0.56
4	***	***	***	0	0.83	0.80	0.78	0.89	0.89	0.57	0.54	0.63	0.71	0.61	0.56	0.54	0.70	0.57	0.68	0.53	0.71	0.66	0.60
5	***	***	***	***	0	0.76	0.74	0.83	0.83	0.52	0.47	0.65	0.70	0.63	0.52	0.49	0.60	0.49	0.52	0.44	0.59	0.51	0.55
6	***	***	***	(**)	***	0	0.46	0.80	0.79	0.51	0.48	0.62	0.65	0.58	0.51	0.48	0.56	0.51	0.60	0.47	0.64	0.58	0.55
7	***	(**)	***	***	***	***	0	0.78	0.76	0.49	0.45	0.60	0.64	0.58	0.49	0.46	0.58	0.49	0.57	0.46	0.63	0.55	0.53
8	***	***	***	***	***	***	***	0	0.90	0.58	0.57	0.69	0.74	0.66	0.59	0.56	0.65	0.59	0.65	0.55	0.73	0.66	0.62
9	***	***	***	***	***	***	***	***	0	0.57	0.54	0.70	0.76	0.68	0.57	0.54	0.69	0.58	0.67	0.53	0.72	0.65	0.60
10	***	***	***	***	***	***	***	***	***	0	0.10	0.38	0.38	0.34	0.09	0.07	0.34	0.12	0.23	0.10	0.32	0.26	0.11
11	***	***	***	***	***	***	***	***	***	***	0	0.32	0.33	0.28	0.11	0.10	0.31	0.11	0.20	0.10	0.32	0.21	0.14
12	***	***	***	***	***	***	***	***	***	***	***	0	0.08	0.03	0.39	0.37	0.51	0.39	0.44	0.35	0.50	0.47	0.43
13	***	(**)	(**)	***	***	***	(**)	***	***	***	***	(*)	0	0.05	0.39	0.37	0.52	0.39	0.45	0.36	0.51	0.48	0.44
14	***	***	***	***	***	***	***	***	***	***	***	(*)	(*)	0	0.35	0.33	0.48	0.36	0.41	0.33	0.45	0.44	0.40
15	***	***	***	***	***	***	***	***	***	***	(**)	***	***	***	0	0.03	0.34	0.10	0.23	0.09	0.33	0.25	0.08
16	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0	0.32	0.10	0.23	0.09	0.30	0.23	0.07
17	***	***	***	***	(**)	***	***	***	(**)	***	***	***	***	***	***	***	0	0.29	0.29	0.26	0.46	0.35	0.37
A (18 to 19)	(**)	***	***	***	***	***	***	***	***	***	***	***	(**)	***	(**)	***	***	0	0.16	0.11	0.33	0.16	0.14
B (20 to 22)	***	***	(**)	***	***	***	***	***	(**)	***	(**)	***	(**)	(**)	***	***	(**)	(**)	0	0.17	0.40	0.25	0.26
C (23 to 26)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0	0.28	0.20	0.12
27	***	***	***	***	***	***	***	***	***	***	***	***	(**)	***	(**)	***	***	***	***	***	0	0.34	0.37
D (28 to 29)	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	0	0.28
30	***	***	***	***	***	***	***	***	***	***	(**)	***	(**)	***	***	***	***	***	***	***	***	***	0

Table S1

Haplotype	Accession number	Distribution – country/drainages
haplotype 1 (ATcs1)	AF273086	[3]Denmark (Skals), Norway (Bjornes Lake, Sima), Spain (hatchery stocks), [5]Spain (Garona), France (Gulf of Biscay), Iceland (Skorradalsvatn), British Isles (Coquet, Wear, Lune, Melvin), [21]continental Italy (IT), Sardinia, Corsica
haplotype 2 (ATcs2)	AF273087	[3]Denmark (Skals, Karup), Norway (Guddal, Sima), Spain (hatchery stocks), [5]France (Gulf of Biscay), British Isles (Coquet, Stour, Rother, Fowey, Teifi, Conwy, Loch Romoch), Russia (Nilima, Vorobiev), [21]Sardinia, Corsica
haplotype 3 (ATcs3)	AF274574	[3]Denmark (Skals), Norway (Bjornes Lake, Guddal, Sima), Spain (hatchery stocks), [5]Spain (Garona), France (Gulf of Biscay), British Isles (Coquet, Wear, Rother, Teifi, Conwy, Melvin), [21]continental Italy, Sardinia, Calabria,
haplotype 4 (ATcs4)	AF274575	[3]Denmark (Skals, Karup), Norway (Bjornes Lake, Guddal, Sima), Spain (hatchery stocks), [5]France (Gulf of Biscay), British Isles (Lune), [21]Sardinia, Calabria.
ATSic	JF297974	[13]Sicily (Ánapo)
clone JE1	AF253557	[14]Iberian (South European Atlantic region), [21]Sicily (Casibile)
*AT-Tyrrh1	KX450263	[21]Italy hatchery stock (Cantino, Marche region)
ATcs14	EF530476	[5]Iceland (Skorradalsvatn)
ATcs33	EF530495	[5]Spain (Minho, Atlantic Ocean, Cantabric Sea)
ATcs35	EF530497	[5]Spain (Atlantic Ocean)
ATcs37	EF530499	[5]Spain (Atlantic Ocean)
ATcs38	EF530500	[5]Spain (Atlantic Ocean)
ATcs47	EF530507	[5]Iceland (Skorradalsvatn)
ATcs50	EF530510	[5]British Isles (Stour)
ATcs51	EF530511	[5]British Isles (Teifi)
ATcs52	EF530512	[5]British Isles (Teifi)
ADcs1	AY836330	[4]Spain (Ter, Ebre, Túria, Segura), [8]Bulgaria (Struma, Mesta, Maritza), [2,9]Macedonia (Prespa Lake, Vardar)
ADcs15	AY836344	[4]France - Corsica (Corsica stream)
A_2	KM216129	[16]Sardinia (Pula)
*AD-Tyrrh1	KX450257	[21]Corsica, Sardinia, Calabria, continental Italy (Aniene River)
*AD-Tyrrh2	KX450258	[21]Corsica, continental Italy (Aniene River)
*AD-Tyrrh3	KX450259	[21]Calabria (Diga Giulia River)
*AD-Tyrrh4	KX450260	[21]Sardinia, continental Italy (Aniene River)
*AD-Tyrrh5	KX450261	[21]continental Italy (Nera River)

*AD-Tyrrh6	KX450262	[21]continental Italy (Nera River)
ADcs11	AY836340	[15]Montenegro (Skadar Lake)
ADcs19	AY836348	[4]Spain (Guadalquivir)
AD-M1	DQ381566	[15]Montenegro (Skadar Lake)
MEcs1	AY836350	[4]Spain (Ter, Llobregat, Ebre, Míjares, Palancia, Túria, Segura), [7]Croatia (Krka)
MEcs4	AY836353	[4]Spain (Ter)
MEcs6	AY836355	[4]Spain (Ebre)
MEcs7	AY836356	[4]Spain (Ter)
MEcs8	AY836357	[4]Spain (Túria)
MAcs1 (Ma1a)	AY836365	[4]Slovenia (Soča), [8]Greece (Aliakmon), [10]Italy (Adige, Po)
MAcs4	JN208022	[12]Italy (Po)
Ma2a	DQ841189	[10]Italy (Adige)
Ma2b	DQ841190	[10]Italy (Adige)
Ma2c	JQ582461	[11]Italy (Adige)
DUcs5	EF530517	[5]Spain (Duero, Minho)
DUcs19	EF530531	[5]Spain (Duero)
DUcs23	EF530535	[5]Spain (Duero)
DUcs27	KM210674	[17]Spain (Upper Miño)
DUcs33	KM210680	[17]Spain (Mandeo, Lago)
Da1a (Iran 3)	AY185568	[1,6]Austria (Drau, Inn, Kamp, Danube), [8]Bulgaria (Cerny Iskar), Serbia (Džepska River), [19]Iran (Tonekabon)
Da2a (Da2)	GQ284834	[1,6]Austria (Inn, Mur, Drau), [10]Italy (Adige)
Da3	AY185571	[6,20]Austria (Kamp)
Da22	AY185573	[1,6,20]Austria (Inn, Kamp, Enns, Drau, Danube), [9]Macedonia (Vardar), [10]Italy (Adige), [18]Bosnia and Herzegovina (Una)
Da23a	AY185574	[6,20]Austria (Kamp)

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[21] Present study (*new haplotypes).

Table S2 Hierarchical steps in estimating K (the number of genetic clusters) from STRUCTURE runs using the ΔK method. L(K) - posterior probability of K; stdev - standard deviation of L(K) from seven independent runs; ΔK - an *ad hoc* quantity, predictor of the real number of clusters (Evanno et al., 2005), best ΔK are highlighted. In the second step of analysis on the Corse-Sardinian dataset (A Tassineta, E Maghjine, Speloncellu, Pozzi, Val d'Ese, Acqua d'Acelli, Lataga, Marroccu, Is Abius & Camboni), ΔK value was strongly affected by two outlier runs for K = 7, which are discarded from the analysis.

	K	L(K)	stdev	ΔK
1st step – All samples	1	-25283.26	0.65	
	2	-21560.37	76.36	22.53
	3	-19558.03	78.29	9.33
	4	-18286.21	159.77	0.56
	5	-17103.79	340.38	0.45
	6	-16072.83	209.98	2.24
	7	-15512.23	346.99	0.89
	8	-14644.31	95.84	5.87
	9	-14338.66	243.51	0.23
	10	-13977.09	671.00	0.27
	11	-13433.76	269.71	1.99
	12	-13427.94	956.70	0.80
	13	-12653.77	332.19	2.29
	14	-12638.71	470.03	0.07
	15	-12590.70	207.52	0.93
	16	-12349.71	313.45	0.72
	17	-12334.23	378.89	0.07
	18	-12290.70	1019.75	0.47
	19	-11764.49	330.06	2.55
	20	-12081.19	529.95	0.52

	21	-12121.57	474.81	0.49
	22	-11927.60	681.48	1.63
	23	-12842.97	1242.83	1.16
	24	-12313.39	619.39	
2nd step – A Tassineta, E Maghjine, Speloncellu, Pozzi, Val d'Ese, Acqua d'Acelli, Lataga, Marroccu, Is Abius & Camboni	1	-7954.80	0.19	
	2	-6059.44	70.35	11.34
	3	-4961.81	107.56	1.68
	4	-4045.29	213.21	1.12
	5	-3367.39	64.76	2.17
	6	-2830.01	143.22	1.61
	7	-2523.24	289.77	1.09
	8	-2530.87	608.84	0.44
	9	-2269.70	216.48	15.83
	10	-5434.84	5154.41	0.79
	11	-4537.20	3798.47	
2nd step – Roya, U Furcone, Cantiano hatchery, Visso hatchery, Ermolinus, Isere hatchery, Sadali, lower Tiber, lower Calabria, Nera, Ancinale, Anapo & south Sicily	1	-13487.59	0.59	
	2	-12204.69	43.49	3.52
	3	-11074.81	25.73	26.79
	4	-10634.14	164.36	0.11
	5	-10174.70	71.59	3.00
	6	-9929.97	113.02	1.25
	7	-9543.69	85.19	1.81
	8	-9311.77	120.03	1.62
	9	-9274.61	274.43	0.90
	10	-8990.30	191.29	0.47
	11	-8796.27	111.50	0.71

	12	-8680.90	154.62	0.04
	13	-8558.93	145.67	1.44
	14	-8646.49	396.96	
3rd step – Marroccu, Is Abius & Camboni	1	-986.37	0.08	
	2	-903.67	1.80	51.68
	3	-913.89	9.17	0.42
	4	-927.93	6.13	5.80
	5	-977.56	7.41	
3rd step – Roya & U Furcone	1	-1400.97	0.52	
	2	-984.26	0.76	646.82
	3	-1059.94	33.94	2.82
	4	-1039.80	34.81	
3rd step – Cantiano hatchery, Visso hatchery, Ermolinus, Isere hatchery, Sadali, lower Tiber & lower Calabria	1	-6676.53	1.02	
	2	-6258.34	1.16	145.92
	3	-6010.09	29.81	0.91
	4	-5789.07	17.21	4.21
	5	-5640.56	26.66	1.69
	6	-5537.01	6.19	5.07
	7	-5402.09	40.88	1.57
	8	-5331.40	38.79	2.84
	9	-5370.70	284.11	0.86
	10	-5167.00	16.51	
3rd step – Nera, Ancinale, Anapo & south Sicily	1	-3036.33	1.26	
	2	-2529.04	55.98	3.32
	3	-2207.90	0.54	196.50
	4	-1993.79	0.47	614.31

	5	-2066.56	31.59	1.52
	6	-2091.26	12.14	0.74
	7	-2106.93	22.61	
4th step – Marroccu	1	-390.20	0.71	
	2	-345.90	1.09	55.26
	3	-362.00	1.79	3.85
	4	-371.30	2.20	
4th step – U Furcone	1	-212.07	0.21	
	2	-144.30	1.54	44.50
	3	-145.23	1.11	0.22
	4	-146.40	2.63	
4th step – Cantiano hatchery, Visso hatchery, Ermolinus & Isere hatchery	1	-3645.03	0.35	
	2	-3448.73	0.92	40.77
	3	-3290.00	7.24	8.98
	4	-3196.29	2.76	20.12
	5	-3158.04	8.24	10.72
	6	-3208.13	122.30	0.83
	7	-3157.06	35.41	
4th step – Sadali, lower Tiber & lower Calabria	1	-2627.00	1.87	
	2	-2509.20	305.16	0.50
	3	-2239.85	9.24	16.54
	4	-2123.31	39.34	1.19
	5	-2053.50	66.01	0.11
	6	-1990.71	57.10	0.04
	7	-1925.44	5.03	
4th step – Nera	1	-538.87	0.70	

	2	-537.10	1.56	3.6033
	3	-540.96	4.03	0.5921
	4	-547.20	3.84	
5th step – Cantiano hatchery, Visso hatchery & Ermolinus	1	-2706.61	0.33	
	2	-2567.81	3.93	10.83
	3	-2471.54	4.84	15.31
	4	-2449.40	10.13	9.30
	5	-2521.51	173.27	
5rd step – lower Tiber	1	-627.07	0.56	
	2	-562.96	8.16	1.7955
	3	-484.19	4.59	8.6897
	4	-445.30	9.63	1.4869
	5	-420.73	7.06	
5rd step – lower Calabria	1	-910.90	0.31	
	2	-792.40	1.19	160.73
	3	-864.99	155.38	0.36
	4	-881.21	109.51	0.36
	5	-857.96	24.51	1.89
	6	-880.94	21.03	
6th step – Cantiano hatchery & Visso hatchery	1	-1831.07	0.33	
	2	-1738.83	0.80	113.84
	3	-1737.83	22.40	1.29

4	-1765.83	14.74	0.56
5	-1802.10	9.03	1.55
6	-1852.33	21.09	

Table S3 Parentage analyses performed with COLONY for Corsican and Sardinian samples. We have chosen the Pair-Likelihood-Score (PLS)/Full-Likelihood (FL) combined (=FPLS) algorithm and used only full-sibs listing to give the results below.

FullSibs families	Prob(Inc.)	Prob(Exc.)	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6	Ind. 7	Ind. 8	Ind. 9	Ind. 10	Ind. 11	Ind. 12	Ind. 13	Ind. 14	Ind. 15	Ind. 16	Ind. 17	Ind. 18	Ind. 19	Ind. 20	Ind. 21
1	1.0000	1.0000	FUR01	FUR02	FUR03	FUR04	FUR05	FUR06	FUR07	FUR08	FUR09	FUR10	FUR11	FUR12	FUR14	FUR15	FUR16	FUR17	FUR19	FUR20			
2	1.0000	0.1236	FUR13	FUR18																			
3	1.0000	1.0000	TAS01	TAS02	TAS03	TAS04	TAS05	TAS06	TAS07	TAS08	TAS09	TAS10	TAS11	TAS12	TAS13	TAS14	TAS15	TAS16	TAS17	TAS18	TAS19		
4	1.0000	1.0000	MAG01	MAG02	MAG03	MAG04	MAG05	MAG06	MAG07	MAG08	MAG09	MAG10	MAG11	MAG12	MAG13	MAG14	MAG15	MAG16	MAG17	MAG18	MAG19	MAG20	MAG21
5	0.9010	0.0882	SPE01	SPE02	SPE04	SPE06	SPE08	SPE11	SPE12	SPE13	SPE14	SPE18	SPE19	SPE20									
6	0.9949	0.0833	SPE03	SPE05	SPE07	SPE09	SPE10	SPE15	SPE16	SPE17													
7	0.7592	0.0251	POZ01	POZ03	POZ05	POZ11																	
8	0.9868	0.0425	POZ02	POZ04	POZ06	POZ07	POZ08	POZ09	POZ10	POZ12	POZ13	POZ14	POZ15	POZ16	POZ17	POZ18	POZ19	POZ20					
9	0.4825	0.0466	ESE01	ESE06	ESE07	ESE08	ESE11	ESE12	ESE13	ESE14	ESE15	ESE16	ESE17	ESE18									
10	0.9859	0.0454	ESE02	ESE03	ESE04	ESE09	ESE10	ESE19	ESE20														
11	1.0000	0.0629	ESE05																				
12	0.9961	0.9961	AQA01	AQA02	AQA03	AQA04	AQA05	AQA06	AQA07	AQA08	AQA09	AQA10	AQA11	AQA12	AQA13	AQA14	AQA15	AQA16	AQA17	AQA18	AQA19	AQA20	
13	1.0000	1.0000	LAT01	LAT02	LAT03	LAT04	LAT05	LAT06	LAT07	LAT08	LAT09	LAT10	LAT11	LAT12	LAT13	LAT14	LAT15	LAT16	LAT17	LAT18	LAT19	LAT20	

Pop. N°	Abbr.	Name
2	FUR =	**U Furcone**
3	TAS =	**A Tassineta**
4	MAG =	**E Maghjine**
5	SPE =	**Speloncellu**
6	POZ =	**Pozzi**
7	ESE =	**Val d'Ese**
8	AQA =	**Acqua d'Acelli**
9	LAT =	**Lataga**

A. In Corsica, each population shows only one to three families. In fact, the polymorphism is so low ($0.08<Hnb<0.26$) that we can deduce that these small populations, totally isolated, have suffered several drastic recent bottlenecks. We can also suppose that one or two pairs of parents have recently re-funded each population making naturally each sample 1 to 3 families. The other interpretation is that such low polymorphism does not allow families detection.

FullSibs families	Prob(Inc.)	Prob(Exc.)	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6	Ind. 7
1	1.0000	1.0000	ERM01	ERM06					
2	1.0000	1.0000	ERM02	ERM03	ERM04	ERM05	ERM07		
3	1.0000	0.7598	ERM08	ERM10	ERM12				
4	1.0000	1.0000	ERM09	ERM14					
5	1.0000	0.3224	ERM11	ERM17					
6	1.0000	0.0935	ERM13						
7	1.0000	1.0000	ERM15	ERM16					
8	0.9140	0.9140	SAD01	SAD07					
9	1.0000	0.0646	SAD02						
10	0.7066	0.7066	SAD03	SAD04					
11	0.4239	0.4239	SAD05	SAD06					
12	0.7525	0.4014	SAD08	SAD10					
13	1.0000	0.1038	SAD09						
14	0.9983	0.9436	SAD11	SAD14					
15	1.0000	0.0857	SAD12						
16	1.0000	0.0364	SAD13						
17	0.6609	0.1047	MAC01	MAC13	MAC18	MAC19			
18	1.0000	0.0949	MAC02	MAC05	MAC12	ABI05	CAM16	CAM17	
19	1.0000	0.0895	MAC03	MAC06	MAC07	MAC16			
20	1.0000	0.0630	MAC04						
21	1.0000	0.0624	MAC08						
22	0.8043	0.0453	MAC09	ABI08	CAM03	CAM06	CAM12		
23	0.9288	0.0997	MAC10	MAC15	MAC20	ABI03	CAM01		
24	0.9815	0.0712	MAC11	CAM10	CAM11	CAM15			
25	0.3707	0.1120	MAC14	ABI01	ABI02	ABI07	ABI09	CAM09	
26	1.0000	0.5833	MAC17	ABI06	ABI11	CAM02	CAM07	CAM13	CAM14
27	0.8060	0.1386	ABI04	ABI10	ABI12	CAM04	CAM05	CAM08	
28	1.0000	0.0406	CAM18						

Pop. N°	Abbr.	Name
10	ERM =	**Ermolinus**
11	SAD =	**Sadali**
12	MAC =	**Marroccu**
13	ABI =	**Is Abius**
14	CAM =	**Camboni**

B. In Sardinia, the landscape is totally different: (i) in north-east populations (Flumendosa basin), mostly composed of Atlantic domestic lineage, the Ermolinus and Sadali populations are composed of 7 to 9 families of generally 1 or 2 individuals (except two families of 3 and 5 trouts). This is classical for highly polymorphic populations (here $0.74<Hnb<0.79$) composed of various origins. (ii) In the south, the same local lineage has been observed in three samples of the same watershed (Cixerri basin) with possible exchanges. The medium level of polymorphism ($0.41<Hnb<0.51$) corresponds to rather large populations and possibly exchanges between them. The whole Cixerri sampling forms 12 families of full-sibs frequently composed of individuals of the three sampled locations.