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

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1 **Brown trout (*Salmo trutta* L.) high genetic diversity**  
2 **around the Tyrrhenian Sea**  
3 **as revealed by nuclear and mitochondrial markers**  
4

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7  
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17  
18 **Abstract**

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

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

34

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

36

37 **Running title:** Tyrrhenian trout genetic structure

38

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41

## 42 **Introduction**

43

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

51 The brown trout is a complex taxon, first investigated through morphology, then with molecular  
52 markers. The term of "*Salmo trutta* complex" (Patarnello et al., 1994; Bernatchez & Osinov, 1995; Giuffra  
53 et al., 1996; Bernatchez, 2001) is used to indicate that the complex is composed of numerous  
54 differentiated geographic forms (without consensus on the taxonomy) that can easily hybridize with each  
55 other (Largiadèr & Scholl, 1996; Meldgaard et al., 2007). Its ecological diversity is also remarkable with  
56 sedentary populations, sometimes in the cold upstream of rivers, and anadromous ones that migrate to seas  
57 or lakes. Based on phenotypes, many species have been described. Kottelat & Freyhof (2007) reviewed  
58 the published nomenclature and reached the number of 28 nominal species. This has been augmented by at  
59 least fifteen new species described during the last twelve years (Delling & Doadrio, 2005; Turan et al.,  
60 2009, 2010, 2011, 2012, 2014a, 2014b; Delling, 2010; Doadrio et al., 2015).

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

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

76 According to the large literature based on morphology and molecular genetics proposing taxonomic  
77 organization of the trout populations inhabiting the Tyrrhenian periphery, a rather confusing picture has  
78 emerged. Nera River (Roma region) was considered to be an indigenous pool, home of the Mediterranean  
79 *S. trutta* lineage (Lucentini et al., 2006). Corsican rivers were proposed to host *S. macrostigma* (Roule,  
80 1933; Guyomard, 1989; Bernatchez et al., 1992) or *S. trutta* (Berrebi, 2015), while Sardinian rivers were  
81 inhabited by *S. macrostigma* (Boulenger, 1901; Mola, 1928; Pomini, 1940; Gandolfi et al., 1991;  
82 Patarnello et al., 1994; Massidda, 1995; Sabatini et al., 2006; Orrú et al., 2010) or *S. trutta* (Sabatini et al.,  
83 2011) or *S. cettii* (Zaccara et al., 2015). South Sicilian trout are of Atlantic *S. trutta* lineage (Schöffmann  
84 et al., 2007) or *S. cettii* (Kottelat, 1997; Kottelat & Freyhof, 2007; Schöffmann et al., 2007; Duchi, 2011 &  
85 2018; Bianco, 2014; Sabatini et al., 2018).

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

90

91

92

93 **Materials and methods**

94

95 Sampling distribution

96

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

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

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

111

112 DNA extraction

113

114 DNA was extracted using the Chelex/proteinase K based method described by Walsh et al. (1991) and  
115 Estoup et al. (1996) then improved by Yue & Orban (2005). A small piece of fin was incubated overnight  
116 at 56°C in 195 µL of 5% Chelex 100 Resin (Biorad) solution containing 50 mM of Tris-HCL (pH 7) and  
117 500 µg/mL of proteinase K. Samples were then incubated at 95°C for 10 min before centrifugation at 3500  
118 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 MgCl<sub>2</sub>, 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-CI*\* genotypes

138

139 Some of the sampled specimens were genotyped at the *LDH-CI*\* 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,

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

154

#### 155 Microsatellite loci

156

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

160 For each marker, one of the 5' ends of the two primers was end-labelled with a fluorescent dye, either  
161 6-FAM, HEX or NED. Polymerase chain reactions (PCR) were performed using the Qiagen multiplex  
162 PCR kit in a final volume of 10  $\mu$ L, containing 3  $\mu$ L of genomic DNA diluted at 10 ng/ $\mu$ L, 5  $\mu$ L of Qiagen  
163 PCR Master Mix, 1  $\mu$ L of Qiagen Q-solution, and 1  $\mu$ L of primer mix at 2  $\mu$ M each (Eurofins MWG  
164 Operon). Amplifications were carried out in a GeneAmp PCR System 2700 thermal cycler (Applied  
165 Biosystems), according to the supplier's instructions (Qiagen multiplex PCR kit): initial denaturation step  
166 (95°C, 15 min;) followed by 35 cycles of denaturation (94°C, 30 s), annealing (55°C for the three  
167 multiplexes, 90 s) and extension (72°C, 60 s); with a final extension step (60°C, 30 min). Amplified PCR  
168 fragments were then diluted and separated on an ABIPRISM 3130/xl/ sequencer (Applied Biosystems)  
169 with GeneScan 500 Rox dye size standards. Allele sizes were determined using the GeneMapper v4.1  
170 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 ( $H_e$ ), unbiased expected  
177 heterozygosity ( $H_{nb}$ : Nei, 1978) and observed heterozygosity ( $H_o$ ) 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 ( $F_{st}$ ) and the intra-river panmixia ( $F_{is}$ ) were  
182 estimated ( $\theta$  and  $f$  estimators of Weir & Cockerham, 1984, respectively). The significance of the  $F_{st}$  and  
183  $F_{is}$  values was tested by random permutation procedures: 5000 individual permutations between samples  
184 for  $F_{st}$  and 5000 allele permutations within samples for  $F_{is}$ , 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  $K$

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

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

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

213

214

## 215 **Results**

216

### 217 Mitochondrial sequences

218

219 A total of 144 mtDNA CR sequences were obtained in this survey corresponding to sixteen different  
220 haplotypes, seven of which are undescribed so far (AD-Tyrrh1 to 6 and AT-Tyrrh1: Tables 3 and S1 for  
221 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

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

256

#### 257 *LDH-C1* \* genotypes

258

259 Genotyping this gene is especially useful to distinguish between natural and domestic origins of the AT  
260 haplotypes of Sicily. Some of the samples were tested, especially those with AT mtDNA haplotypes  
261 (Table 1). Hatchery samples were nearly all characterized by the *LDH-C1*\*90 allele except for one  
262 \*90/100 heterozygote genotype among the 20 Cantiano hatchery trouts (sample 15). This hatchery \*90  
263 allele was also found in 8/8 in the Allaro River and 7/10 in the Diga Giulia River, both in Calabria, and  
264 just 1/10 in the Anapo River in Sicily. The domestic allele *LDH-C1*\*90 was not found in Corsica (but only  
265 four fishes analyzed) nor in the Manghisi River in Sicily (five trouts).

266

#### 267 Microsatellite genotypes

268

269 For samples numbering over ten specimens (20 of the 30 considered), the Micro-Checker software  
270 made no drop-out detection, two tests among 240 had suspect stuttering and 17 among 240 suggested the  
271 occurrence of null alleles. The 17 possible null allele detections were spread among 8 of the 12 loci, with  
272 0 to 3 cases each, showing that there was no systematic presence of null alleles in a given marker,  
273 probably not disturbing the calculations.

274 The first representation of overall microsatellite genetic diversity is given by multidimensional  
275 analyses. Fig. 3a first isolates Corsican populations at the negative part of the first axis, with very low  
276 intra-population and very high inter-population diversity, together with absence of Atlantic influence. The  
277 southern samples of Sardinian trout (numbers 12 to 14) clustered in one group. Note that because this is a  
278 2D representation of a 3D simplification of the 273D hyperspace (274 x 324 matrix), Sardinian  
279 autochthonous trout (green envelope) are clearly separated from E Maghjine Corsican trout (red circles)  
280 along axis 3 despite the apparent overlapping in the diagram (see Fig. S4). The same is true between  
281 Lataga (grey circles) and Acqua d'Acelli (dark green circles) fishes (Fig. S4).

282 In contrast, several trout populations seemed to be introgressed or hybridized with the commercial  
283 international Atlantic strain represented here by the Italian and French hatcheries (Cantiano, Visso and  
284 Isère hatcheries, samples 15, 16 and 30). They are positioned at the very end of the positive part of axis  
285 one. In order to understand this part of the diagram, Atlantic-like samples were reanalyzed alone. The  
286 second analysis (Fig. 3b) showed clear separation of the Anapo population (Sicily, sample 27) from the  
287 remaining populations. Finally, the third analysis (Fig. 3c) that considered the Atlantic-like samples  
288 without the Anapo one, gathered at the left the domestic Atlantic strains and the Ermolinus and Sadali  
289 samples of central-eastern Sardinia (numbers 10 and 11 in Table 1) and those from Allaro and Precariti in  
290 Calabria (23 and 26), probably of domestic origin. The remaining samples collected around two clusters:  
291 first, numbers 18, 19, 28 and 29 (respectively from the Tiber River in Lazio province and the southern  
292 samples in Sicily), and second, numbers 20 to 25 corresponding to most samples from Calabria (except for  
293 23, the Allaro River, probably of full domestic origin).

294 Summarizing, the multidimensional analyses highlighted several types of populations:

295 - insular/endemic differentiated populations: all Corsican (samples 2 to 9) and southern Sardinian  
296 samples (12 to 14); the Nera population in continental Italy (17) (Fig. 3a);

297 - the Anapo River population (27) clearly isolated from the remaining Atlantic populations (Fig. 3b);

298 - southern populations, close to the domestic cluster: downstream Tiber River samples (numbers 18  
299 and 19); two Sicilian samples (28 and 29); most Calabrian samples (numbers 20 to 24, except for sample  
300 23 from the Allaro River that clustered with domestic trout);

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

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

318 Unlike the hierarchical Structure analysis and  $\Delta K$  method (Evanno et al., 2005) which estimated 22  
319 groups, method based on maximizing the mean estimated ln probability of data (ln P(D); Pritchard et al.,  
320 2000) show that the most probable  $K = 19$  (Fig. S5 & S6). This last analysis method is less informative  
321 than hierarchical Structure, and, unlike hierarchical method, some of populations are not detected as  
322 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-Appennine 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).

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

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

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

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

363

364 Population parameters

365

366 Population parameters add some biological information. Heterozygosity, as measured with various H  
367 parameters and with the mean number of alleles A (Table 4), was very low in Corsica ( $H_{nb}=0.17$ ), but  
368 very high in Sardinia (0.58). Parameter A, the mean number of alleles by locus, was also very distinctive  
369 (respectively 1.9 and 4.7). Hatchery populations are considered to be highly polymorphic (Berrebi et al.,  
370 2000; Bohling et al., 2016) and displayed large values:  $0.67 < H_{nb} < 0.78$  and  $5.7 < A < 8.3$ .

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



374 which is a very high value among neighboring rivers ( $0.47 > F_{st} > 0.90$ ). The mean  $F_{st}$  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 ( $0.03 < F_{st} < 0.08$ ). In Calabria, the small sample sizes limited the estimations  
378 between groupings to an opposition between the Ancinale and Alaro/Assi/Stilaro/Preariti basins ( $F_{st}$  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 ( $0.47 > F_{st} > 0.90$ ; 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 ( $0.08 < H_{nb} < 0.26$ , the lowest values among all the samples) compared to  
406 native grouped Tiber populations ( $0.51 < H_{nb} < 0.75$ ), native Sardinian trout ( $0.45 < H_{nb} < 0.51$ ), and even the  
407 grouped Calabrian ( $0.62 < H_{nb} < 0.78$ ) and Sicilian populations ( $0.49 < H_{nb} < 0.59$ ). 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 ( $0.08 < H_{nb} < 0.26$ ) 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  $0.74 < H_{nb} < 0.79$ ) 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 ( $0.41 < H_{nb} < 0.51$ ) 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).

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

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

438 Tiber River trout are probably influenced by the numerous upstream hatcheries settled in the Marche  
439 (samples 15 and 16) and Umbria regions. However, this influence seems limited in the wild according to  
440 the mitochondrial marker (no AT haplotype upstream in the Nera River and 33% downstream). Nuclear  
441 markers clearly isolate a first natural group, the upstream Nera River sample alone, visible outside the  
442 central part of the multidimensional diagram (Fig. 3a) in the B1 lineage (Fig. 4) and with haplotypes AD-  
443 Tyrrh5 and 6, not observed elsewhere (Table 3). According to microsatellites (Fig. 3c and the B2 cluster  
444 in Fig 4.), another lower Tiber group is genetically closer to the domestic samples (haplotypes AD-Tyrrh  
445 1, 2 and 4, all three not endemic to the Tiber River).

446 Calabria is a dry area cut at the south by small parallel coastal rivers flowing into the Ionian Sea.  
447 Domestic Atlantic strains were introduced into this region, and totally replaced the Allaro population  
448 (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-CI\*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-CI\*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 *CI\*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.

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

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

484

485 Conservation purposes

486

487 The brown trout is generally accepted as a diversified and complex assemblage of salmonids of the  
488 genus *Salmo*. It has been investigated for a long time by several methods, but without a clear description  
489 of its taxonomy. According to Sanz (2018), who reviewed the entire *S. trutta* complex data, the origin of  
490 this difficulty is due to the multiple episodes of colonization and secondary contacts. This high  
491 phylogenetic complexity with large genetic differences within lineages is accompanied by morphological  
492 and life-history diversity that has led to the description of dozens of nominal species (Kottelat & Freyhof,  
493 2007).

494 The increase in the number of new trout species, sometimes elevating known subspecies to the species  
495 rank (Isaac et al., 2004; Zachos et al., 2013), sometimes accepting ancient morphological species as true  
496 species (Ninua et al., 2018), is a strategy to be considered as a threatened taxon in the IUCN Red List  
497 criteria. This should be a help for conservation (Garnet & Christidis, 2007) but such a strategy can lead to  
498 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 (Avice 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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586

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876

877 **Table captions**

878

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

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

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

888 **Table 4:** Parameters estimating the genetic diversity and the panmixia. Map = see Fig. 1,  $H_e$  = calculated  
889 heterozygosity under the hypothesis of Hardy-Weinberg Equilibrium,  $H_{nb}$  = similar to  $H_e$  parameter  
890 but weighted depending on the sample size (Nei, 1978),  $H_o$  = observed heterozygosity,  $A$  = mean  
891 number of allele by locus. Values of  $F_{is}$  between parentheses have no real sense as several samples  
892 were grouped to obtain a sample size over 10.

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

899

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 dotted 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  $\Delta K$  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



932 **Supporting information**

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

935 **Table S2:** Hierarchical steps in estimating K (the number of genetic clusters) from STRUCTURE runs  
936 using the  $\Delta K$  method. L(K) - posterior probability of K; stdev - standard deviation of L(K) from seven  
937 independent runs;  $\Delta K$  - an ad hoc quantity, predictor of the real number of clusters (Evanno et al.,  
938 2005), best  $\Delta K$  are highlighted.

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

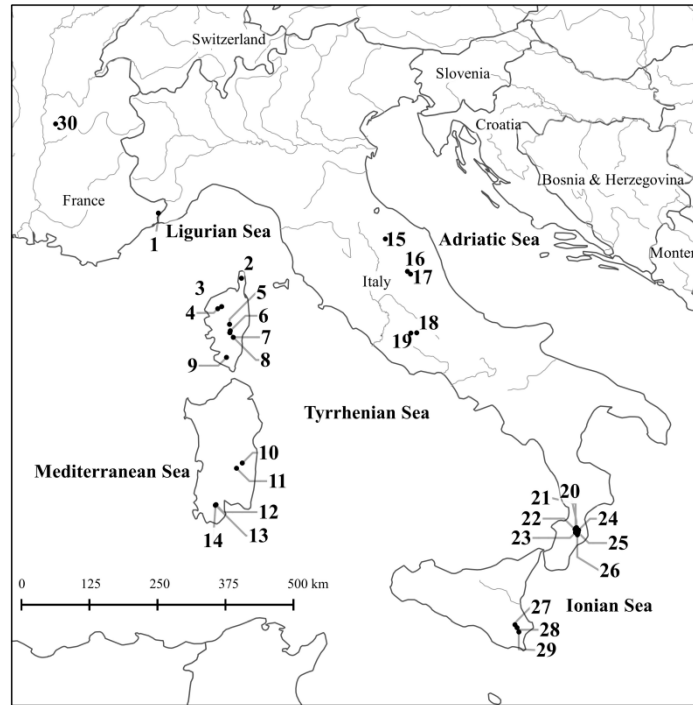
940 **Fig. S4:** New projection of the FCA analysis presented in Fig. 3a according to axes 1 and 3. In this new  
941 perspective, Sardinian samples and E Maghine Corsican one, together with the Corsican samples  
942 Lataga and Aqua d'Acelli, which seemed similar in Fig. 3a, are in fact clearly different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

944 **Fig. S5:** Estimation of the number of genetic clusters (K) for the first level, from STRUCTURE runs using  
945 the  $\Delta K$  method.

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

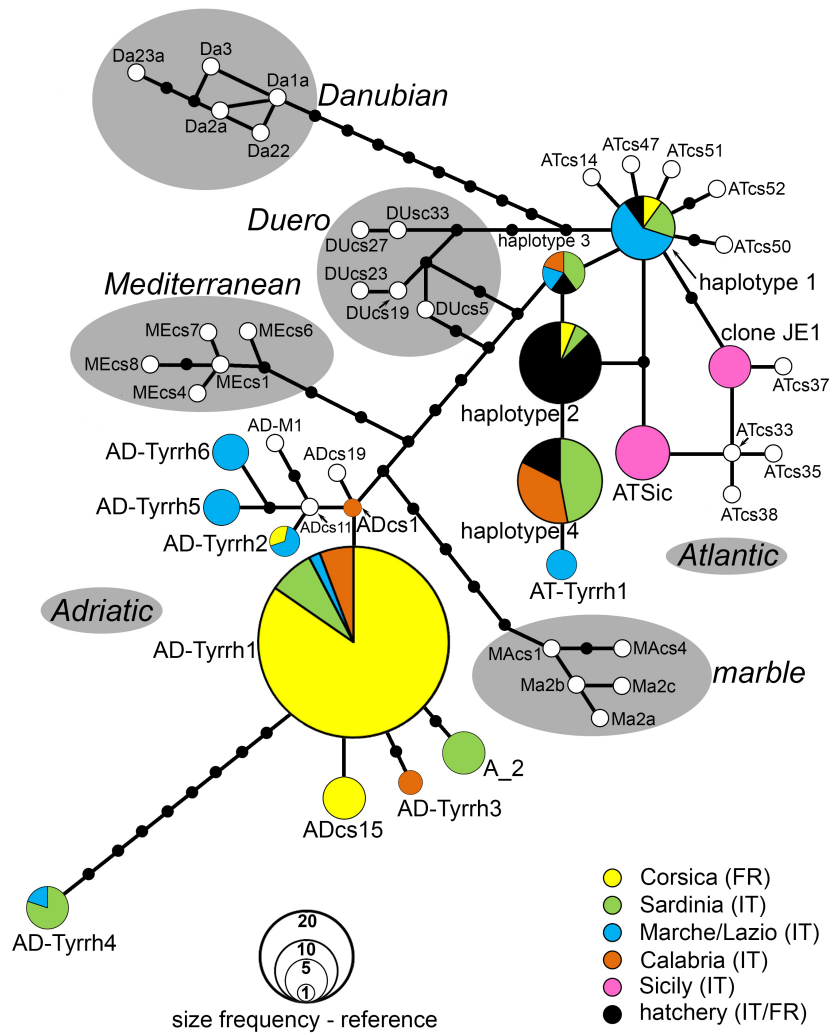
950

951 **Figure 1**



952

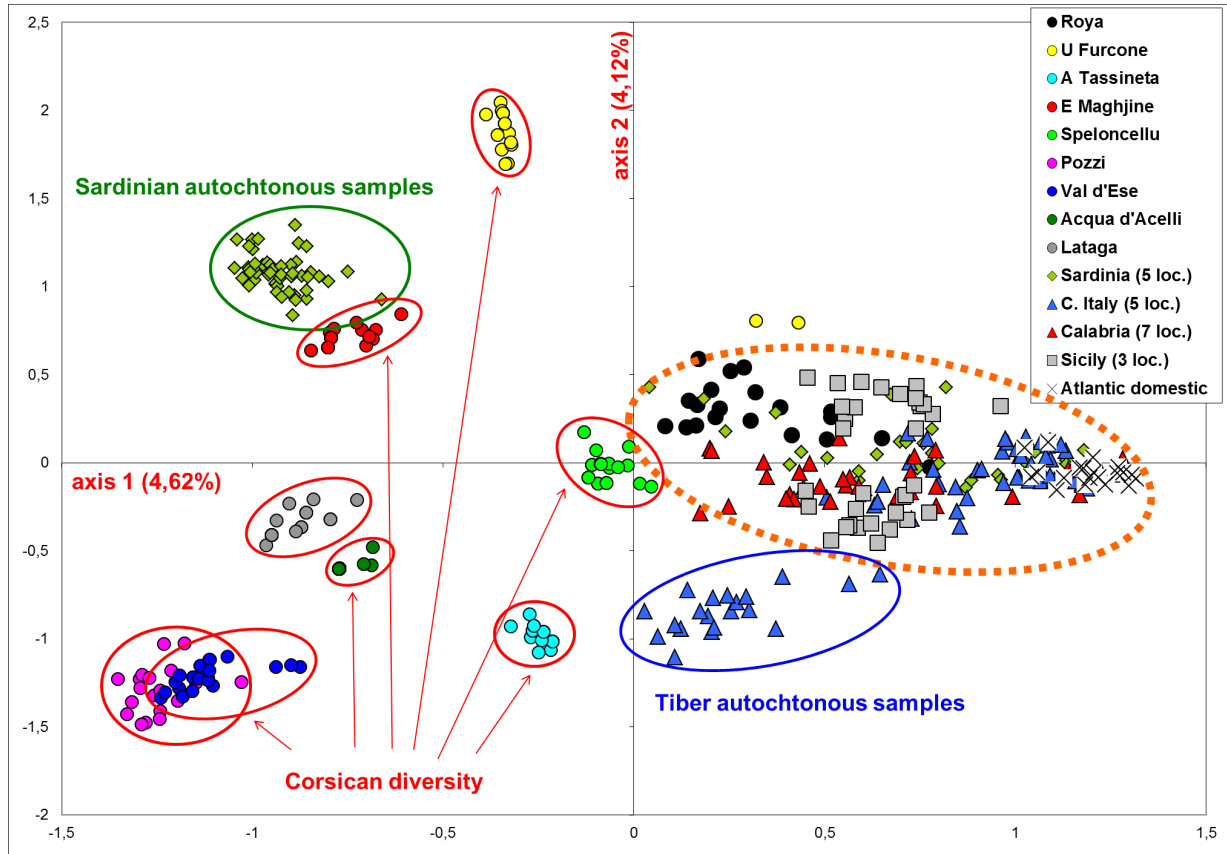
953



955

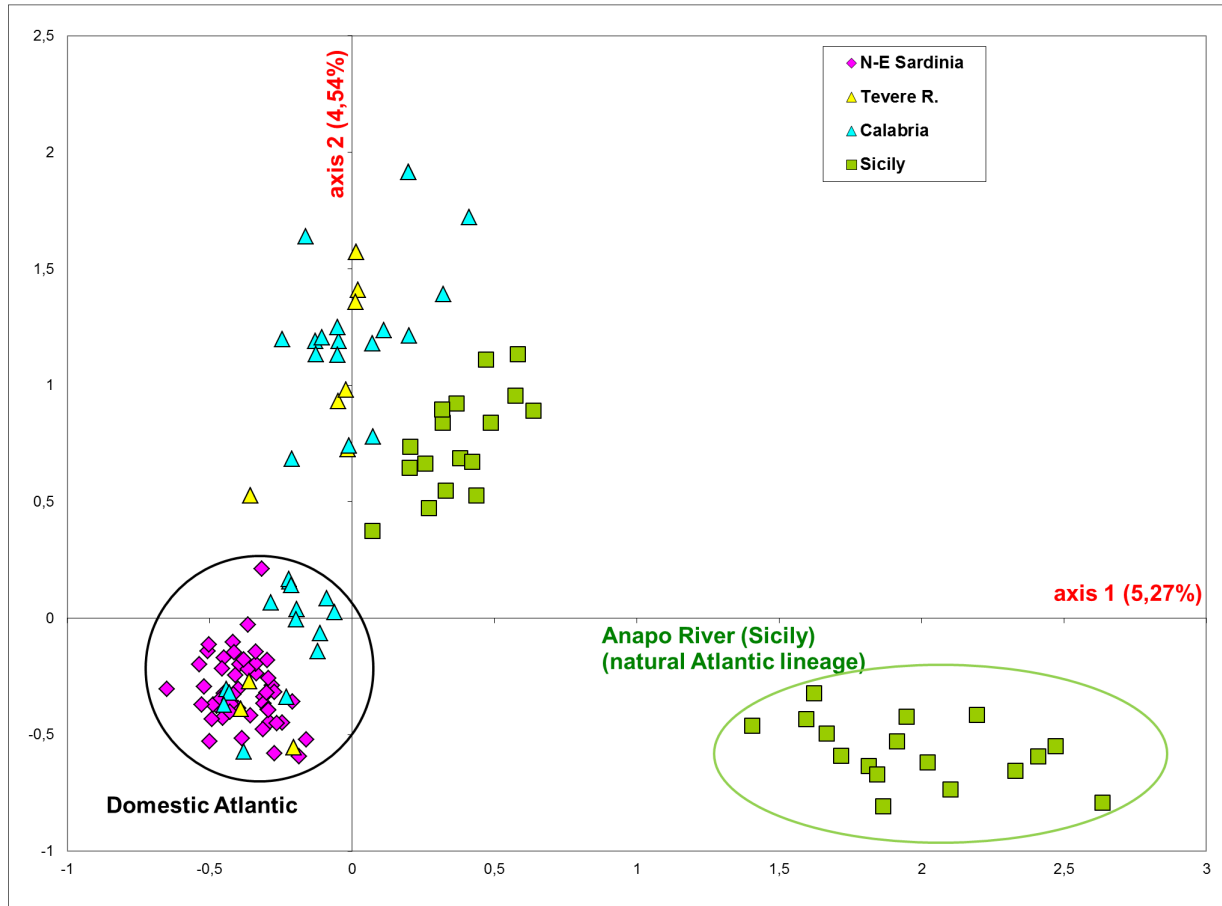
956

957 Figure 3a



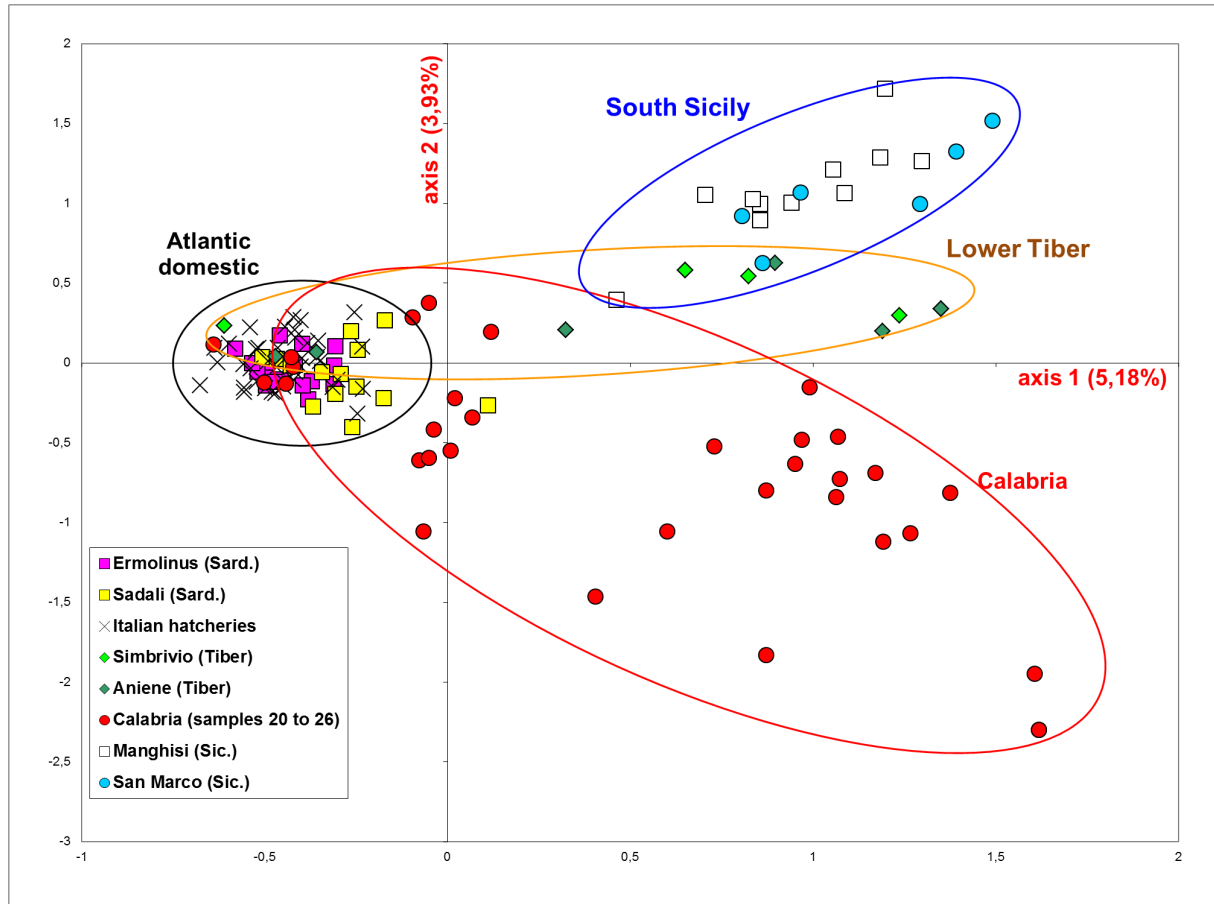
958  
959

960 Figure 3b



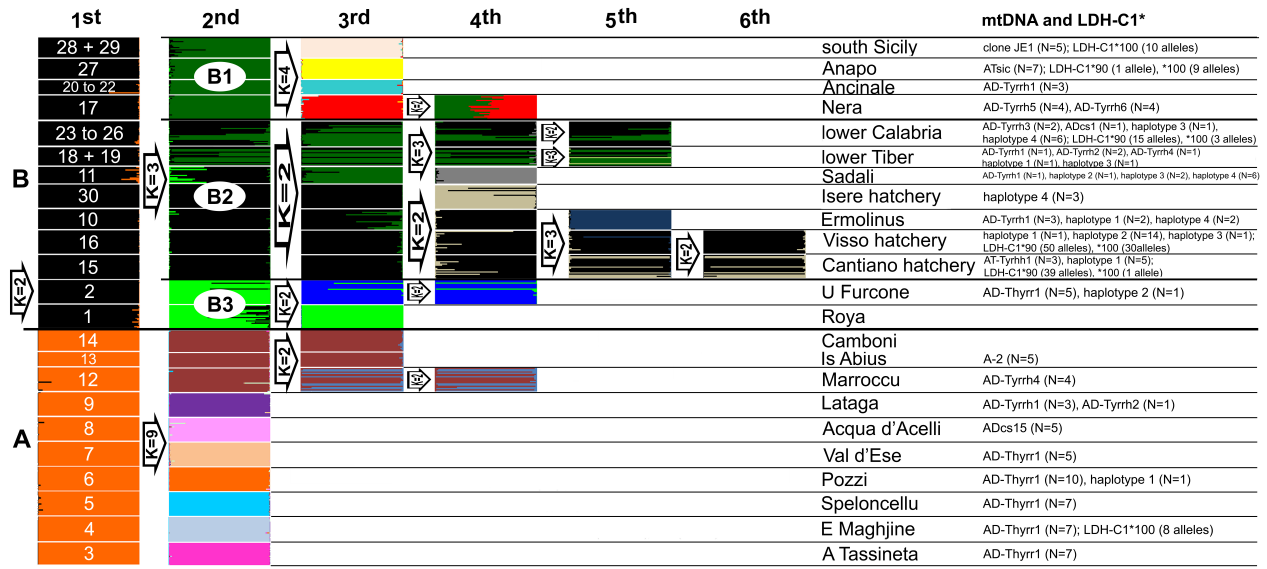
961  
962

963 Figure 3c



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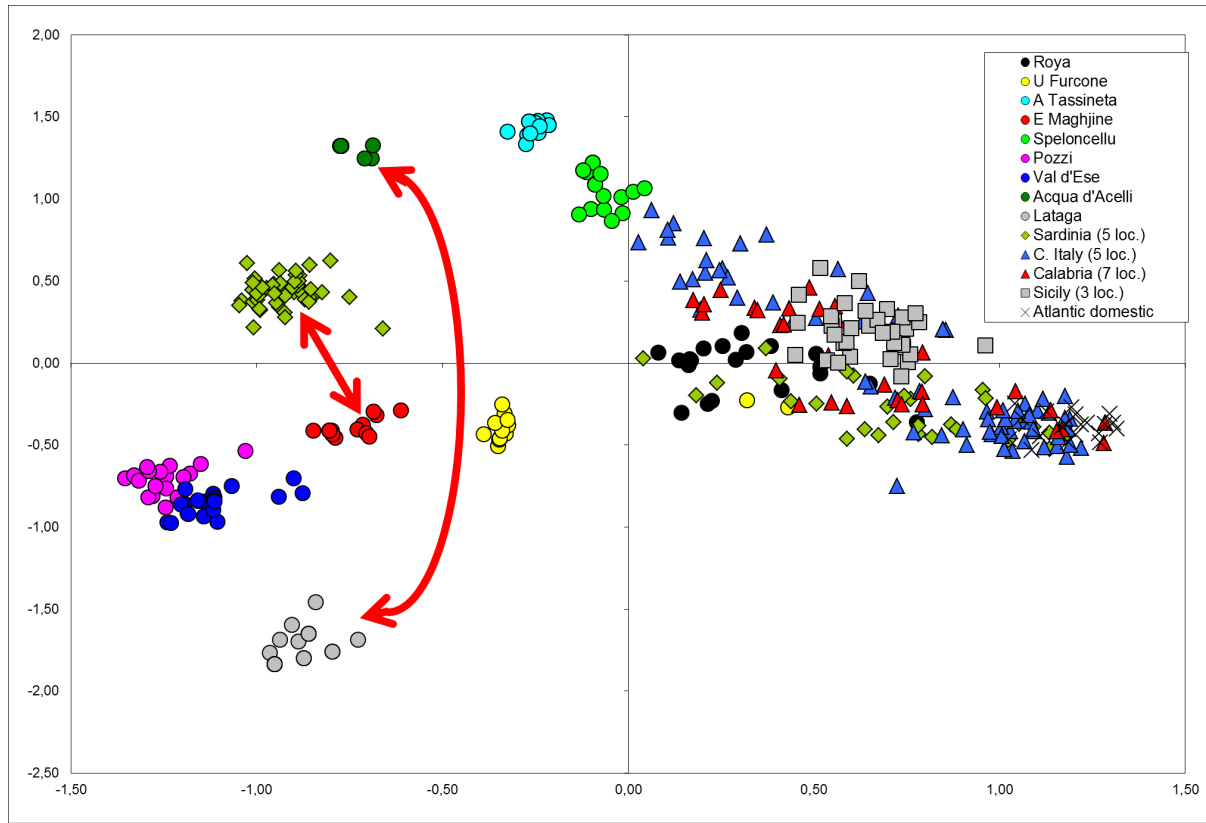
966 Figure 4



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968

969 Figure S4

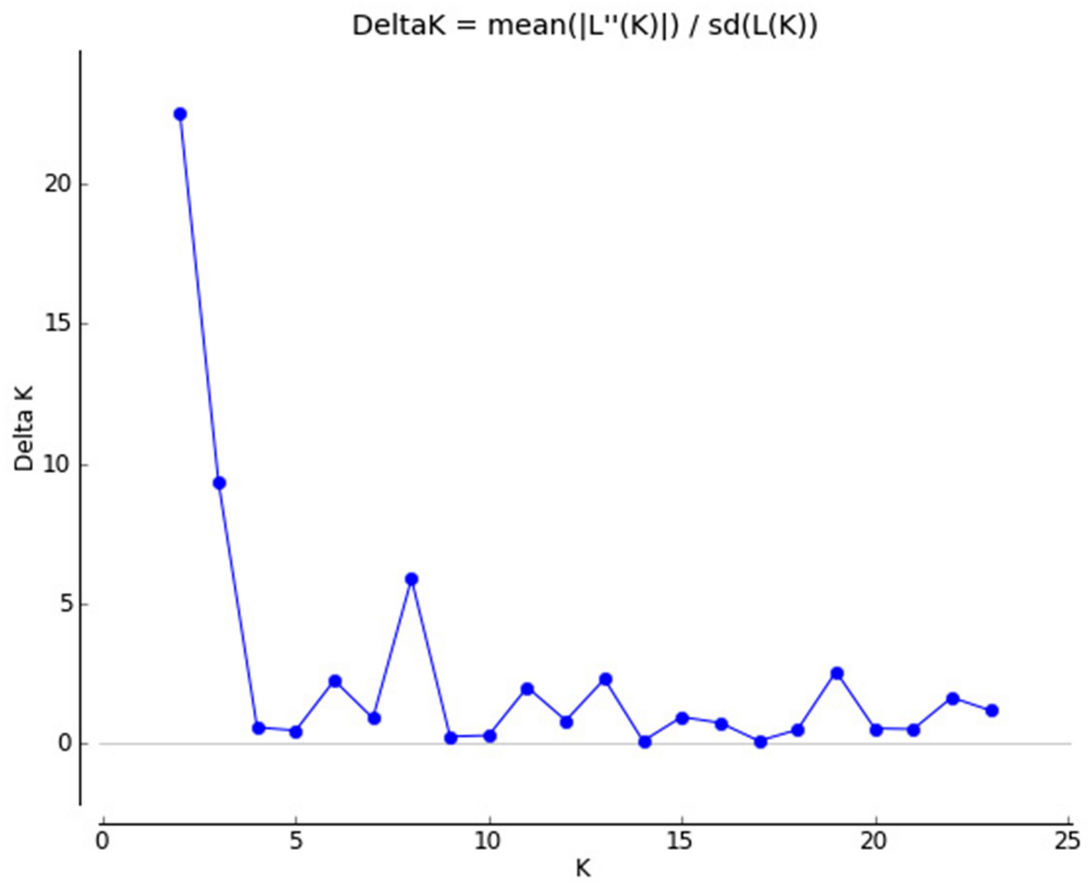


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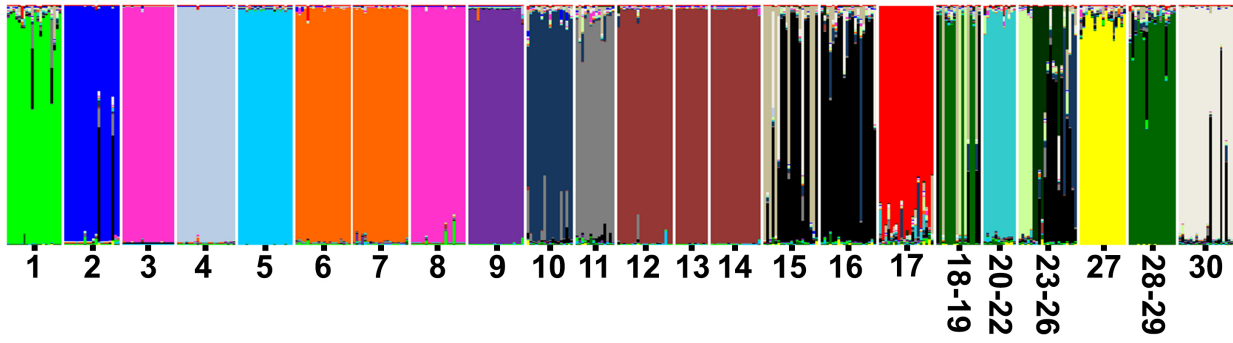
972 Figure S5



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975 Figure S6



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

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

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

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

Region	Map N°	River or hatchery	sequence numbers	Haplotype frequency														LDH-CI*90	LDH-CI*100		
				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
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

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992 **Table 4.** Parameters estimating the genetic diversity and the panmixia. Map = see Fig. 1, He = calculated  
 993 heterozygosity under the hypothesis of Hardy-Weinberg Equilibrium, Hnb = similar to He parameter but  
 994 weighted depending on the sample size (Nei, 1978), Ho = observed heterozygosity, A = mean number of allele by  
 995 locus. Values of Fis between parentheses have no real sense since several samples have been grouped for a  
 996 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
20to22	Ancinale	12	0.59	0.62	0.57	5.00	(0.082)	ns
23to26	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

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999 **Table 5.** Double matrix indicating the Fst ( $\Theta$ , Weir & Cockerham, 1984) values (upper triangle) and their  
 1000 significance (lower triangle). All Fst were very highly significant \*\*\*. After Bonferroni correction, only the level  
 1001 of significance was reduced, indicated between parentheses, for some comparisons simply significant (\*) or  
 1002 highly significant (\*\*). Med. = Roya River flowing to the Ligurian Sea, Dom. = domestic Atlantic commercial  
 1003 strain. Numbers refer to the first column of Table 1 and Fig. 1. A, B, C and D are grouped samples in order to  
 1004 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

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Table S1

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



*AD-Tyrrh6	KX450262	<sup>21</sup> continental Italy (Nera River)	1007
ADcs11	AY836340	<sup>15</sup> Montenegro (Skadar Lake)	<del>1008</del>
ADcs19	AY836348	<sup>4</sup> Spain (Guadalquivir)	
AD-M1	DQ381566	<sup>15</sup> Montenegro (Skadar Lake)	1009
MEcs1	AY836350	<sup>4</sup> Spain (Ter, Llobregat, Ebre, Mijares, Palancia, Túria, Segura), <sup>7</sup> Croatia (Krka)	<del>1010</del>
MEcs4	AY836353	<sup>4</sup> Spain (Ter)	1011
MEcs6	AY836355	<sup>4</sup> Spain (Ebre)	<del>1012</del>
MEcs7	AY836356	<sup>4</sup> Spain (Ter)	
MEcs8	AY836357	<sup>4</sup> Spain (Túria)	1013
MAcs1 (Ma1a)	AY836365	<sup>4</sup> Slovenia (Soča), <sup>8</sup> Greece (Aliakmon), <sup>10</sup> Italy (Adige, Po)	<del>1014</del>
MAcs4	JN208022	<sup>12</sup> Italy (Po)	
Ma2a	DQ841189	<sup>10</sup> Italy (Adige)	1015
Ma2b	DQ841190	<sup>10</sup> Italy (Adige)	
Ma2c	JQ582461	<sup>11</sup> Italy (Adige)	1016
DUcs5	EF530517	<sup>5</sup> Spain (Duero, Minho)	1017
DUcs19	EF530531	<sup>5</sup> Spain (Duero)	
DUcs23	EF530535	<sup>5</sup> Spain (Duero)	1018
DUcs27	KM210674	<sup>17</sup> Spain (Upper Miño)	1019
DUcs33	KM210680	<sup>17</sup> Spain (Mandeo, Lago)	
Da1a (Iran 3)	AY185568	<sup>1,6</sup> Austria (Drau, Inn, Kamp, Danube), <sup>8</sup> Bulgaria (Cerny Iskar), Serbia (Džepska River), <sup>19</sup> Iran (Tonekabon)	<del>1020</del>
Da2a (Da2)	GQ284834	<sup>1,6</sup> Austria (Inn, Mur, Drau), <sup>10</sup> Italy (Adige)	1021
Da3	AY185571	<sup>6,20</sup> Austria (Kamp)	1022
Da22	AY185573	<sup>1,6,20</sup> Austria (Inn, Kamp, Enns, Drau, Danube), <sup>9</sup> Macedonia (Vardar), <sup>10</sup> Italy (Adige), <sup>18</sup> Bosnia and Herzegovina (Una)	<del>1023</del>
Da23a	AY185574	<sup>6,20</sup> Austria (Kamp)	1024

- 1025 <sup>1</sup> Baric, S., Riedl, A., Meraner, A., Medgyesy, N., Lackner, R., Pelster, B. & Via, J. D. 2010. Alpine headwater  
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- 1081

1082

1083 Table S2 Hierarchical steps in estimating K (the number of genetic clusters) from STRUCTURE runs  
 1084 using the  $\Delta K$  method. L(K) - posterior probability of K; stdev - standard deviation of L(K) from seven  
 1085 independent runs;  $\Delta K$  - an *ad hoc* quantity, predictor of the real number of clusters (Evanno et al., 2005),  
 1086 best  $\Delta K$  are highlighted. In the second step of analysis on the Corse-Sardinian dataset (A Tassineta, E  
 1087 Maghjine, Speloncellu, Pozzi, Val d'Ese, Acqua d'Acelli, Lataga, Marroccu, Is Abius & Camboni),  $\Delta K$   
 1088 value was strongly affected by two outlier runs for K = 7, which are discarded from the analysis.

1089

	<b>K</b>	<b>L(K)</b>	<b>stdev</b>	<b><math>\Delta K</math></b>
1 <sup>st</sup> step – All samples	1	-25283.26	0.65	
	<b>2</b>	<b>-21560.37</b>	<b>76.36</b>	<b>22.53</b>
	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	
<hr/>				
2 <sup>nd</sup> 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	
<hr/>				
2 <sup>nd</sup> 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	
<hr/>				
3 <sup>rd</sup> 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	
<hr/>				
3 <sup>rd</sup> 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	
<hr/>				
3 <sup>rd</sup> 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	
<hr/>				
3 <sup>rd</sup> 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	
<hr/>				
4 <sup>th</sup> 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	
<hr/>				
4 <sup>th</sup> 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	
<hr/>				
4 <sup>th</sup> 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	
<hr/>				
4 <sup>th</sup> 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	
<hr/>				
4 <sup>th</sup> 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	
<hr/>				
5 <sup>th</sup> 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	
<hr/>				
5 <sup>rd</sup> 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	
<hr/>				
<hr/>				
5 <sup>rd</sup> 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	
<hr/>				
6 <sup>th</sup> step – Cantiano hatchery & Visso hatchery	1	-1831.07	0.33	
	2	-1738.83	0.80	113.84
	3	-1737.83	22.40	1.29

1090		4	-1765.83	14.74	0.56
1091		5	-1802.10	9.03	1.55
1092		6	-1852.33	21.09	
1093	<hr/>				



1094 Table S3 Parentage analyses performed with COLONY for Corsican and Sardinian samples. We have  
 1095 chosen the Pair-Likelihood-Score (PLS)/Full-Likelihood (FL) combined (=FPLS) algorithm and used only  
 1096 full-sibs listing to give the results below.

1097

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		

1098

1099 **A.** In Corsica, each population shows only one to three families. In fact, the polymorphism is so low  
 1100 (0.08<Hnb<0.26) that we can deduce that these small populations, totally isolated, have suffered several  
 1101 drastic recent bottlenecks. We can also suppose that one or two pairs of parents have recently re-funded  
 1102 each population making naturally each sample 1 to 3 families. The other interpretation is that such low  
 1103 polymorphism does not allow families detection.

1104

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						

1105

1106 **B.** In Sardinia, the landscape is totally different: (i) in north-east populations (Flumendosa basin), mostly  
1107 composed of Atlantic domestic lineage, the Ermolinus and Sadali populations are composed of 7 to 9  
1108 families of generally 1 or 2 individuals (except two families of 3 and 5 trouts). This is classical for highly  
1109 polymorphic populations (here  $0.74 < H_{nb} < 0.79$ ) composed of various origins. (ii) In the south, the same  
1110 local lineage has been observed in three samples of the same watershed (Cixerri basin) with possible  
1111 exchanges. The medium level of polymorphism ( $0.41 < H_{nb} < 0.51$ ) corresponds to rather large populations  
1112 and possibly exchanges between them. The whole Cixerri sampling forms 12 families of full-sibs  
1113 frequently composed of individuals of the three sampled locations.

1114

1115