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1 **The Role of the south-western Alps as a unidirectional corridor for**  
2 **Mediterranean brown trout (*Salmo trutta* complex) lineages**

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22 **Running title:** The south-western Alps: a corridor for *Salmo trutta*

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31        **Abstract**

32        The role of south-western Alps as a corridor for Mediterranean trout (*Salmo trutta* complex Linnaeus,  
33 1758) was evaluated in order to understand the influence of the last glacial events in shaping the spatial  
34 distribution of the genetic diversity of this salmonid. For this, the allochthonous hypothesis of a man-  
35 mediated French origin (19th century) of the Mediterranean trout inhabiting the Po tributaries in the Italian  
36 side of south-western Alps was tested. A total of 412 individuals were analyzed at the mitochondrial control  
37 region. The phylogenetic classification was carried out by using a Median-Joining Network analysis.  
38 Mismatch pair-wise analysis, molecular dating and Kernel density distribution analysis of the main  
39 mitochondrial lineages were evaluated to compare past demographic dynamics with the current spatial  
40 distribution of genetic diversity. The main outcomes resulted strongly in agreement with a biogeographic  
41 scenario where the south-western Alps acted as a unidirectional corridor that permitted the colonization of  
42 the upper Durance (Rhône River basin) by trout from the Po River basin. Therefore, the Mediterranean trout  
43 should be considered as native also along the Italian side of the south-western Alps and the allochthonous  
44 hypothesis should be rejected.

45        **Key words:** *Salmo trutta* complex, Alpine barrier, ice cover, conservation genetics, biological corridors

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## 61 INTRODUCTION

62 The study of the phylogeographic history of a species represents a fundamental step to understand the  
63 factors producing genetic diversity both within and among species, with particular reference to the role  
64 played by past environmental and climatic changes in shaping high levels of genetic complexity as well as  
65 their current spatial distribution (Purvis & Hector, 2000). The reconstruction of the recent evolutionary  
66 history of an organism is therefore essential to set-up concrete management and conservation actions aiming  
67 at protecting its evolutionary potential (Losos *et al.*, 2013). This is particularly true for those organisms  
68 impacted by socio-economic interests, where conservation induces conflict with commercial interests  
69 (Redpath *et al.*, 2013). In addition, the tentative to state the natural occurrence of an organism on the basis of  
70 phylogenetic studies could be made difficult by the long history of the human-mediated natural species range  
71 alterations, that may act as a confounding factor that adds further complexity in the study of the already  
72 complex evolutionary pathway of an organism (Fernández-García *et al.*, 2014; Sanz, 2018).

73 Although, the human ability in shaping plant and animal distribution is rooted in ancient culture and  
74 traditions (Larson & Fuller, 2014), nowadays, human-mediated transport beyond biogeographic barriers has  
75 led to the introduction and establishment of alien species (sometimes invasive) in new regions worldwide  
76 (Shackleton *et al.*, 2019; Berrebi *et al.*, 2020). About freshwater fish, the first written historical records date  
77 back the major human-mediated introductions at Roman time and successively at medieval period (Sønstebø,  
78 Borgstrøm, & Heun, 2007; Miró & Ventura, 2013). Basically, the above ancient freshwater fish  
79 translocations were promoted by food purposes, whereas, nowadays, freshwater fish represents one of the  
80 most important group of animals introduced for sport purposes worldwide (Leprieur *et al.*, 2008). In  
81 particular, brown trout (*Salmo trutta* complex) is listed as the 100 of the world's worst invasive alien species  
82 (Global Invasive Species Database [2019], downloaded from [http://193.206.192.138/gisd/100\\_worst.php](http://193.206.192.138/gisd/100_worst.php) on  
83 [13-03-2019](http://193.206.192.138/gisd/100_worst.php)). At the same time, its natural diversity is imperilled in much of its native range (Budy *et al.*,  
84 2013).

85 The Italian IUCN red list of vertebrates (Rondinini *et al.*, 2013) classified its conservation status as near  
86 threatened, and the French red list (IUCN, 2010) considers the trout status as low concern. It is probably  
87 wildly optimistic statements considering that along the Apennine chain less than 3% of native populations  
88 were free of genetic introgressive hybridization with Atlantic genes of domestic origin (Splendiani *et al.*,  
89 2016a). Further, in the Alpine area, its conservation value appears at least chaotic. The brown trout  
90 (regardless of its phylogenetic origin) is “on paper” considered as allochthonous in the Piemonte Region and  
91 native in the neighbouring Lombardia Region (based on Regional lists of freshwater fishes). In addition, the  
92 AIIAD (Italian Association of Freshwater Fish Ichthyologists) guideline for Italian salmonid management  
93 proposes to adopt a passive conservation approach for the putative native populations of south-western Alps  
94 (Zanetti, Nonnis Marzano & Lorenzoni 2013). Based on the opinions expressed in this document, the

95 Mediterranean trout populations recognizable in this region should be the consequence of historical human-  
96 mediated translocations from the Rhône River basin to the Po River basin. The reasons of these trout  
97 translocations would be related with the well-known passion for trout fishing of the Queen Elena of Italy  
98 (1873 – 1952) (e.g., Siccardi, 1996). Unfortunately, in the above AIIAD document, there is no citation of  
99 historical records to sustain this hypothesis. On the contrary, bibliographic records collected in the  
100 Geographic dictionary of the Sardinian State (Casalis, 1833, 1852), evidenced a widespread presence of  
101 brown trout in the occidental Alps long before Elena’s reign (1900-1946). Interesting, as far as it is known,  
102 there are no pieces of evidence of trout translocations from France to Italy, taking into account the above  
103 historical literature. On the other hand, historical translocation of freshwater fish across the Alps have been  
104 well documented, as the case of the historical (16<sup>th</sup> century) translocation of *Salvelinus alpinus* from Austria  
105 to Trentino Alto Adige Region (Italy) (Tiberti & Splendiani, 2019).

106 Here, a comprehensive molecular data set is used to describe wild Mediterranean brown trout population  
107 genetic diversity encompassing from the Rhône River basin to the Po River basin to try to reach several  
108 objectives. The first aim of the present study is to provide, for the first time, a focus on brown trout  
109 mitochondrial DNA (mtDNA) genetic diversity distribution along a putative contact zone between several  
110 lineages of this species complex. The second aim is to find solid hypotheses to correlate spatial distribution  
111 of genetic diversity with the main evolutionary forces that could have played a role in shaping the  
112 Mediterranean brown trout lineage distribution observed in the study area, that are: i) effects of the last  
113 glacial maximum (LGM, that are, ice cover extension, effects of ice flow pattern during deglaciation  
114 episodes, localization of glacial refuges, etc), and ii) the role of geomorphologic and hydrogeological  
115 characteristics of the mountain relief in the south-western Alps in shaping genetic diversity. Finally, the third  
116 aim is to test the hypothesis of non autochthony proposed by AIIAD and as a consequence provide  
117 recommendations to preserve, in a rational manner, what remains of the native genetic diversity of brown  
118 trout in south-western Alps watercourses.

## 119 MATERIAL AND METHODS

### 120 SAMPLING DESIGN

121 The *S. trutta* complex is characterized by a puzzling pattern of geographical forms probably underlying  
122 the taxonomic inflation reported in literature with the description of nearly 50 *Salmo* species (Tougaard *et al.*,  
123 2018). The description of the native trout taxa previously identified (e.g., Kottelat & Freyof, 2007) in the  
124 study area have been summarised in Table S1. In this study, for practical reasons, the following terms will be  
125 used hereafter: (1) “marble trout” for *S. marmoratus* specimens (generally fixed for MA haplotypes), (2)  
126 “native brown trout” for the individuals showing both a fario phenotype and native haplotypes of the  
127 Mediterranean area (that are, haplotypes belonging to the lineages AD, ME or MA), (3) “Lake Garda  
128 Carpione” to indicate *S. carpio* specimens and (4) “Atlantic brown trout” to indicate *S. trutta* specimens  
129 hosting domestic and non-native haplotypes (AT lineage). The sampling efforts were mainly focused along

130 the south-western Alps, as for example, i) in the upper Durance, that belongs to the Rhône basin, but is  
131 located adjacent to the Po River basin along the French side of the south-western Alps, and ii) along the main  
132 sub-basins of the Po River basin, as regards the Italian side of the south-western Alps. For comparisons,  
133 further samples from other sites of the Rhône River basin and from neighbour minor Mediterranean  
134 independent rivers were also included, for example, the rivers Var, Loup, Roya and Sansobbia. The sampling  
135 size ranged from 4 to 24 with a mean value of 10 trout per sample. The total number of analysed *S. trutta*  
136 was 412 specimens that were collected from 42 sites (Figure 1, Table 1).

## 137 DNA EXTRACTION

138 From each fish, a small fin clip was removed and conserved in 95% ethanol until DNA extraction. Due to  
139 the union of genetic data obtained from two different laboratories (*i.e.*, from the ISEM, Université de  
140 Montpellier and from the DiSVA, Università Politecnica delle Marche, hereafter, respectively, Lab.1 and  
141 Lab. 2), total genomic DNA was extracted using two methods. The first one (Lab. 1) consisted in a  
142 Chelex/proteinase K-based protocol described by Estoup, Largiader, Perrot & Chourrot (1996). A small  
143 piece of fin was incubated overnight at 56°C in 195 µl of 5% Chelex 100 Resin (Biorad) solution containing  
144 50 mM of Tris-HCL (pH 7) and 500 µg/ml of proteinase K. Samples were then incubated at 95°C for 10 min  
145 before centrifugation at 3500 g for 5 min. Supernatants were recovered and frozen at – 20°C until required  
146 for use. In the second method (Lab. 2), total genomic DNA was extracted using an automated DNA extractor  
147 (MagCore® Automated Nucleic acid Extractor in combination with the Genomic DNA Tissue Kit 401).

148 In Lab. 1, the mtDNA control region (CR) was amplified by PCR using the PST (5'-  
149 CCCAAAGCTAAAATTCTAAAT-3') and FST (5'-GCTTTAGTTAAGCTACGC-3') primers (Cortey &  
150 García-Marín, 2002). Each 50 µL reaction included 0.4 µM of each primer (Eurofins MWG Operon), dNTP  
151 (2 mM each), 2 mM of MgCl<sub>2</sub>, 10 µL of 5 9 PCR buffer, 1 U of Taq polymerase (GoTaq® Promega), and  
152 about 50 ng of genomic DNA. The PCR conditions included initial denaturation (95°C, 5 min), followed by  
153 30 cycles of strand denaturation (94° C, 1 min), primer annealing (52° C, 1 min), and DNA extension (72° C,  
154 1 min) cycles, and then by a final extension (72° C, 5 min). All PCR amplifications were performed in  
155 Eppendorf Mastercycler thermocyclers. The amplified DNA fragments were run on a 0.8% agarose gel to  
156 verify the amplification efficiency. The amplified products were purified and sequenced in both directions to  
157 confirm the polymorphic sites in an ABIPRISM 3130/xl/sequencer (Applied Biosystems). In Lab. 2, the  
158 mtDNA CR was PCR-amplified according to Bernatchez & Danzmann (1993) (primer sequences: LN20, 5'  
159 ACCACTAGCACCCAAAGCTA; HN20, 5' GTGTTATGCTTTAGTTAAAGC). Screening of mtDNA  
160 genetic variability was conducted through Single-Strand Conformation Polymorphism (SSCP) analysis.  
161 Because shorter fragments are better suited for detection of mutations in SSCP gels (Hayashi, 1991), the CR  
162 PCR products of *c.* 1000 bp were first digested with *AluI* restriction enzyme and then run on a non-  
163 denaturing polyacrylamide gel for 12-h at 5 W in a cool chamber. Finally, the non-digested segment of *c.*  
164 1000 bp was sequenced in a sub-sample of individuals with the same SSCP profile (that is, three-four trout  
165 per each SSCP morph detected).

167 The mtDNA CR sequences were aligned using Clustal W (Larkin *et al.*, 2007). In order to assign the  
168 sequence haplotypes observed in this study to each of the main brown trout mtDNA lineages, several  
169 reference *S. trutta* CR sequences were downloaded from GenBank (belonging to the mtDNA lineages ME,  
170 AD, MA and AT) (see Table S2 for more details). The genealogical relationship among haplotypes was  
171 depicted using a Median-Joining Network (Bandelt, Forster, & Rohl, 1994) constructed using Network 5  
172 (Fluxus Technology Ltd., www.fluxus-engineering.com), considering also gaps and missing nucleotides. The  
173  $\epsilon$  parameter was set to zero. Historical demography inferences were drawn from three neutrality tests  
174 implemented in DnaSP 6 (Rozas *et al.*, 2017): i) Fu's  $F_S$  (Fu, 1997), ii) Tajima's  $D$  (Tajima, 1989) and iii)  $R_2$   
175 (Ramos-Onsins & Rozas, 2002), and from mismatch distribution analysis by using Arlequin 3.5 (Excoffier &  
176 Lischer, 2010). Briefly, a significantly negative Tajima's  $D$  and  $F_S$ , and a significantly positive  $R_2$  indicate a  
177 scenario of demographic expansion. In the mismatch analysis, a curve displaying the observed distribution of  
178 pair-wise differences within each lineage is compared to an expected curve under a model of population  
179 growth-decline. Generally, a curve with a single peak associated with a low number of pair-wise differences  
180 indicates expansion, while a curve with two or multiple peaks indicates stability. Differences between  
181 observed and expected pair-wise mismatch distribution were evaluated using the sum of squared deviations  
182 (SSD) and the raggedness index ( $r$ ) as implemented in Arlequin 3.5.

183 The estimation of time to the most recent ancestor (TMRCA) of the AD, ME and MA lineages was  
184 carried out with a Bayesian coalescent analysis using BEAST 1.10.4 (Suchard *et al.*, 2018) under a HKI + I  
185 model as inferred by using jModeltest (Posada, 2008). We adopted two fixed values with a normal prior  
186 distribution (0.75 - 1%) of divergence rates (*e.g.*, Sanz, 2018 and references therein), and taking into account  
187 that we were interested in the estimation of the separation time between the Mediterranean lineages AD, ME  
188 and MA, we adopted the basic strict clock model, as implemented in BEAST, because, potentially  
189 outperforming for trees with shallow roots (Brown & Yang, 2011). The strict molecular clock was used in  
190 combination with three coalescent models (constant size, exponential growth, expansion growth). To  
191 determine the best fitting model of the data (Brandley, Schmitz, & Reeder, 2005), a modified Akaike  
192 Information Criterion (AICM) as provided in TRACER 1.6 (Rambaut *et al.*, 2018) was used. The models  
193 were run five times for fifty million generation with a 10% burn-in stage. Markov chain convergence was  
194 checked visually by the inspection of the traces, while the run stability was measured using the effective  
195 sample size ( $ESS > 200$  for all parameters) using Tracer. Results of the independent convergent runs were  
196 combined with LogCombiner v 1.10.4 (auxiliary program implemented in the BEAST package) to estimate  
197 TMRCA and 95% highest probability density intervals (HPD). A consensus tree was then generated using  
198 TreeAnnotator 1.10.4 (auxiliary program implemented in the BEAST package) with the following options:  
199 maximum clade credibility and mean node heights.

200 Hierarchical analysis of molecular variance (AMOVA) was used to test how the effects of the last glacial  
201 events, occurred in the study area, could explain the current spatial genetic distribution. Groupings included:

202 i) Rhône samples vs Po samples and ii) Rhône samples vs Po and Durance samples. This latter grouping was  
203 set-up to test the hypothesis of a recent post-glacial origin of brown trout samples from upper Durance  
204 related to slope failure phenomena due to last deglaciation events. The above tests were carried out by using  
205 Arlequin 3.5, using conventional F-statistics and testing the statistical significance of the tests with 5,000  
206 permutations.

207 To depict the biogeographic scenarios underlying the observed haplotype spatial distribution, the mtDNA  
208 lineage distribution observed in each population was both analysed by mapping pie charts geographically  
209 and using Kernel density (KD) analysis along an elevation, longitudinal and latitudinal gradient. The KD  
210 analysis was conducted partitioning the samples as i) samples from the Rhône River basin and ii) samples  
211 from the Po River basin. For these tests, brown trout samples were grouped in the following categories: i)  
212 samples fixed for AD haplotypes, ii) samples fixed for ME haplotypes, iii) samples fixed for MA haplotypes  
213 and mixed samples, iv) samples sharing AD and ME haplotypes, v) AD-MA, vi) ME-MA and vi) AD-ME-  
214 MA. The rationale of the above partitioning was to verify if haplotype distribution can match with plausible  
215 scenarios of extinction and recolonization events connected with the environmental changes occurred during  
216 the last glacial maximum in the south-western Alps. The KD analysis was carried out by using the density  
217 function in R software (R Development Core Team, 2017).

## 218 RESULTS

### 219 HAPLOTYPE CLASSIFICATION

220 Before starting with sequence analyses, the PolyT region of the CR was considered of a constant length of  
221 14bp in all sequences. In fact, such region is likely to be unstable and thus characterized by a high mutation  
222 rate, showing frequent 14-T variants. In this circumstance, sequence stretch length identity, could be the  
223 mere consequence of homoplasy and would not represent a real phylogenetic signal. On the whole, an  
224 alignment of 981 bp was obtained, from which 21 haplotypes emerged (Table 2). The observed haplotypes  
225 belonged to four main mtDNA lineages: AD, ME, MA and AT (*sensu* Bernatchez *et al.*, 1992 or Bernatchez,  
226 2001). The AD lineage was represented by three haplotypes: ADporh-1 and ADrh-1, observed for the first  
227 time in this study (GenBank accession numbers, respectively, MK948034 and MK948035) and ADcs-1  
228 already described in literature (Cortey, Pla & García-Marín, 2004). As far for the ME lineage, seven  
229 haplotypes were observed, two of which already detected in other studies, that are: MEcs-1 and MEcs-15  
230 (*e.g.*, Cortey *et al.*, 2004) and the other five were detected for the first time in this study and named as  
231 follow: MEcs-28 to MEcs-32 (from MK948029 to MK948033). The MA lineage displayed a new haplotype  
232 named MAsl-1 (MK948036), and three mtDNA variants already detected in previous studies, namely Ma2a,  
233 Ma2b and Ma2c (Meraner, Baric, Pelster, & Dalla Via, 2007; Meraner, Gratton, Baraldi & Gandolfi, 2013).  
234 Finally, the Atlantic lineage was represented by six haplotypes, five of them were already detected in  
235 Mediterranean rivers and classified as haplotypes of hatchery origin, that are: haplotype 1 to haplotype 4  
236 (Cortey & García-Marín, 2002) and the haplotype At1e, (Meraner *et al.*, 2007), while one haplotype was



237 described for the first time in this study (haplotype 3b, MK948037). This latter haplotype should be also  
238 considered of hatchery origin due to high similarity with the domestic variant haplotype 3 (Table 2, Figure  
239 2).

#### 240 HAPLOTYPE SPATIAL DISTRIBUTION

241 The ME lineage dominated in the Rhône River drainage and in the other minor French rivers included in  
242 this study. The most common ME haplotype was MEcs-1, the rest of the ME haplotypes (MEcs-28 – 32) was  
243 endemic of French Mediterranean rivers. On the other hand, within the Po River drainage and in the  
244 Sansobbia River, the ME lineage was represented by the haplotype MEcs-1.

245 The AD lineage was quite common in the Po River drainage, however, the ancestral haplotype of this  
246 lineage (ADcs-1, see Figure 2) was observed only in a sole trout (in sample GIU, Po River basin). However,  
247 in other studies within the Po River drainage, the haplotype ADcs-1 was detected in both 19<sup>th</sup> century trout  
248 (Splendiani *et al* 2017), as well as in recent samples (Gratton *et al.*, 2014; Stefani, Anzani, & Marieni, 2019).  
249 Toward the west part of the Po River drainage, the haplotype ADporh-1 was newly detected (one Mutational  
250 Step from ADcs-1). This latter haplotype presented a spatial distribution confined to the upper reaches of  
251 both upper Durance River (Rhône basin) and Po River along a transect of the south-western Alps extended  
252 from the Cottian to the Maritime Alps (Figure 1 and Table 2, see also next paragraph). Finally, the haplotype  
253 ADrh-1 consistently with its position in the Median-Joining Network (one MS from the haplotype ADporh-  
254 1) was found only in the western part of the Rhône River drainage, in the Petit Buëch stream.

255 The MA haplotypes were found only within the Po River drainages. The most common haplotype  
256 detected for this lineage was Ma2b. This mtDNA variant occupied a central position within the MA lineage  
257 (Figure 2). In line with its haplotype network position, this haplotype was observed elsewhere within the Po  
258 River drainages in both modern (Meraner *et al.*, 2007, 2013) and museum specimens (from 19<sup>th</sup> century, *e.g.*,  
259 Splendiani *et al.*, 2017).

260 Finally, as expected, the AT haplotypes formed a separate cluster in the Median-Joining Network (Figure  
261 2). These non-native haplotypes for the Mediterranean area showed an evident greater abundance within the  
262 Italian samples (Table 2, Figure 1). Here, a mean value of AT haplotypes of 38% and a maximum value of  
263 70% (in the locality MAIb) were observed. In four localities (TRO, RIP, FER and GES), the AT haplotypes  
264 were not observed. In the French samples, the AT haplotypes were observed only in two localities out of 21,  
265 GLU (60%) and ROY (20%).

#### 266 MISMATCH ANALYSIS AND DIVERGENCE TIME ESTIMATES

267 Mismatch distribution analysis indicated (see Table S3 and Figure S1) a scenario consistent with a model  
268 of demographic expansion (Excoffier, 2004) for the brown trout mtDNA lineages ME and AD and a stable  
269 demographic trend for the lineage MA.

270 The AICM suggested that a strict clock under a constant size coalescent model best-fits our data. The  
271 TMRCA estimations placed the origin of the AD lineage from 278,000 (95% HPD 170,000 – 391,000) to  
272 212,000 (95% HPD 129,000 – 298,000) years ago by adopting, respectively, a substitution rate of 0.75 and  
273 1%, the origin of the ME lineage from 267,000 (95% HPD 166,000-372,000) to 191,000 (95% HPD  
274 122,000-265,000) years ago, and the origin of the MA lineage from 122,000 (95% HPD 172,000-205,000) to  
275 117,000 (95% HPD 51,000-193,000) years ago (see Table S4). MA lineage appears so as the youngest one.  
276 Finally, the origin of the AD branch composed by the haplotypes ADporh-1 and ADrh-1 was placed around  
277 151,000 (95% HPD 11,000-99,000) and 120,000 (95% HPD 16,000-86,000) years.

## 278 AMOVA

279 With both two grouping options (Rhône samples vs Po Samples and Rhône samples vs Durance and Po  
280 samples), the AMOVA analyses showed that most of the genetic variation was explained at the within  
281 population level (53.26 and 51.62%, respectively) and among populations within group level (33.84 and  
282 26.84%, respectively). However, the AMOVA analyses showed also that grouping the Durance samples  
283 within the Po River group explained much more genetic variation (21.54 %) than grouping the Durance  
284 within its main river basin (*i.e.*, the Rhône River basin) (14.92 %). In both cases, the statistical significance  
285 of the source of variation represented among groups was highly significant ( $P = 0.0000$ ).

## 286 KERNEL DENSITY (KD) BROWN TROUT LINEAGE DISTRIBUTION

287 Within the Rhône River drainage, the highest density of samples characterized by the sole presence of  
288 ME haplotypes was found between 4.5 – 5.5 E longitudes (Figure 3A). Unfortunately, only one sample was  
289 characterized by the sole presence of AD haplotypes (BUË), therefore, KD analysis was not applicable in  
290 this case. On the contrary, the rest of the Rhône samples (*i.e.*, samples from the Durance sub-basin) were  
291 composed by a mixture of AD and ME haplotypes. In this study, a total of 91 trouts originated from the  
292 Rhône River basin in France, among them, 31 specimens showed the AD haplotypes. All these latter trouts  
293 came from the Durance sub-basin (samples BUË, BIA, CLA, UBA and GUI, see Table 2). The last two  
294 rivers (UBA and GUI) are flowing in France directly from the France-Italy boundary. This kind of  
295 populations peaked around 7.0 E longitude, corresponding with the upper part of the Durance River (Figure  
296 3A). When KD was carried out to relate brown trout lineages distribution with elevation, a similar net  
297 separation between different categories of samples was observed. For example, in the Rhône River,  
298 populations fixed for ME haplotypes were most abundant around 0 – 500 m (Figure 3B), whereas admixed  
299 populations (AD – ME) showed higher values of probability between 1500 – 2000 m. Within the Po River  
300 drainage, the different categories of populations defined based on mtDNA lineage composition, appeared  
301 clearly stratified along an altitudinal cline. Pure ME populations were detected only in one case (BAR,  
302 Tanaro River, Ligurian Apennine). Admixed AD – ME populations were, however, the most common and  
303 reached a density peak around 1600 – 2000 m. Pure AD populations peaked slightly lower, around 1400 m  
304 (Figure 3C). Around this latter quote, peaked both pure brown trout samples fixed for MA haplotypes and

305 admixed MA – AD brown trout samples. Further downstream (c. 450 m), pure marble trout samples (MA)  
306 were abundant (data from Giuffra *et al.*, 1994). Finally, a pattern of brown trout mtDNA lineage density  
307 distribution along the south-western Alps was also evident along a latitudinal gradient (Figure 3D). For  
308 example, pure AD samples appeared most abundant around 44.0 – 44.5 N, roughly corresponding with the  
309 Maritime Alps (Italian side), while admixed populations (AD – ME) peaked around 45.0 N (*i.e.*, Cottian  
310 Alps).

## 311 DISCUSSION

312 In the following chapters of this study, a comprehensive phylogeographic analysis of brown trout  
313 populations inhabiting adjacent tributaries of the Rhône and Po River basins is proposed for the first time.  
314 Based on the biogeographic scenario reconstructed, the allochthonous hypothesis proposed by the Italian  
315 Association of Freshwater Fish Ichthyologists (Zanetti *et al.*, 2013) about Mediterranean brown trout  
316 populations inhabiting the south-western Po tributaries was evaluated. Substantially, the shared haplotype  
317 diversity (AD and ME haplotypes) observed along the two sides of the south-western Alps and, at the same  
318 time, the presence of an important ice cover along the western part of the Rhône basin (*i.e.*, the Durance  
319 glacier) during the glacial phases, incompatible with the presence of freshwater fish, suggest that native  
320 brown trout survived the adverse phases of the upper Pleistocene just in the tributaries of the south-western  
321 Po basin. Probably, the erosional events related with the deglaciation phenomena permitted the opening of a  
322 biological corridor for brown trout from the Po basin toward the Rhône basin.

## 323 THE ORIGIN OF THE *S. TRUTTA* ME LINEAGE IN THE STUDY AREA

324 According to previous studies (Cortey *et al.*, 2004; Vera *et al.*, 2019), the Iberian Peninsula would  
325 represent the ideal candidate as the centre of the origin of the ME lineage (*e.g.*, Sanz, 2018). However, the  
326 ME haplotype diversity detected in the present study partially contrast with the above hypothesis. Within the  
327 Rhône River basin and neighbour rivers, five new ME haplotypes were detected. This fact appears in  
328 accordance with a western Mediterranean origin of the ME lineage as previously proposed (*e.g.*, Bernatchez,  
329 2001; Cortey *et al.*, 2004; Oliver, 2014) but also suggests that the Rhône basin area acted as an important  
330 evolutionary centre for ME genetic diversity.

331 Although, the use of the sole mitochondrial control region could be taken into account with caution for  
332 inferring isolation time accurately (Schenekar, Lerceteau-Köhler, & Weiss, 2014), an attempt was, however,  
333 tried in this study. In this sense, it is interesting to indicate that the pre-defined divergence rates of 0.75 - 1%  
334 adopted in this study designed a time since expansion of the major Mediterranean lineages (ME, AD and  
335 MA) in accordance with their altitudinal distribution observed in the study area (see below).

336 The TMRCA analysis suggest a main expansion of the ME lineage around 191,000-267,000 years ago,  
337 roughly corresponding with the last (III) Mindel-Riss Interglacial, and resulting slightly more ancient respect  
338 previous estimations (Cortey *et al.*, 2004). Pleistocene Interglacial warming periods have been regarded as

339 phases of isolation in small headwaters for Mediterranean brown trout populations, thus promoting genetic  
340 signatures within lineages (*e.g.*, Sanz, 2018 and references therein). On the other hand, during glaciations,  
341 colder climate conditions may have triggered sea-ward migratory tactics in Mediterranean brown trout  
342 populations, as highlighted by several paleontological evidences (Muñoz & Casadevall, 1997; Splendiani *et*  
343 *al.*, 2016b; Splendiani *et al.*, 2020). Therefore, thanks to a seaward migratory route, the expansion of brown  
344 trout in the Mediterranean area was possible. The colonisation of northern Corsica Island by the ME lineage  
345 during the last glaciation is an example of this expansion (Gauthier & Berrebi, 2007). The spatial distribution  
346 of the MEcs-1 haplotype suggests a potential eastward dispersion, from the Rhône River outlet to the Var  
347 River (SAL, Gulf of Lion) where this haplotype was found and more eastern to the Sansobbia River (SAN,  
348 Ligurian Sea) (Figure 1, Table 2). Then, when the ME lineage reached the upper part of the Ligurian rivers,  
349 the colonization of the Po River basin was likely possible thanks to river capture events occurred along the  
350 Ligurian Apennine chain. This scenario could explain the finding of the MEcs-1 haplotype in the Rio  
351 Baracca (BAR, Po River basin, see also Figure 1, Table 2). Interestingly, the role played by the  
352 hydrographical captures of the upstream portions of Mediterranean rivers was also proposed in the literature  
353 to explain, for example, the exchange of Duero haplotypes between rivers flowing along opposite slope of  
354 the Cantabrian mountains (Iberian Peninsula) (Vera *et al.*, 2015), as well as to explain the native occurrence  
355 of the Danubian haplotypes within marble trout populations of the Sôca River (Berrebi, Jesenšek, & Crivelli,  
356 2017). Concerning the study area, the phylogeographic history of the Italian vairone (*Telestes muticellus*) in  
357 populations of west Liguria (Marchetto *et al.*, 2010) could also be explained invoking the presence of  
358 biological corridors opened by ancient Mediterranean river captures.

359 Once reached the Po River basin, it can be reasonably expected that the ME lineage tried to colonize  
360 available salmonid habitats. However, when the climate conditions went colder and the Alpine ice cover  
361 expanded, this lineage survived only in refuge areas as, for example, in south-western Alps. Milder  
362 conditions in this part of the Alpine chain and the absence of other brown trout lineages may have permitted  
363 the colonization of the upper reaches of the western Alps by the MEcs-1 haplotype (see the Kernel density  
364 analysis in the Results section and Figure 3).

#### 365 THE ORIGIN OF THE *S. TRUTTA* AD LINEAGE IN THE STUDY AREA

366 The Adriatic-Balkan part of the Mediterranean basin is considered the centre of the origin of the AD  
367 lineage (Sanz, 2018). The main expansion of the AD lineage seems to take place around 267,000 – 212,000  
368 year ago and therefore nearly simultaneous with the last expansion proposed above for the ME lineage.  
369 Although, as already stressed, divergence estimations should be interpreted with caution, mainly with regard  
370 to the absolute date of expansion values, more reliable, on the contrary, appears the simultaneous time of  
371 expansion of the AD and ME lineages observed in this study. The simultaneous expansion of these two  
372 lineages fits well with both their similar peri-Mediterranean spatial distribution and with their phylogenetic  
373 complexity (*e.g.*, Sanz, 2018).

374 In north Italy, the central haplotype of the AD lineage (ADcs-1) was observed in two museum specimens  
375 (dating back to the end of 19<sup>th</sup> century) of Lakes Garda and Maggiore and in a modern sample of the Adige  
376 Adriatic River (Meraner *et al.*, 2013). According to Splendiani *et al.* (2016a, 2017), the spatial distribution of  
377 the Mediterranean trout genetic diversity in north Italy represents a sort of “map” of the potential Alpine  
378 peripheral refuges where brown trout survived during the extreme glacial phases. In addition, the role played  
379 by the area of the Lake Garda as an important glacial refuge for the genus *Salmo* is also evidenced by the  
380 detection of two endemic AD and MA haplotypes in Lake Garda Carpione, an endemic trout of this major  
381 Italian lake (Gratton *et al.*, 2014) (Figure 2). Further, the finding of a new AD haplotypes (ADporh-1),  
382 endemic of the south-western Alps, suggests that also this area could have acted as both an important glacial  
383 and interglacial refuge for brown trout. In the south-western Alps' part of the Po River basin, the haplotype  
384 ADporh-1 was the sole AD haplotype observed. This haplotype was found as fixed in samples collected  
385 around 1000 – 1500 m (Figure 3), that is slightly lower than the quote where AD-ME mixed populations  
386 peaked. Thus, the observed spatial distribution suggests that the ME lineage colonized first the headwaters of  
387 the south-western Po River basin, whereas the AD lineage tried to do the same later. Based on molecular  
388 dating analyses, both ME and AD lineages showed a similar divergence time and thus it is hard to explain  
389 their different altitudinal distribution. However, the proximity of the south-western Alps to an important  
390 centre of origin of the lineage ME, as can be considered the Rhône River basin, could explain why the ME  
391 lineage reached the highest sites of the south-western part of the Po River basin first.

392 The peculiar AD haplotypes detected across the south-western Alps (*i.e.*, ADporh-1 and ADrh-1  
393 haplotypes) probably split from the ADcs-1 ancestor when warmer climate condition promoted phases of  
394 isolation. The estimated origin for this AD branch of *c.* 151,000 – 120,000 years ago, corresponding  
395 approximatively with the Riss-Würm Interglacial. During the warmer phases, brown trout population may  
396 have survived in high altitude habitats of the south-western part of the Po River basin. This region would  
397 also have been used as refuge during the colder phases (*i.e.*, the Younger Dryas stadial, *c.* 12,800 and 11,600  
398 years BP) when the rest of the high-altitude Alpine streams was covered by a massive ice cover. Later, at the  
399 beginning of the Holocene, the extreme erosional events produced by massive episodes of deglaciation  
400 promoted the colonization of the upper Durance basin from the adjacent high altitude brown trout  
401 populations survived in the south-western Po streams by river captures (see the next paragraph for more  
402 information: The role of the south-western Alps as an asymmetrical biological corridor for brown trout  
403 lineages).

404 The Italian side of the Maritime Alps represented also an isolated refuge. Interestingly, in this region,  
405 both the AD-Tyrrh-1, very common elsewhere within the Tyrrhenian watercourses (*e.g.*, Berrebi *et al.*, 2019)  
406 and ADcs-11, very common around the Adriatic Sea rivers (Sušnik *et al.*, 2007; Snoj *et al.*, 2009, 2010),  
407 haplotypes were not found. Based on the network haplotype topology (Figure 2), we can hypothesized that  
408 ADcs-1 colonized first the Po River, and then, during phases of geographic isolation within refugia, new  
409 haplotypes, as ADporh-1 within the Maritime Alps refuge and the *S. carpio* AD haplotypes (ScarAD-1 and

410 ScarAD-2), within the Lake Garda refuge, could split. According to Sanz (2018), the AD lineage was  
411 characterized by multiple waves of expansions. Successive expansion opportunities were probably used by  
412 individuals carrying haplotypes ADcs-11 (around the Adriatic Sea) and AD-Tyrrh-1 around the Tyrrhenian  
413 Sea, these latter ones, however, were unable to colonize the Po River as it was already occupied by both  
414 brown trout (showing the ADcs-1, ADporh-1 and MEcs-1 haplotypes) and marble trout (showing the Ma2a,  
415 Ma2b, Ma2c and MAsl-1 haplotypes).

416

#### 417 THE ORIGIN OF THE *S. TRUTTA* MA LINEAGE OBSERVED IN THE STUDY AREA

418 Within the Po River basin, the MA lineage showed an evolutionary pathway like that observed in the case  
419 of the AD lineage. For example, in the Lake Garda Carpione, Gratton *et al.* (2014) exhibited two endemic  
420 MA haplotypes (named here ScarMA1 and ScarMA2, see also Figure 2). Further west, in brown trout  
421 samples, a new MA haplotype (MAsl-1) was found, although in a sole specimen (Table 2). In this study, the  
422 most diffuse MA haplotype detected in the south-western part of the Po River was Ma2b, also common  
423 elsewhere in the Po River basin both in native brown trout and Lake Garda Carpione specimens (*e.g.*,  
424 Meraner *et al.*, 2007; 2013). However, the most relevant result was represented by the spatial distribution of  
425 the MA lineage as detailed below. In this study, the native brown trout samples from the south-western  
426 tributaries of the Po River basin, characterized by the sole presence of MA haplotypes, showed an altitudinal  
427 range of distribution intermediate between marble trout (*c.* 450 m) and native brown trout populations  
428 characterized by a mix of AD and ME haplotypes (*c.* 1600 – 2000 m). Therefore, based on the altitudinal  
429 distribution of the MA lineage it could be hypothesized that this lineage tried to reach the upstream thermal  
430 refuges for last. This hypothesis seems to accord well with the younger origin of MA lineage respect to ME  
431 and AD lineages emerged in this study and proposed also by Oliver (2014). In addition, the altitudinal  
432 distribution of the MA lineage appear congruent with the stable or declining demographic scenario emerged  
433 by mismatch analysis. The reduced habitat availability could have contrasted the demographic expansion of  
434 this lineage (*e.g.*, Lavery *et al.*, 1996; Bernatchez, 2001). Probably, this mtDNA lineage was fixed in marble  
435 trout that inhabited the lower part of the Po River basin, then, when climate become warmer, this salmonid  
436 tried to reach colder habitats at higher elevations. A similar palaeohistorical scenario was previously  
437 proposed (Berrebi *et al.*, 2000) to explain the spatial distribution of both brown trout and marble trout within  
438 the Sôca River basin (Slovenia). The detection, in the present study, of only native brown trout phenotypes in  
439 samples characterized by the sole presence of MA haplotypes suggests that the contact between brown trout  
440 and marble trout occurred within an ecological contact zone (*i.e.*, ecotonal zone) where the parental  
441 Mediterranean phenotype outcompeted (*e.g.*, Arnold, 1997). Alternatively, the evolution of habitat  
442 preference of the MA lineage for lower river sections could explain its altitudinal distribution observed in the  
443 south-western Alps. However, elsewhere, as for example in the Adige River basin, the MA lineage was able  
444 to reach, accessible and formerly glaciated, high altitude sites (around 1000 – 1600 m) (Meraner *et al.*, 2007,  
445 2010; Splendiani *et al.*, 2016a).

446 THE ROLE OF THE SOUTH-WESTERN ALPS AS AN ASYMMETRICAL BIOLOGICAL  
447 CORRIDOR FOR BROWN TROUT LINEAGES

448 The comparison between the brown trout mtDNA genetic diversity observed along the two sides (east and  
449 west) of the south-western Alps highlights the lack of a substantial genetic differentiation between the  
450 samples collected in the upper Durance River (Rhône River basin) and the upper reaches of the Po River.  
451 Most samples from upper Durance and Po basins were composed by a mixture of the ADporh-1 and MEcs-1  
452 haplotypes (Table 2). The similarity in haplotype composition between the native brown trout populations of  
453 the two-opposite sides of the south-western Alps was also supported by the hierarchical analysis of  
454 molecular variance (AMOVA). In fact, when the samples of the upper Durance were grouped together with  
455 the Po River group, the level of genetic variation explained between groups of populations increased from 14  
456 to 21%, suggesting that the vicariant events between upper Durance and Po brown trout populations occurred  
457 recently. In this regard, it is important to note that all the Durance collection sites extended in elevation from  
458 1117 to 2077 m, that is, an altitudinal range occupied by the ice cover during the last glacial maximum (*e.g.*,  
459 Figure 1), which implies the arrival of trouts after this period. Interestingly, the private ADrh-1 haplotype, a  
460 mtDNA variant distant of one MS only from the haplotype ADporh-1, was detected in the Petit Buëch  
461 stream, sited at the margin of the Durance glacier. Here, the milder climate condition of this part of Durance  
462 basin could have allowed the maintenance of trout populations and represented a refuge for the haplotype  
463 ADporh-1 and the centre of origin for the haplotype ADrh-1.

464 A possible explanation for the high genetic affinity observed between brown trout samples from the upper  
465 Durance and Po River basins could be deduced when taking into account the effects, in terms of fish  
466 exchange along the Alpine barrier, provided by the last deglaciation events occurred in the south-western  
467 Alps. Likely, the first important factor that has drawn the present geographic structure of trout populations is  
468 the spatial distribution of the ice cover during the LGM. During this period, the Durance paleo-glacier was  
469 one of the most important Alpine glacier (Cossart, Braucher, Fort, Bourlès, & Carcaillet, 2008) (Figure 1).  
470 On the other hand, along the Italian side of the south-western Alps, the ice cover appeared less extended  
471 (Hughes, Woodward, & Gibbard, 2006; Szövényi *et al.*, 2009) (Figure 1). An explanation for the formation  
472 of an unidirectional corridor between the two side of the south-western Alps could be related with the  
473 formation of small ephemeral lakes and/or the swelling of connecting streams at the retreating edge of a  
474 glacier that may allow watershed crossing and drainage switching by freshwater fish (Waters *et al.*, 2001).  
475 This scenario was proposed to explain the colonization of the Lake Geneva (Rhône River basin) by bullhead  
476 *Cottus gobio* migrants from the Rhine River basin during the last glacial retreat (Vonlanthen *et al.*, 2007).  
477 Further, also the spatial distribution of the genetic diversity of *Galaxias platei* in Patagonia along the Andes  
478 also represents a similar example showing the role of glacial retreat events in promoting fish migrations  
479 across watersheds (Zemlak *et al.*, 2008; Habit *et al.*, 2010). Therefore, a scenario can be suggested where,  
480 first, during the colder phases, brown trout survived in the ice-free tributaries of the south-western Alps (*i.e.*,  
481 Maritime and Cottian Alps), and second, during the erosional events related to the ice melting (early

482 Holocene), an unidirectional corridor opened and permitted the colonization of the empty habitats of the  
483 adjacent upper Durance river catchment from the Po watershed. Interestingly, along the south-western Alps,  
484 the haplotype MEcs-1 was recently detected (Splendiani *et al.*, 2017) in a museum specimen collected in  
485 1876 in the Lake Mont Cenis (1974 m a.s.l.) (Figure 1), a former small Alpine Lake (since 1921 the lake was  
486 artificialized by the construction of a weir) of post-glacial origin belonging to the Dora Riparia River and  
487 located near the divide between the Po and Rhone catchments.

488 Finally, the above scenario could be also proposed to explain the spatial pattern of genetic diversity that  
489 has been observed in other freshwater organisms inhabiting the two sides of the south-western Alps. For  
490 example, as in the case of the high genetic similarity observed between adjacent populations of *Cottus gobio*  
491 (Šlechtová *et al.*, 2004), or similarly, the lack of genetic differentiation observed between adjacent  
492 populations of *Austrapotamobius pallipes* (Stefani *et al.*, 2011).

#### 493 TAXONOMIC IMPLICATIONS

494 As suggested above, in material and methods section, the main aim of the present study was not related  
495 with the attempt to solve the well-known problem of the *S. trutta* complex systematic (Splendiani *et al.*,  
496 2019b). However, the phylogeographic scenario of Mediterranean brown trout that emerged here represents  
497 an opportunity to partially face with the above taxonomic issues. The trout from the two sides of the south-  
498 western Alps are traditionally classified into three-four nominal species that we have used here for practical  
499 reasons: *S. rhodanensis* (Rhône River basin) a contested species (Berrebi *et al.*, in prep.), *S. cettii* (a non-  
500 valid name when used to indicate trout from the Tyrrhenian and Ligurian Sea draining rivers) and *S.*  
501 *farioides* or *S. cenerinus* (depending on the authors, Adriatic draining rivers) (Figure 1). At mtDNA level,  
502 none of the above nominal species showed a genetic distinctiveness able to justify the recognition of  
503 different species. For example, the two Ligurian samples (SAN, putative *S. cettii* and BAR, putative *S.*  
504 *farioides* - *S. cenerinus*) collected from the two sides of the Apennine chain, were both fixed for the  
505 haplotype MEcs-1, that is a haplotype quite widespread in the study area, as well in the rest of the  
506 Mediterranean rivers. More north, along the contact zone of the Rhône – Po River basins, the samples of the  
507 Durance River (putative *S. rhodanensis*) showed a haplotype composition more similar to that observed  
508 along the Italian side (putative *S. farioides* - *S. cenerinus*), respect to that shown by rest of the Rhône  
509 samples as highlighted by the AMOVA analyses. Obviously, more sound conclusions should be drawn by  
510 analysing also nuclear and morphological markers (see Ninua, Tarkhnishvili, & Gvazava, 2018 for similar  
511 arumentations). These preliminary results however refute the traditional taxonomic position adopted until  
512 now for the Mediterranean trout of the study area.

#### 513 CONCLUSIONS

514 The main findings of this study highlight that brown trout should be considered native in the south-  
515 western tributaries of the Po River basin. In this area, native brown trout survived the extreme climate phases  
516 of Pleistocene. In this respect, the biological value of the south-western Alps for the conservation of the last  
15



517 wild native Mediterranean trout population should be considered of primary importance. As a consequence,  
518 the non-native statement and the non-intervention approach proposed by the Italian Association of  
519 Freshwater Fish Ichthyologists (Zanetti *et al.*, 2013), based on a human conjectural man-mediated origin of  
520 Italian slope trout from the Rhône River basin should be rejected. In addition, the weakness of the  
521 allochthonous hypothesis is also sustained by the lack of historical records describing the occurrence of such  
522 practices in the study area (*e.g.*, Splendiani *et al.*, 2019a).

523 In conclusion, caution must be exercised when planning conservation actions. For example, elsewhere, in  
524 the Italian Alpine region, the massive introduction of domestic Mediterranean brown trout of Apennine  
525 origin (AD, ME and MA haplotypes) started in the last five-ten years. In most cases, the outcomes of pivotal  
526 genetic screening on local brown trout populations involved in these projects were not published, or even  
527 never done. This probably occurred (and still occurs) in Italy because local administrations have transferred  
528 to sport fishing associations the fully management of these practices. In these circumstances, the rationale of  
529 these putative conservation actions has not been evaluable by the scientific community. Paradoxically,  
530 conservation plans can even represent a further threat for the protection of wild native trout. As far as we  
531 know, Mediterranean trout hatchery managers in Italy have not published (even considering grey  
532 bibliography) the genetic description of their stocks. Recently, the genetic analysis of one of these putative  
533 domestic Mediterranean stocks actually turned out as a mix of Mediterranean and Atlantic brown trout  
534 (Splendiani *et al.*, 2019b). The irrational planning of massive stocking activities, even if carried out with  
535 native brown trout, can introduce further risks related to the potential deleterious genetic effects of  
536 supplementation programs (Fernández-Cebrián *et al.*, 2014). These latter ones can result in the breakdown of  
537 the delicate equilibrium persisting in the incipient parapatric speciation process subsisting between native  
538 brown trout and marble trout, as for example in south-western Alps (Giuffra *et al.*, 1994), and can affect the  
539 adaptive genetic architecture of native genomes (Caputo, Giovannotti, Nisi Cerioni, Splendiani, & Olmo,  
540 2009; Schenekar & Weiss, 2017). The situation is far better in the French side of the investigated area. In  
541 France, more and more administrative organizations, under the supervision of Ecology Ministry, adopted  
542 "patrimonial" management during the last twenty years. For this, a large part of the trout populations have  
543 been analysed and published in France (<https://data.oreme.org/trout/home>) with nuclear and mitochondrial  
544 markers driving conservation and stocking.

#### 545 REFERENCES

- 546 **Arnold ML. 1997.** *Natural Hybridization and Evolution*. Oxford University Press, Oxford, UK
- 547 **Bandelt HJ, Forster P & Rohl A. 1994.** Median-Joining Networks for Inferring Intraspecific Phylogenies.  
548 *Molecular Biology* **16**: 37–48.
- 549 **Bernatchez L. 2001.** The evolutionary history of brown trout (*Salmo trutta* L.) inferred from  
550 phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution;*  
551 *international journal of organic evolution* **55**: 351–79.
- 552 **Bernatchez L, Guyomard R & Bonhomme F. 1992.** DNA sequence variation of the mitochondrial control  
553 region among geographically and morphologically remote European brown trout *Salmo trutta* populations.  
554 *Molecular Ecology* **1**: 161–173.
- 555 **Bernatchez L & Danzmann G. 1993.** Congruence in control-region sequence and restriction-site variation

556 in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Molecular Biology and Evolution* **10**,  
557 1002-1014.

558 **Berrebi P, Povz M, Jesensek D, Cattaneo-Berrebi G & Crivelli AJ. 2000.** The genetic diversity of native,  
559 stocked and hybrid populations of marble trout in the Soca river, Slovenia. *Heredity* **85**: 277–287.

560 **Berrebi P, Caputo Barucchi V, Splendiani A, Muracciole S, Sabatini A, Palmas F, Tougard C, Arculeo**  
561 **M & Marić S. 2019.** Brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as  
562 revealed by nuclear and mitochondrial markers. *Hydrobiologia* **826**:209–231.

563 **Berrebi P, Marić S, Snoj A & Hasegawa K. 2020.** Brown trout in Japan -introduction history, distribution  
564 and genetic structure. *Knowledge and Management of Aquatic Ecosystems* **421**, 18.

565 **Berrebi P, Jesenšek D & Crivelli AJ. 2017.** Natural and domestic introgressions in the marble trout  
566 population of Soča River (Slovenia). *Hydrobiologia* **785**: 277–291.

567 **Brandley MC, Schmitz A & Reeder TW. 2005.** Partitioned Bayesian analyses, partition choice, and the  
568 phylogenetic relationships of scincid lizards. *Systematic Biology* **54**: 373–390.

569 **Brown RP & Yang Z. 2011.** Rate variation and estimation of divergence times using strict and relaxed  
570 clocks. *BMC Evolutionary Biology* **11**.

571 **Budy P, Thiede GP, Lobón-Cerviá J, Fernandez GG, Mchugh P, Mcintosh A, Voøllestad LA, Becares**  
572 **E & Jellyman P. 2013.** Limitation and facilitation of one of the world’s most invasive fish: An  
573 intercontinental comparison. *Ecology* **94**: 356–367.

574 **Caputo V, Giovannotti M, Nisi Cerioni P, Splendiani A & Olmo E. 2009.** Chromosomal study of native  
575 and hatchery trouts from Italy (*Salmo trutta* complex, Salmonidae): Conventional and FISH analysis.  
576 *Cytogenetic and Genome Research* **124**.

577 **Casalis G. 1833.** Dizionario geografico storico-statistico-commerciale degli Stati di S.M. il Re di Sardegna.  
578 Vol. I. Torino: Maspero.

579 **Casalis G. 1852.** Dizionario geografico storico-statistico-commerciale degli Stati di S.M. il Re di Sardegna.  
580 Vol. XXII. Torino:Maspero.

581 **Cortey M & García-Marín JL. 2002.** Evidence for phylogeographically informative sequence variation in  
582 the mitochondrial control region of Atlantic brown trout. *Journal of Fish Biology* **60**: 1058–1063.

583 **Cortey M, Pla C & García-Marín JL. 2004.** Historical biogeography of Mediterranean trout. *Molecular*  
584 *Phylogenetics and Evolution* **33**: 831–844.

585 **Cossart E, Braucher R, Fort M, Bourlès DL & Carcaillet J. 2008.** Slope instability in relation to glacial  
586 debuitting in alpine areas (Upper Durance catchment, southeastern France): Evidence from field data and  
587 <sup>10</sup>Be cosmic ray exposure ages. *Geomorphology* **95**: 3–26.

588 **Estoup A, Largiadier CR, Perrot E & Chourrot D. 1996.** Rapid one-tube DNA extraction for reliable PCR  
589 detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology* **5**:  
590 295-298.

591 **Excoffier L. 2004.** Patterns of DNA sequence diversity and genetic structure after a range expansion:  
592 Lessons from the infinite-island model. *Molecular Ecology* **13**: 853–864.

593 **Excoffier L & Lischer HEL. 2010.** Arlequin suite ver 3.5: A new series of programs to perform population  
594 genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564–567.

595 **Fernández-Cebrián R, Araguas RM, Sanz N, García-Marín JL & Fraser D. 2014.** Genetic risks of  
596 supplementing trout populations with native stocks: a simulation case study from current Pyrenean  
597 populations. *Canadian Journal of Fisheries and Aquatic Sciences* **71**: 1243–1255.

598 **Fernández-García JL, Carranza J, Martínez JG & Randi E. 2014.** Mitochondrial D-loop phylogeny  
599 signals two native Iberian red deer (*Cervus elaphus*) Lineages genetically different to Western and Eastern  
600 European red deer and infers human-mediated translocations. *Biodiversity and Conservation* **23**: 537–554.

601 **Fu YX. 1997.** Statistical tests of neutrality of mutations against population growth, hitchhiking and  
602 background selection. *Genetics* **147**: 915–925.

603 **Gauthier A & Berrebi P. 2007.** La colonisation de l’île par différentes souches de truite. In: *Guide de*  
604 *gestion de la truite macrostigma*, 4-10.

605 **Giuffra E, Bernatchez L & Guyomard R. 1994.** Mitochondrial Control Region and Protein-Coding Genes  
606 Sequence Variation Among Phenotypic Forms of Brown Trout *Salmo-Trutta* From Northern Italy. *Molecular*  
607 *Ecology* **3**: 161–171.

608 **Gratton P, Allegrucci G, Sbordoni V & Gandolfi A. 2014.** The evolutionary jigsaw puzzle of the  
609 surviving trout (*Salmo trutta* L. complex) diversity in the Italian region. a multilocus Bayesian approach.  
610 *Molecular Phylogenetics and Evolution* **79**: 292–304.

611 **Habit EM, Walde SJ, Zemplak TS, Ruzzante DE & Carrea C. 2010.** Surviving historical Patagonian

612 landscapes and climate: molecular insights from *Galaxias maculatus*. *BMC Evolutionary Biology* **10**: 67.

613 **Hayashi K. 1991.** PCR-SSCP: A simple and sensitive method for detection of mutations in the genomic

614 DNA. *Genome Research* **1**: 34–38.

615 **Hughes PD, Woodward JC & Gibbard PL. 2006.** Quaternary glacial history of the Mediterranean

616 mountains. *Progress in Physical Geography* **30**: 334–364.

617 **Kottelat M & Freyhof J (2007)** Handbook of European fresh-water fishes. Publications Kottelat, Cornol

618 **Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace**

619 **IM, Wilm A, Lopez R, Thompson JD, Gibson TJ & Higgins DG. 2007.** Clustal W and Clustal X version

620 2.0. *Bioinformatics* **23**: 2947–2948.

621 **Larson G & Fuller DQ. 2014.** The Evolution of Animal Domestication. *Annual Review of Ecology,*

622 *Evolution, and Systematics* **45**: 115–136.

623 **Lavery S, Moritz C, & Fielder DR. 1996.** Genetic patterns suggest exponential population growth in a

624 declining species. *Molecular Biology and Evolution* **13**:1106–1113.

625 **Leprieur F, Beauchard O, Blanchet S, Oberdorff T & Brosse S. 2008.** Fish invasions in the world's river

626 systems: When natural processes are blurred by human activities. *PLoS Biology* **6**: 0404–0410.

627 **Losos JB, Arnold SJ, Bejerano G, Brodie ED, Hibbett D, Hoekstra HE, Mindell DP, Monteiro A,**

628 **Moritz C, Orr HA, Petrov DA, Renner SS, Ricklefs RE, Soltis PS & Turner TL. 2013.** Evolutionary

629 Biology for the 21st Century. *PLoS Biology* **11(1)**: 1-8.

630 **Marchetto F, Zaccara S, Muenzel FM & Salzburger W. 2010.** Phylogeography of the Italian vairone

631 (*Telestes muticellus*, Bonaparte 1837) inferred by microsatellite markers: Evolutionary history of a

632 freshwater fish species with a restricted and fragmented distribution. *BMC Evolutionary Biology* **10**.

633 **Meraner A, Baric S, Pelster B & Dalla Via J. 2007.** Trout (*Salmo trutta*) mitochondrial DNA

634 polymorphism in the centre of the marble trout distribution area. *Hydrobiologia* **579**: 337–349.

635 **Meraner A, Gratton P, Baraldi F & Gandolfi A. 2013.** Nothing but a trace left? Autochthony and

636 conservation status of Northern Adriatic *Salmo trutta* inferred from PCR multiplexing, mtDNA control

637 region sequencing and microsatellite analysis. *Hydrobiologia* **702**: 201–213.

638 **Miró A & Ventura M. 2013.** Historical use, fishing management and lake characteristics explain the

639 presence of non-native trout in Pyrenean lakes: Implications for conservation. *Biological Conservation* **167**:

640 17–24.

641 **Muñoz M & Casadevall M. 1997.** Fish remains from Arbreda Cave (Serinya Girona), northeast Spain, and

642 their palaeoecological significance. *Journal of Quaternary Science* **12**: 111–115.

643 **Ninua L, Tarkhishvili D & Gvazava E. 2018.** Phylogeography and taxonomic status of trout and salmon

644 from the Ponto-Caspian drainages, with inferences on European Brown Trout evolution and taxonomy.

645 *Ecology and Evolution* **8**: 2645–2658.

646 **Oliver J. 2014.** Evolutionary history of the brown trout, *Salmo trutta* L., in France. First-year MEME

647 research project Université Montpellier II April-June 2014.

648 **Posada D. 2008.** jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**: 1253–

649 1256.

650 **Purvis A & Hector A. 2000.** Getting the measure of biodiversity. *Nature* **405**: 212–219.

651 **Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA. 2018.** Posterior summarization in Bayesian

652 phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901–904.

653 **Ramos-Onsins SE & Rozas J. 2002.** Statistical properties of new neutrality tests against population growth.

654 *Molecular Biology and Evolution* **19**: 2092–2100.

655 **Redpath SM, Young J, Evely A, Adams WM, Sutherland WJ, Whitehouse A, Amar A, Lambert RA,**

656 **Linnell JDC, Watt A & Gutiérrez RJ. 2013.** Understanding and managing conservation conflicts. *Trends*

657 *in Ecology and Evolution* **28**: 100–109.

658 **Rondinini C, Battistoni A, Peronace V, Teofili (compilatori) C. 2013.** Lista Rossa IUCN dei Vertebrati

659 Italiani. Roma: Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

660 **Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE &**

661 **Sanchez-Gracia A. 2017.** DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular*

662 *Biology and Evolution* **34**: 3299–3302.

663 **Sanz N. 2018.** Phylogeographic history of brown trout: A review. In: Lobón Cerviá J, Sanz N, editors.

664 Brown trout: Biology, ecology and management. Hoboken, NJ: John Wiley & Sons Ltd. pp. 65–102.

665 **Schenekar T, Lerceteanu-Köhler E & Weiss S. 2014.** Fine-scale phylogeographic contact zone in Austrian

666 brown trout *Salmo trutta* reveals multiple waves of post-glacial colonization and a pre-dominance of natural

667 versus anthropogenic admixture. *Conservation Genetics* **15**: 561–572.

668 **Schenekar T & Weiss S. 2017.** Selection and genetic drift in captive versus wild populations: an assessment  
669 of neutral and adaptive (MHC-linked) genetic variation in wild and hatchery brown trout (*Salmo trutta*)  
670 populations. *Conservation Genetics* **18**: 1011–1022.

671 **Shackleton RT, Larson BMH, Novoa A, Richardson DM & Kull CA. 2019.** The human and social  
672 dimensions of invasion science and management. *Journal of Environmental Management* **229**: 1–9.

673 **Siccardi S. 1996.** Elena la Regina mai dimenticata. Torino: Paoline.

674 **Šlechtová V, Bohlen J, Freyhof J, Persat H & Delmastro GB. 2004.** The Alps as barrier to dispersal in  
675 cold-adapted freshwater fishes? Phylogeographic history and taxonomic status of the bullhead in the Adriatic  
676 freshwater drainage. *Molecular Phylogenetics and Evolution* **33**: 225–239.

677 **Snoj A, Marić S, Berrebi P, Crivelli AJ, Shumka S & Sušnik S. 2009.** Genetic architecture of trout from  
678 Albania as revealed by mtDNA control region variation. *Genetics Selection Evolution* **41**: 22.

679 **Snoj A, Glamuzina B, Razpet A, Zablocki J, Bogut I, Lerceteau-Köhler E, Pojskić N & Sušnik S. 2010.**  
680 Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout  
681 community. *Hydrobiologia* **651**: 199–212.

682 **Sønstebø JH, Borgstrøm R & Heun M. 2007.** Genetic structure of brown trout (*Salmo trutta* L.) from the  
683 Hardangervidda mountain plateau (Norway) analyzed by microsatellite DNA: A basis for conservation  
684 guidelines. *Conservation Genetics* **8**: 33–44.

685 **Splendiani A, Ruggeri P, Giovannotti M, Pesaresi S, Occhipinti G, Fioravanti T, Lorenzoni M, Nisi  
686 Cerioni P & Caputo Barucchi V. 2016a.** Alien brown trout invasion of the Italian peninsula: the role of  
687 geological, climate and anthropogenic factors. *Biological Invasions* **18**: 2029–2044.

688 **Splendiani A, Fioravanti T, Giovannotti M, Negri A, Ruggeri P, Olivieri L, Cerioni PN, Lorenzoni M  
689 & Barucchi VC. 2016b.** The effects of paleoclimatic events on mediterranean trout: Preliminary evidences  
690 from ancient DNA. *PLoS ONE* **11**.

691 **Splendiani A, Fioravanti T, Giovannotti M, Olivieri L, Ruggeri P, Nisi Cerioni P, Vanni S, Enrichetti  
692 F & Caputo Barucchi V. 2017.** Museum samples could help to reconstruct the original distribution of  
693 *Salmo trutta* complex in Italy. *Journal of Fish Biology* **90**: 2443–2451.

694 **Splendiani A, Palmas F, Sabatini A & Caputo V 2019a.** The name of the trout: considerations on the  
695 taxonomic status of the *Salmo trutta* L., 1758 complex (Osteichthyes: Salmonidae) in Italy. *The European  
696 Zoological Journal* **86**: 432–442.

697 **Splendiani A, Giovannotti M, Righi T, Fioravanti T, Cerioni PN, Lorenzoni M, Carosi A, La Porta G  
698 & Barucchi VC. 2019b.** Introgression despite protection: the case of native brown trout in Natura 2000  
699 network in Italy. *Conservation Genetics*.

700 **Splendiani A, Fioravanti T, Ruggeri P, Giovannotti M, Carosi A, Marconi M, Lorenzoni M, Righi T,  
701 Nisi Cerioni P & Caputo Barucchi V. 2020.** Life history and genetic characterisation of sea trout *Salmo  
702 trutta* in the Adriatic Sea. *Freshwater Biology* **65**: 460–473.

703 **Stefani F, Anzani A & Marieni A. 2019.** Echoes from the past: a genetic trace of native brown trout in the  
704 Italian Alps. *Environmental Biology of Fishes* **102**: 1327–1335.

705 **Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ & Rambaut A. 2018.** Bayesian phylogenetic  
706 and phylodynamic data integration using BEAST 1.10. *Virus Evolution* **4**: 1–5.

707 **Sušnik S, Weiss S, Odak T, Delling B, Treer T & Snoj A. 2007.** Reticulate evolution: Ancient  
708 introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei:  
709 Salmonidae). *Biological Journal of the Linnean Society* **90**: 139–152.

710 **Szövényi P, Arroyo K, Guggisberg A & Conti E. 2009.** Effects of Pleistocene glaciations on the genetic  
711 structure of *Saxifraga florulenta* (Saxifragaceae), a rare endemic of the Maritime Alps. *Taxon* **58**: 532–543.

712 **Tajima F. 1989.** Statistical model for testing the neutral mutation hypothesis by DNA  
713 polymorphism. *Genetics* **123**: 585–595.

714 **Tiberti R & Splendiani A. 2019.** Management of a highly unlikely native fish: The case of arctic charr  
715 *Salvelinus alpinus* from the Southern Alps. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**:  
716 312–320.

717 **Tougard C, Justy F, Guinand B, Douzery EJP & Berrebi P. 2018.** *Salmo macrostigma* (Teleostei,  
718 Salmonidae): Nothing more than a brown trout (*S. trutta*) lineage? *Journal of Fish Biology* **93**: 302–310.

719 **Vera M, García-Marín JL, Martínez P & Bouza C. 2015.** Phylogenetic diversity within the endemic  
720 brown trout Duero lineage: Implications for conservation and management. *Marine and Freshwater  
721 Research* **66**: 1066–1071.

722 **Vera M, Bouza C, Casanova A, Heras S, Martínez P & García-Marín JL. 2019.** Identification of an  
723 endemic Mediterranean brown trout mtDNA group within a highly perturbed aquatic system, the Llobregat

724 River ( NE Spain ). **9**: 277–291.  
725 **Vonlanthen P, Excoffier L, Bittner D, Persat H, Neuenschwander S & Largiadèr CR. 2007.** Genetic  
726 analysis of potential postglacial watershed crossings in Central Europe by the bullhead (*Cottus gobio* L.).  
727 *Molecular Ecology* **16**: 4572–4584.  
728 **Waters JM, Crow D, Youngson JH & Wallis GP. 2001.** Genes meet geology: Fish phylogeographic  
729 pattern reflects ancient, rather than modern, drainage connections. *Evolution* **55**: 1844–1851.  
730 **Zanetti M, Nonnis Marzano F, Lorenzoni M. 2013.** I salmonidi italiani: Linee guida per la conservazione  
731 della biodiversità. Technical report. Available:  
732 [http://www.aiiad.it/sito/images/docs/sistemica/GRUPPO%20DI%20LAVORO%20SALMONIDI\\_RELAZ](http://www.aiiad.it/sito/images/docs/sistemica/GRUPPO%20DI%20LAVORO%20SALMONIDI_RELAZIONE%20FINALE.pdf)  
733 [IONE%20FINALE.pdf](http://www.aiiad.it/sito/images/docs/sistemica/GRUPPO%20DI%20LAVORO%20SALMONIDI_RELAZIONE%20FINALE.pdf).  
734 **Zemlak TS, Habit EM, Walde SJ, Battini MA, Adams EDM & Ruzzante DE. 2008.** Across the southern  
735 Andes on fin: Glacial refugia, drainage reversals and a secondary contact zone revealed by the  
736 phylogeographical signal of *Galaxias platei* in Patagonia. *Molecular Ecology* **17**: 5049–5061.  
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### 739 **Figure captions**

#### 741 Figure 1

742 Map showing the sampling locations of brown trout throughout the Rhône and the western part of the Po  
743 River basins. Color pie charts indicate the mtDNA haplotype frequency distribution of the main brown trout  
744 lineages. Numbers in bold within pie charts represent the Atlantic haplotype frequency (%). The distribution  
745 of the Alpine ice cover during the last glacial maximum is represented by the light blue area. Box at the top  
746 right shows the range of the nominal Mediterranean trout species as reported in Kottelat & Freyof (2007).  
747 † The geographic range of *Salmo cettii* according to Kottelat & Freyhof (2007), # the revised geographic  
748 range of *S. cettii* according to Splendiani *et al.* (2019b and references therein). Locations code colored  
749 differentially based on the river basin of origin.

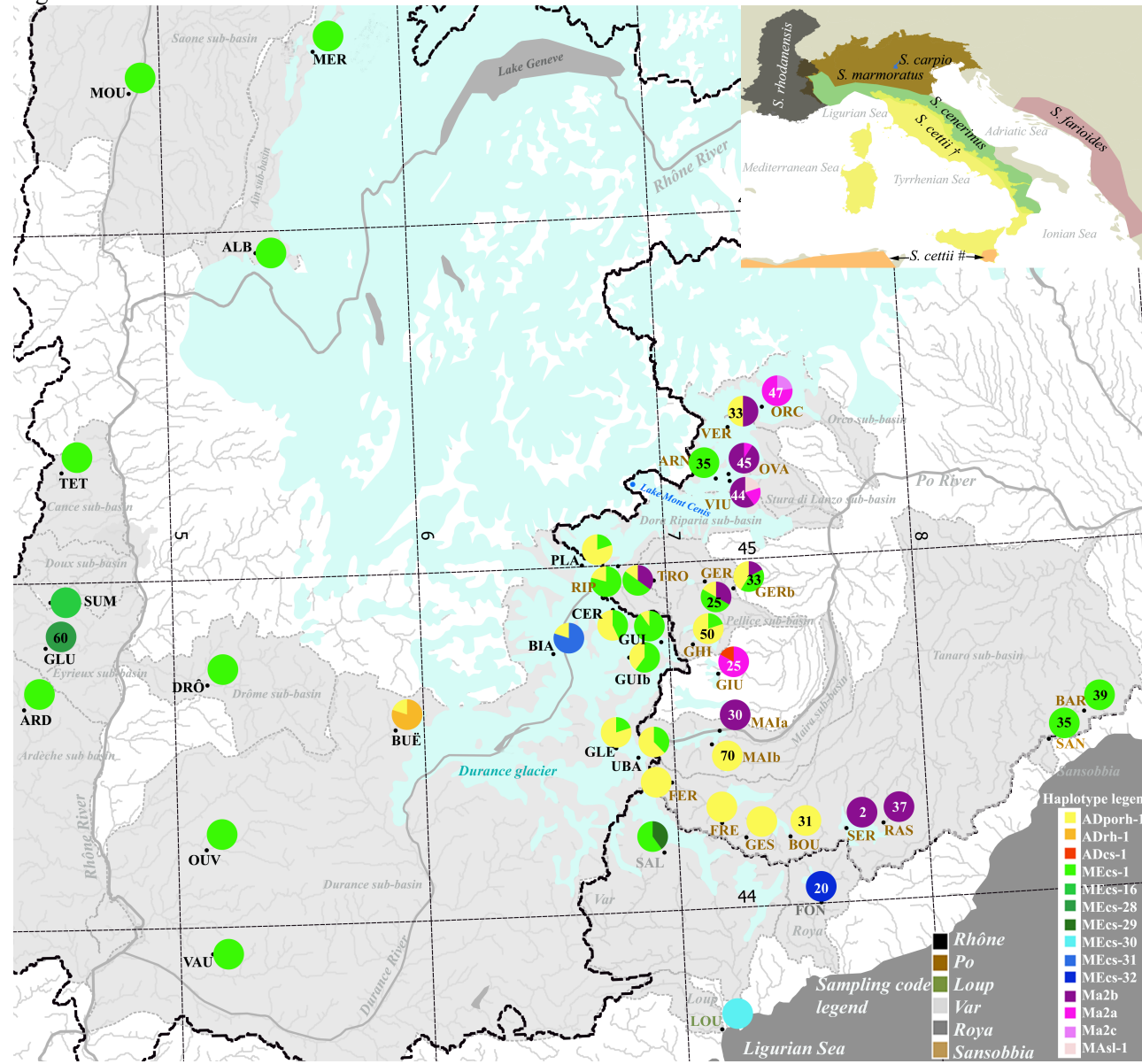
#### 751 Figure 2

752 Median-Joining Network showing the phylogenetic relationships subsisting between the 18 native brown  
753 trout haplotypes detected in this study (colored circles) and the brown trout haplotypes observed in  
754 previously published studies (grey circles, see also Table 1; the position of the haplotypes ADcs-11 and AD-  
755 Tyrrh-1 was reported because commented in the text). As regards the haplotypes observed in the study area,  
756 the size of each circle is proportional with the haplotype absolute frequency. Locations code colored  
757 differentially based on the river basin of origin.

#### 759 Figure 3

760 Plot showing the probability density function (density) obtained comparing elevation, longitude and latitude  
761 with the mtDNA genetic composition of brown trout populations from the Rhône River basin (A and B) and  
762 from the Po River basin (C and D). The mtDNA lineage composition was represented by the following  
763 colored scheme: population characterized by the sole presence of ME haplotypes (Pure ME), in green; pure  
764 AD populations, in yellow; admixed AD-ME populations in orange; admixed AD-MA populations, in light  
765 blue and marble trout samples (from Giuffra *et al.*, 1994) in blue. To avoid confusion, other minor admixed  
766 population were not showed.  
767

Fig. 1



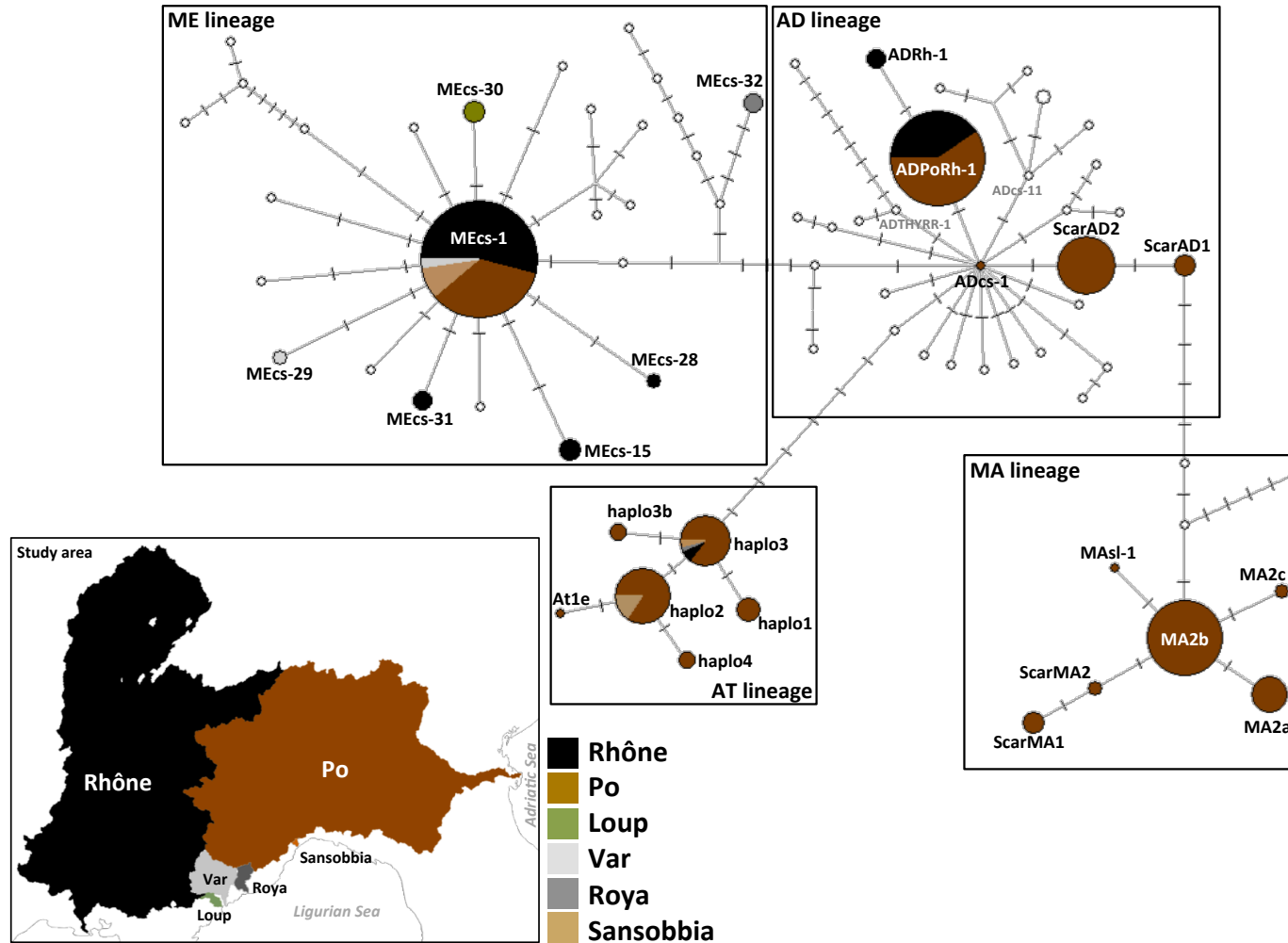
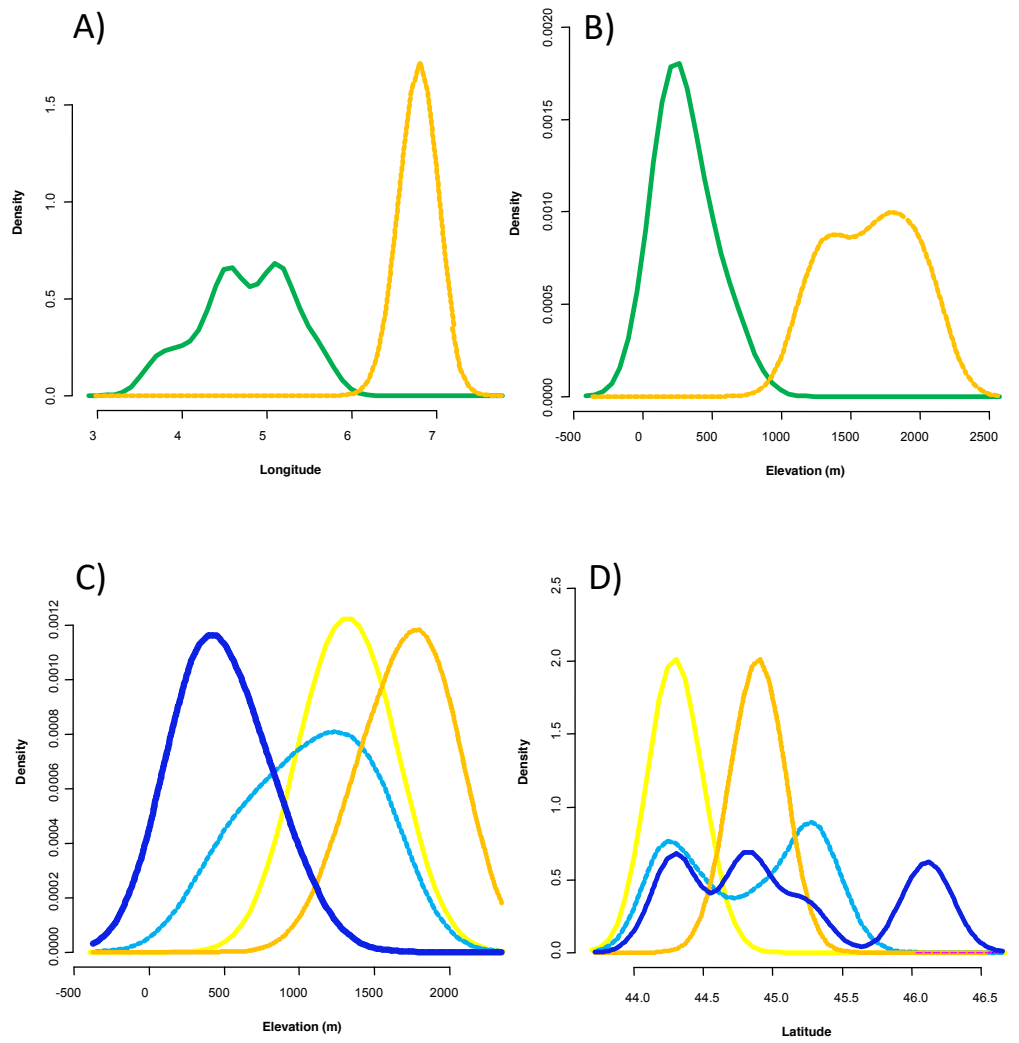


Fig. 3





**Table 1.** Collection site information. Sampling code; Nominal taxon: *Salmo rhodanensis*, *S.rod*; *Salmo cenerinus*, *S.cen*; *Salmo fariodes*, *S. far.*; *Salmo cettii*, *S.cet.*; ?, no nominal proposed in literature; Sea drainage, Gulf of Lion, G; Ligurian, L, Adriatic, A; hierarchical description of the river network investigated (main-basin, sub-basin, stream); Sample size, No; Laboratories (Lab.) involved in genetic analyses, F = ISEM, Université de Montpellier (Lab.1) (Reynaud, Tougard & Berrebi 2011), and I = DiSVA,

| Code | Taxon              | Sea drainage | Main-basin | Sub-basin      | Stream       | No | Lab. | Lat.  | Lon. | Elevation (m) |
|------|--------------------|--------------|------------|----------------|--------------|----|------|-------|------|---------------|
| MER  | <i>S. rod</i>      | G            | Rhône      | Ain            | Merlue       | 5  | F    | 46.51 | 5.64 | 433           |
| MOU  | <i>S. rod</i>      | G            | Rhône      | Saône          | Mouge        | 5  | F    | 46.40 | 4.87 | 176           |
| ALB  | <i>S. rod</i>      | G            | Rhône      | Ain            | Albarine     | 5  | F    | 45.94 | 5.38 | 258           |
| ORC  | <i>S.cen/S.far</i> | A            | Po         | Orco           |              | 17 | I    | 45.43 | 7.42 | 700           |
| VER  | <i>S.cen/S.far</i> | A            | Po         | Stura di Lanzo | Rio          | 18 | I    | 45.38 | 7.28 | 1220          |
| TET  | <i>S. rod</i>      | G            | Rhône      | Cance          | Riotet       | 5  | F    | 45.32 | 4.56 | 668           |
| OVA  | <i>S.cen/S.far</i> | A            | Po         | Stura di Lanzo | Rio          | 20 | I    | 45.24 | 7.27 | 1480          |
| ARN  | <i>S.cen/S.far</i> | A            | Po         | Stura di Lanzo | Rio Arnas    | 10 | I    | 45.24 | 7.20 | 1370          |
| VIU  | <i>S.cen/S.far</i> | A            | Po         | Stura di Lanzo | Stura di Viù | 9  | I    | 45.23 | 7.28 | 1540          |
| RIP  | <i>S.cen/S.far</i> | A            | Po         | Dora Riparia   | Ripa         | 24 | I    | 45.00 | 6.81 | 1900          |
| PLA  | <i>S. rod</i>      | G            | Rhône      | Durance        | Clarée       | 5  | F    | 45.00 | 6.66 | 1484          |
| TRO  | <i>S.cen/S.far</i> | A            | Po         | Pellice        | Chisone      | 20 | I    | 44.95 | 6.95 | 1835          |
| GER  | <i>S.cen/S.far</i> | A            | Po         | Pellice        | Germanasca   | 9  | I    | 44.94 | 7.15 | 680           |
| GERb | <i>S.cen/S.far</i> | A            | Po         | Pellice        | Germanasca   | 16 | I    | 44.92 | 7.27 | 750           |
| CER  | <i>S. rod</i>      | G            | Rhône      | Durance        | Cerveyrette  | 6  | I    | 44.87 | 6.78 | 2077          |
| GLU  | <i>S. rod</i>      | G            | Rhône      | Eyrieux        | Gluyère      | 5  | F    | 44.81 | 4.48 | 568           |
| GUIb | <i>S. rod</i>      | G            | Rhône      | Durance        | Guil         | 20 | I    | 44.77 | 6.97 | 1779          |
| GHI  | <i>S.cen/S.far</i> | A            | Po         | Pellice        | Ghiacciard   | 10 | I    | 44.76 | 7.09 | 1440          |
| BIA  | <i>S. rod</i>      | G            | Rhône      | Durance        | Biaisse      | 5  | F    | 44.75 | 6.53 | 1200          |
| GUI  | <i>S. rod</i>      | G            | Rhône      | Durance        | Guil         | 5  | F    | 44.73 | 6.84 | 1693          |
| DRÔ  | <i>S. rod</i>      | G            | Rhône      | Drôme          | Drôme        | 5  | F    | 44.70 | 5.13 | 229           |
| GIU  | <i>S.cen/S.far</i> | A            | Po         |                | Rio Giulian  | 10 | I    | 44.67 | 7.19 | 1120          |
| ARD  | <i>S. rod</i>      | G            | Rhône      | Ardèche        | Thines       | 5  | F    | 44.64 | 4.39 | 217           |
| BUË  | <i>S. rod</i>      | G            | Rhône      | Durance        | Petit Buëch  | 5  | F    | 44.55 | 5.88 | 1117          |
| MAIa | <i>S.cen/S.far</i> | A            | Po         | Maira          | Bedale di    | 10 | I    | 44.51 | 7.18 | 950           |
| BAR  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Baracca      | 18 | I    | 44.50 | 8.65 | 570           |
| MAIb | <i>S.cen/S.far</i> | A            | Po         | Maira          | Bedale       | 10 | I    | 44.47 | 7.15 | 1180          |
| GLE  | <i>S. rod</i>      | G            | Rhône      | Durance        | Gleizolles   | 5  | F    | 44.47 | 6.77 | 1319          |
| UBA  | <i>S. rod</i>      | G            | Rhône      | Durance        | Ubayette     | 15 | I    | 44.44 | 6.85 | 1953          |
| SAN  | <i>S. cet</i>      | L            | Sansobbi   |                |              | 20 | I    | 44.43 | 8.50 | 660           |
| FER  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Rio Ferriere | 8  | I    | 44.37 | 6.98 | 1480          |
| FRE  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Rio Freddo   | 8  | I    | 44.24 | 7.17 | 1550          |
| RAS  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Rio          | 14 | I    | 44.22 | 7.82 | 1100          |
| OUV  | <i>S. rod</i>      | G            | Rhône      | Ouvèze         | Ouvèze       | 5  | F    | 44.22 | 5.11 | 222           |
| SER  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Rio          | 13 | I    | 44.21 | 7.67 | 1280          |
| GES  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Gesso        | 4  | I    | 44.20 | 7.27 | 1450          |
| BOU  | <i>S.cen/S.far</i> | A            | Po         | Tanaro         | Bousset      | 13 | I    | 44.20 | 7.45 | 1170          |
| SAL  | ?                  | G            | Var        | Tinée          | Tinée        | 5  | F    | 44.17 | 6.94 | 1930          |
| FON  | ?                  | G            | Roya       | Roya           | Roya         | 5  | F    | 44.00 | 7.55 | 426           |
| SUM  | <i>S. rod</i>      | G            | Rhône      | Doux           | Sumène       | 5  | F    | 43.98 | 3.72 | 203           |
| VAU  | <i>S. rod</i>      | G            | Rhône      | Ouvèze         | Sorgue       | 5  | F    | 43.92 | 5.13 | 89            |
| LOU  | ?                  | G            | Loup       | Loup           | Loup         | 5  | F    | 43.65 | 7.13 | 7             |

**Table 2.** Haplotype frequency distribution at mtDNA control region in wild brown trout populations from the Rhône, Po and other neighbour Mediterranean rivers. Sampling code as in Table 1.

| Code | Basin   | MEcs1 | MEcs15 | MEcs28 | MEcs29 | MEcs30 | MEcs31 | MEcs32 | ADp orh-1 | ADrh-1 | ADcs-1 | MA2b | Ma2a | MA2c | MAsl-1 | hap1 | hap2 | hap3 | hap4 | At1e | hap3b |
|------|---------|-------|--------|--------|--------|--------|--------|--------|-----------|--------|--------|------|------|------|--------|------|------|------|------|------|-------|
| MER  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| MOU  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| ALB  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| ORC  | Po      |       |        |        |        |        |        |        |           |        |        |      | 7    | 2    |        |      | 5    |      |      |      | 3     |
| VER  | Po      |       |        |        |        |        |        |        | 6         |        |        | 6    |      |      |        | 6    |      |      |      |      |       |
| TET  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| OVA  | Po      |       |        |        |        |        |        |        |           |        |        | 10   | 1    |      |        |      | 9    |      |      |      |       |
| ARN  | Po      | 3     |        |        |        |        |        |        |           |        |        | 2    |      |      |        |      | 1    | 4    |      |      |       |
| VIU  | Po      |       |        |        |        |        |        |        |           |        |        | 3    | 1    |      | 1      |      | 3    | 1    |      |      |       |
| PLA  | Rhône   | 1     |        |        |        |        |        |        | 4         |        |        |      |      |      |        |      |      |      |      |      |       |
| RIP  | Po      | 19    |        |        |        |        |        |        | 5         |        |        |      |      |      |        |      |      |      |      |      |       |
| TRO  | Po      | 10    |        |        |        |        |        |        | 3         |        |        | 7    |      |      |        |      |      |      |      |      |       |
| GER  | Po      | 3     |        |        |        |        |        |        | 1         |        |        | 2    |      |      |        |      |      | 3    |      |      |       |
| GERb | Po      | 5     |        |        |        |        |        |        | 5         |        |        | 2    |      |      |        |      | 2    |      | 1    | 1    |       |
| CER  | Rhône   | 9     |        |        |        |        |        |        | 11        |        |        |      |      |      |        |      |      |      |      |      |       |
| GLU  | Rhône   |       |        | 2      |        |        |        |        |           |        |        |      |      |      |        |      |      | 3    |      |      |       |
| GUIb | Rhône   | 18    |        |        |        |        |        |        | 2         |        |        |      |      |      |        |      |      |      |      |      |       |
| GHI  | Po      | 1     |        |        |        |        |        |        | 4         |        |        |      |      |      |        |      |      | 4    | 1    |      |       |
| BIA  | Rhône   |       |        |        |        |        | 4      |        | 1         |        |        |      |      |      |        |      |      |      |      |      |       |
| GUI  | Rhône   | 3     |        |        |        |        |        |        | 2         |        |        |      |      |      |        |      |      |      |      |      |       |
| DRÔ  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| GIU  | Po      |       |        |        |        |        |        |        |           |        | 1      |      | 5    |      |        |      | 2    | 2    |      |      |       |
| ARD  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| BUË  | Rhône   |       |        |        |        |        |        |        | 1         | 4      |        |      |      |      |        |      |      |      |      |      |       |
| MA1a | Po      |       |        |        |        |        |        |        |           |        |        | 7    |      |      |        |      |      | 3    |      |      |       |
| BAR  | Po      | 11    |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      | 7    |      |      |       |
| GIE  | Rhône   | 1     |        |        |        |        |        |        | 4         |        |        |      |      |      |        |      |      |      |      |      |       |
| MA1b | Po      |       |        |        |        |        |        |        | 3         |        |        |      |      |      |        |      | 1    | 5    | 1    |      |       |
| UBA  | Rhône   | 8     |        |        |        |        |        |        | 13        |        |        |      |      |      |        |      |      |      |      |      |       |
| SAN  | Sansob. | 13    |        |        |        |        |        |        |           |        |        |      |      |      |        |      | 6    | 1    |      |      |       |
| FER  | Po      |       |        |        |        |        |        |        | 8         |        |        |      |      |      |        |      |      |      |      |      |       |
| FRE  | Po      |       |        |        |        |        |        |        | 8         |        |        |      |      |      |        |      |      |      |      |      |       |
| OUV  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| RAS  | Po      |       |        |        |        |        |        |        |           |        |        | 10   |      |      |        |      | 4    |      |      |      |       |
| SER  | Po      |       |        |        |        |        |        |        |           |        |        | 12   |      |      |        |      |      | 0    |      |      |       |
| GES  | Po      |       |        |        |        |        |        |        | 4         |        |        |      |      |      |        |      |      |      |      |      |       |
| BOU  | Po      |       |        |        |        |        |        |        | 9         |        |        |      |      |      |        |      | 4    |      |      |      |       |
| SAL  | Var     | 3     |        |        | 2      |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| FON  | Roya    |       |        |        |        |        |        |        | 4         |        |        |      |      |      |        |      |      |      | 1    |      |       |
| SUM  | Rhône   |       | 5      |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| VAU  | Rhône   | 5     |        |        |        |        |        |        |           |        |        |      |      |      |        |      |      |      |      |      |       |
| LOU  | Loup    |       |        |        |        |        | 5      |        |           |        |        |      |      |      |        |      |      |      |      |      |       |

Table S1. Schematic summary of the Mediterranean taxa in the *Salmo trutta* complex of the study area. Reference to mtDNA lineages as follows: 1, Bernatchez 2001; 2, Berrebi et al. 2019; 3, Fabiani et al. 2017; 4, Fruciano et al. 2014; 5, Giuffra et al. 1994; 6, 1996; 7, Gratton et al. 2014; 8, Lerceteanu-Köhler et al. 2013; 9, Marić et al. (2017) 9, Meraner & Gandolfi 2018; 10, Sabatini et al. 2011; 11, Schöffmann et al. 2007; 12, Snoj et al. 2011; 13, Splendiani et al. 2006; 14, 2007; 15, 2017; 16, Zaccara et al. 2015; 17, Marić et al. 2017; 18, Tougard et al. 2018.; 19, this study.

| Taxon                                     | Geographical range   | mtDNA lineages  | mtDNA references  | Nomenclature change   |
|---|--|---|-------------------|---|
| <i>Salmo rhodanensis</i> Fowler, 1974     | Rhône River basin  | AD, ME  | 1, 19             | Questioned the validity of specie rank in this study and in Berrebi et al. (in prep.).  |
| <i>S. cettii</i> Rafinesque Schmaltz 1810 | Described for the S.E. Sicily but extended to the Apennines (Tyrrhenian side) by Kottelat & Freyhof (2007)                               | AT (southern clade)   | 2, 4, 11, 18      | Considered a senior synonym of <i>S. macrostigma</i> by Splendiani et al. (2019).   |
| <i>S. macrostigma</i> (Dumeril, 1858)     | Described for Algeria, but extended also to Apennines (Tyrrhenian side), Sicily, Sardinia (Italy) and Corsica (France) by Sommani (1951) | AD, MA, ME (According to Sommani, 1951)<br>AT (southern clade) (according to Splendiani et al., 2019) | 1-4,7-13, 16      | Considered a junior synonym of <i>S.cettii</i> by Splendiani et al. (2019).   |
| <i>S. cenerinus</i> Chiereghini, 1847     | Described for the Gulf of Trieste, but extended to the Apennines (Adriatic side) by Kottelat & Freyhof (2007)                            | AD, MA, ME (according to Kottelat & Freyhof, 2007)<br>MA (according to Bianco & Delmastro (2011))     | 1, 2, 7, 9, 13-15 | Considered by Bianco & Delmastro (2011) as a junior synonym for <i>Salmo marmoratus</i> Cuvier, 1829.   |
| <i>S. farioides</i> Karaman, 1938         | Described for south-western Balkans but extended to the Padano-Venetian district by Bianco & Delmastro (2011)                            | AD  | 17                | Considered a valid name for the native trout of the Drin River basin (S.E. Balkans) by Marić et al. (2017) and extended by Bianco & Delmastro (2011) to the Adriatic slope of the Apennine. |
| <i>S. carpio</i> L., 1758                 | Lake Garda   | AD, ME, MA  | 1, 5-7, 9, 13     | Considered a subspecies of <i>S. trutta</i> by Tortonese (1970)   |
| <i>S. marmoratus</i> Cuvier, 1829         | Po River Basin and Balkan Peninsula  | MA  | 1, 5-7, 9, 13-15  | Considered a subspecies of <i>S. trutta</i> by Tortonese (1970)   |

Table S1 Bibliography

- Bernatchez L. 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* 55:351–379.
- Berrebi P, Caputo Barucchi V, Splendiani A, Muracciole S, Sabatini A, Palmas F, Tougard C, Arculeo M, Marić S. 2019. Brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. *Hydrobiologia* 826:209–231. DOI: 10.1007/s10750-018-3734-5.
- Bianco PG, Delmastro GB. 2011. Recenti novità tassonomiche riguardanti i pesci d'acqua dolce autoctoni in Italia e descrizione di una nuova specie di luccio. In: De Filippo G, editor. *Researches on wildlife conservation*, Vol. 2. USA: IGF publication. pp. 14.(suppl.).
- Fabiani A, Gratton P, Zappes IA, Seminara M, D'Orsi A, Sbordoni V, Allegrucci G. 2017. Investigating the genetic structure of trout from the Garden of Ninfa (central Italy): Suggestions for conservation and management. *Fisheries Management and Ecology* 2017:1–11.
- Fruciano C, Pappalardo AM, Tigano C, Ferrito V. 2014. Phylogeographical relationships of Sicilian brown trout and the effects of genetic introgression on morphospace occupation. *Biological Journal of the Linnean Society* 112:387–398.
- Giuffra E, Bernatchez L, Guyomard R. 1994. Mitochondrial control region and protein-coding genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. *Molecular Ecology* 3:161–171.
- Giuffra E, Guyomard R, Forneris G. 1996. Phylogenetic relationships and introgression patterns between incipient parapatric species of Italian brown trout (*Salmo trutta* L. complex). *Molecular Ecology* 5:207–220.

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|--|
| Gratton P, Allegrucci G, Sbordoni V, Gandolfi A. 2014. The evolutionary jigsaw puzzle of the surviving trout ( <i>Salmo trutta</i> L. complex) diversity in the Italian region. A multilocus Bayesian approach. <i>Molecular Phylogenetics and Evolution</i> 79:292–304. DOI: 10.1016/j.ympev.2014.06.022.   |
| Kottelat M, Freyhof J. 2007. Handbook of European fresh-water fishes. Publications Kottelat, Cornol  |
| Lerceteau-Köhler E, Schliewen U, Kopun T, Weiss S. 2013. Genetic variation in brown trout <i>Salmo trutta</i> across the Danube, Rhine, and Elbe headwaters: A failure of the phylogeographic paradigm? <i>BMC Evolutionary Biology</i> 13:176.  |
| Marić S, Sušnik S, Schöffmann J, Kostov V, Snoj A. 2017. Phylogeography of stream-dwelling trout in the Republic of Macedonia and a molecular genetic basis for revision of the taxonomy proposed by S. Karaman. <i>Hydrobiologia</i> 785:249–260.   |
| Meraner A, Gandolfi A. 2018. Genetics of the genus <i>Salmo</i> in Italy: Evolutionary history, population structure, molecular ecology and conservation. In: Lobón-Cerviá J, Sanz N, editors. <i>Brown trout: Biology, ecology and management</i> . Hoboken, NJ: John Wiley & Sons Ltd. pp. 65–102.   |
| Sabatini A, Cannas R, Follasa MC, Palmas F, Manunza A, Matta GA, Pendugiu A, Serra P, Cau A. 2011. Genetic characterization and artificial reproduction attempt of endemic Sardinian trout <i>Salmo trutta</i> L., 1758 (Osteichthyes, Salmonidae): Experiences in captivity. <i>Italian Journal of Zoology</i> 78:20–26. DOI: 10.1080/11250003.2010.497171. |
| Schöffmann J, Sušnik S, Snoj A. 2007. Phylogenetic origin of <i>Salmo trutta</i> L 1758 from Sicily, based on mitochondrial and nuclear DNA analyses. <i>Hydrobiologia</i> 575:51–55.  |
| Snoj A, Marić S, Sušnik Bajec S, Berrebi P, Janjani S, Schöffmann J. 2011. Phylogeographic structure and demographic patterns of brown trout in North-West Africa. <i>Molecular Phylogenetics and Evolution</i> 61:203–211.  |
| Splendiani A, Giovannotti M, Nisi Cerioni P, Caniglia ML, Caputo V. 2006. Phylogeographic inferences on the native brown trout mtDNA variation in central Italy. <i>Italian Journal of Zoology</i> 72:179–189.   |
| Splendiani A, Giovannotti M, Caniglia ML, Nisi Cerioni P, Battistella S, Caputo V. 2007. Presenza di aplotipi “marmoratus” in popolazioni di trota fario ( <i>Salmo trutta</i> L., 1758) dell’Italia centrale: Transfaunazione o paleointrogressione? <i>Atti Del Congresso Congiunto AIOL-SItE 2007</i> :89–93.   |
| Splendiani A, Fioravanti T, Giovannotti M, Olivieri L, Ruggeri P, Nisi Cerioni P, Vanni S, Enrichetti F, Caputo Barucchi V. 2017. Museum samples could help to reconstruct the original distribution of <i>Salmo trutta</i> complex in Italy. <i>Journal of Fish Biology</i> 90:2443–2451.   |
| Tougaard C, Justy F, Guinand B, et al. 2018. <i>Salmo macrostigma</i> (Teleostei, Salmonidae): Nothing more than a brown trout ( <i>S. trutta</i> ) lineage? <i>Journal of Fish Biology</i> 93:302–310.  |
| Zaccara S, Trasforini S, Antognazza CM, Puzzi C, Robert Britton J, Crosa G. 2015. Morphological and genetic characterization of Sardinian trout <i>Salmo cettii</i> Rafinesque, 1810 and their conservation implications. <i>Hydrobiologia</i> 760:205–223.  |

Table S2: List of haplotypes retrieved from GenBank, with accession numbers and their distributions.

| Haplotype           | Accession number | Distribution – country/drainages  |
|---------------------|------------------|---|
| haplotype 1 (ATcs1) | AF273086         | <sup>2</sup> Denmark (Skals), Norway (Bjornes Lake, Sima), Spain (hatchery stocks), <sup>4</sup> Spain (Garona), France (Gulf of Biscay), Iceland (Skorradalsvatn), British Isles (Coquet, Wear, Lune, Melvin), <sup>11</sup> continental Italy (IT), Sardinia, Corsica |
| haplotype 2 (ATcs2) | AF273087         | <sup>2</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>11</sup> Sardinia, Corsica          |
| haplotype 3 (ATcs3) | AF274574         | <sup>2</sup> Denmark (Skals), Norway (Bjornes Lake, Guddal, Sima), Spain (hatchery stocks), <sup>4</sup> Spain (Garona), France (Gulf of Biscay), British Isles (Coquet, Wear, Rother, Teifi, Conwy, Melvin), <sup>11</sup> continental Italy, Sardinia, Calabria       |
| haplotype 4 (ATcs4) | AF274575         | <sup>2</sup> Denmark (Skals, Karup), Norway (Bjornes Lake, Guddal, Sima), Spain (hatchery stocks), <sup>4</sup> France (Gulf of Biscay), British Isles (Lune), <sup>11</sup> Sardinia, Calabria   |
| Hap3b               | MK948037         | <sup>20</sup> Italy (Po)  |
| At1e                | DQ841192         | <sup>8</sup> Italy (Adige), <sup>20</sup> Italy (Po)  |
| ADcs1               | AY836330         | <sup>3</sup> Spain (Ter, Ebre, Túría, Segura), <sup>6</sup> Bulgaria (Struma, Mesta, Maritza), <sup>1, 7</sup> Macedonia (Prespa Lake, Vardar), <sup>20</sup> Italy (Po)  |
| ADcs2               | AY836331         | <sup>3</sup> Spain (Guadalfeo)  |
| ADcs3               | AY836332         | <sup>3</sup> Spain (Ebre)   |
| ADcs4               | AY836333         | <sup>3</sup> Spain (Ter)  |
| ADcs5               | AY836334         | <sup>3</sup> Spain (Guadalfeo)  |
| ADcs6               | AY836335         | <sup>3</sup> Spain (Ebre, Guadalfeo)  |
| ADcs7               | AY836336         | <sup>3</sup> Spain (Ebre)   |
| ADcs8               | AY836337         | <sup>3</sup> Spain (Ebre)   |
| ADcs9               | AY836338         | <sup>3</sup> Spain (Turia)  |
| ADcs10              | AY836339         | <sup>3</sup> Spain (Guadalfeo)  |
| ADcs11              | AY836340         | <sup>3</sup> Greece (Alfios), <sup>10</sup> Montenegro (Skadar Lake), <sup>12</sup> Albania (Drin, Skumbini, Cermit), <sup>13</sup> Montenegro (Zeta, Morača, Cijevna)  |
| ADcs15              | AY836344         | <sup>4</sup> France - Corsica (Corsica stream)  |
| ADcs16              | AY836345         | <sup>3</sup> Spain (Ebre)   |
| ADcs17              | AY836346         | <sup>3</sup> Spain (Ebre)   |
| ADcs18              | AY836347         | <sup>3</sup> Spain (Guadalquivir)   |
| ADcs19              | AY836348         | <sup>3</sup> Spain (Guadalquivir)   |
| ADcs20              | AY836349         | <sup>3</sup> Greece (Tripotamos), <sup>13</sup> Bulgaria (Maritza)  |
| ADporh-1            | MK948034         | <sup>20</sup> France (Rhône), Italy (Po)  |
| ADrh-1              | MK948035         | <sup>20</sup> France (Rhône)  |
| AD-Tyrrh1           | KX450257         | <sup>11</sup> Corsica, Sardinia, Calabria, continental Italy (Aniene River)   |
| AD-Tyrrh2           | KX450258         | <sup>11</sup> Corsica, continental Italy (Aniene River)   |
| AD-Tyrrh3           | KX450259         | <sup>11</sup> Calabria (Diga Giulia River)  |

|                 |             |   |
|-----------------|-------------|---|
| AD-Tyrrh4       | KX450260    | <sup>11</sup> Sardinia, continental Italy (Aniene River)  |
| AD-Tyrrh5       | KX450261    | <sup>11</sup> continental Italy (Nera River)  |
| AD-Tyrrh6       | KX450262    | <sup>11</sup> continental Italy (Nera River)  |
| AD-zls-01       | MG194729    | <sup>15</sup> Italy (Liri)  |
| AD-zls-01       | MG194729    | <sup>15</sup> Italy (Liri)  |
| ScarAD-1 (C208) | KJ834848    | <sup>16</sup> Italy (Garda Lake)  |
| ScarAD-2 (C021) | KJ834822    | <sup>16</sup> Italy (Garda Lake)  |
| MEcs1           | AY836350    | <sup>3</sup> Spain (Ter, Llobregat, Ebre, Mijares, Palancia, Túria, Segura),<br><sup>5</sup> Croatia (Krka), <sup>20</sup> France (Rhône, Var), Italy (Po, Sansobbia) |
| MEcs4           | AY836353    | <sup>3</sup> Spain (Ter)  |
| MEcs6           | AY836355    | <sup>3</sup> Spain (Ebre)   |
| MEcs7           | AY836356    | <sup>3</sup> Spain (Ter)  |
| MEcs8           | AY836357    | <sup>3</sup> Spain (Túria)  |
| MEcs9           | AY836358    | <sup>3</sup> Spain (Túria)  |
| MEcs10          | AY836359    | <sup>3</sup> Spain (Ebre)   |
| MEcs11          | AY836360    | <sup>3</sup> Spain (Túria)  |
| MEcs12          | AY836361    | <sup>3</sup> Spain (Ter)  |
| MEcs13          | AY836362    | <sup>3</sup> Spain (Ebre)   |
| MEcs14          | AY836363    | <sup>3</sup> Spain (Ebre, Guadalfeo)  |
| MEcs15          | AY836364    | <sup>3</sup> Spain (Ebre), <sup>20</sup> France (Rhône)   |
| MEcs16          | unpublished | <sup>17</sup> Spain   |
| MEcs17          | unpublished | <sup>17</sup> Spain   |
| MEcs18          | unpublished | <sup>17</sup> Spain   |
| MEcs20          | unpublished | <sup>17</sup> Spain   |
| MEcs21          | unpublished | <sup>17</sup> Spain   |
| MEcs22          | unpublished | <sup>17</sup> Spain   |
| MEcs23          | MG970273    | <sup>18</sup> Spain (Cardener)  |
| MEcs25          | MG970274    | <sup>18</sup> Spain (Cardener)  |
| MEcs26          | MG970275    | <sup>18</sup> Spain (Cardener)  |
| MEcs27          | MG970276    | <sup>18</sup> Spain (Cardener)  |
| MEcs28          | MK948029    | <sup>20</sup> France (Rhône)  |
| MEcs29          | MK948030    | <sup>20</sup> France (Var)  |
| MEcs30          | MK948031    | <sup>20</sup> France (Loup)   |
| MEcs31          | MK948032    | <sup>20</sup> France (Rhône)  |
| MEcs32          | MK948033    | <sup>20</sup> France (Roya)   |
| ME-nin63        | MG194732    | <sup>15</sup> Italy (Sisto)   |
| MAcs1           | AY836365    | <sup>3</sup> Slovenia (Soča), <sup>6</sup> Greece (Aliakmon), <sup>8</sup> Italy (Adige), Italy (Po)  |
| Ma2a            | DQ841189    | <sup>8</sup> Italy (Adige), <sup>20</sup> North Italy (Po)  |
| Ma2b            | DQ841190    | <sup>8</sup> Italy (Adige), <sup>20</sup> North Italy (Po)  |
| Ma2c            | JQ582461    | <sup>9</sup> Italy (Adige), <sup>20</sup> North Italy (Po)  |
| ScarMA1 (C201)  | KJ834841    | <sup>16</sup> Italy (Garda Lake)  |

|                |          |  |
|----------------|----------|--|
| ScarMA2 (C024) | KJ834825 | <sup>16</sup> Italy (Garda Lake)                             |
| marm1          | KJ834770 | <sup>16</sup> North Italy (Po, Adige), Slovenia (Soca River) |
| Mak1           | JX846931 | <sup>19</sup> Croatia (Krka)                                 |
| MAsl-1         | MK948036 | <sup>20</sup> North Italy (Po)                               |

- <sup>1</sup> Berrebi, P., Tougard, C., Dubois, S., Shao, Zh., Koutseri, I., Petkovski, S. & Crivelli, A. J. 2013. Genetic diversity and conservation of the Prespa trout in the Balkans. *International Journal of Molecular Sciences* 14: 23454–23470.
- <sup>2</sup> Cortey, M. & García-Marín, J. L. 2002. Evidence for phylogeographically informative sequence variation in the mitochondrial control region of Atlantic brown trout. *Journal of Fish Biology* 60: 1058–1063.
- <sup>3</sup> Cortey, M., Pla, C. & García-Marín, J. L. 2004. Historical biogeography of Mediterranean trout. *Molecular Phylogenetics and Evolution* 33: 831–844.
- <sup>4</sup> Cortey, M., Vera, M., Pla, C. & García-Marín, J. L., 2009. Northern and Southern expansions of Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. *Biological Journal of the Linnean Society* 97: 904–917.
- <sup>5</sup> Jadan, M., Strunjak-Perović, I., Topić Popović, N. & Čož-Rakovac, R. 2015. Three major phylogenetic lineages of brown trout (*Salmo trutta* Linnaeus, 1758) in the Krka River system (Croatia) revealed by complete mitochondrial DNA control region sequencing. *Journal of Applied Ichthyology* 31: 192–196.
- <sup>6</sup> Kohout, J., Šedivá, A., Apostolou, A., Stefanov, T., Marić, S., Gaffaroğlu, M. & Šlechta, V. 2013. Genetic diversity and phylogenetic origin of brown trout *Salmo trutta* populations in eastern Balkans. *Biologia* 68: 1229–1237.
- <sup>7</sup> Marić, S., Sušnik Bajec, S., Schöffmann, J., Kostov, V. & Snoj, A. (2017). Phylogeography of stream-dwelling trout in the Republic of Macedonia and a molecular genetic basis for revision of the taxonomy proposed by S. Karaman. *Hydrobiologia* 785: 249–260.
- <sup>8</sup> Meraner, A., Baric, S., Pelster, B. & Dalla-Via, J. 2007. Trout (*Salmo trutta*) mitochondrial DNA polymorphism in the centre of the marble trout distribution area. *Hydrobiologia* 579: 337–349.
- <sup>9</sup> Meraner, A., Gratton, P., Baraldi, F. & Gandolfi, A. 2013. Nothing but a trace left? Autochthony and conservation status of Northern Adriatic *Salmo trutta* inferred from PCR multiplexing, mtDNA control region sequencing and microsatellite analysis. *Hydrobiologia* 702: 201–213.
- <sup>10</sup> Sušnik, S., Snoj, A., Wilson, I. F., Mrdak, D. & Weiss, S. 2007. Historical demography of brown trout (*Salmo trutta*) in the Adriatic drainage including the putative *S. letnica* endemic to Lake Ohrid. *Molecular Phylogenetics and Evolution* 44: 63–76.
- <sup>11</sup> Berrebi P, Caputo Barucchi V, Splendiani A, Muracciole S, Sabatini A, Palmas F, Tougard C, Arculeo M, Maric S (2019) Brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. *Hydrobiologia* 826:209–231.
- <sup>12</sup> Snoj, A., S. Marić, P. Berrebi, A. Crivelli, S. Shumka & S. Sušnik, 2009. Genetic architecture of trout from Albania as revealed by mtDNA control region variation. *Genetics Selection Evolution*, 41: 22
- <sup>13</sup> Snoj, A., Glamuzina, B., Razpet, A., Zablocki, J., Bogut, I., Lerceteau-Köhler, E., Pojskić, N., Sušnik, S., 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. *Hydrobiologia* 651, 651, 199–212.
- <sup>14</sup> Kohout J., Šedivá A., Apostolou A., Stefanov T., Marić S., Gaffaroğlu M., et al. (2013). Genetic diversity and phylogenetic origin of brown trout *Salmo trutta* populations in eastern Balkans. *Biologia*, 68, 1229–1237.
- <sup>15</sup> Fabiani A, Gratton P, Zappes IA, Seminara M, D’Orsi A, Sbordoni V, Allegrucci G (2018) Investigating the genetic structure of trout from the Garden of Ninfa (central Italy): suggestions for conservation and management. *Fishery Management and Ecology* 25:1–11.
- <sup>16</sup> Gratton, P., Allegrucci, G., Sbordoni, V. & Gandolfi, A. (2014). The evolutionary jigsaw puzzle of the surviving trout (*Salmo trutta* L. complex) diversity in the Italian region. A multilocus Bayesian approach. *Molecular Phylogenetics and Evolution* 79, 292–304.
- <sup>17</sup> Vera, M. (2006). Estudio de las variaciones espaciales y temporales de la diversidad genética de la trucha común, *Salmo trutta*, en ríos de la Península Ibérica. PhD thesis, Girona, 22 de Marzo de 2006.
- <sup>18</sup> Vera, M., Bouza, A., Casanova, S., Heras, P., Martínez, J., García-Marín, L. (2019). Identification of an endemic Mediterranean brown trout mtDNA group within a highly perturbed aquatic system, the Llobregat River (NE Spain). *Hydrobiologia* 827, 277–291.
- <sup>19</sup> Jadan, M., Strunjak-Perović, I., Topić Popović, N. & Čož-Rakovac, R. (2015). Three major phylogenetic lineages of brown trout (*Salmo trutta* Linnaeus, 1758) in the Krka River system (Croatia) revealed by complete mitochondrial DNA control region sequencing. *Journal of Applied Ichthyology*, 31, 192–196.
- <sup>20</sup> Present study (new haplotypes)

**Table S3. Demographic indices calculated for three brown trout mtDNA lineages based on control region sequence analysis.**

|           | Neutrality test |                |                 | Mismatch analysis |                | Model     |
|-----------|-----------------|----------------|-----------------|-------------------|----------------|-----------|
|           | F <sub>s</sub>  | D              | R <sub>2</sub>  | SSD               | r              |           |
| <b>ME</b> | <b>-2.874*</b>  | <b>-1.897*</b> | <b>0.124***</b> | <b>0.027**</b>    | <b>0.066*</b>  | Expansion |
| <b>AD</b> | -2.126          | <b>-2.147*</b> | <b>0.121***</b> | <b>0.023**</b>    | <b>0.076**</b> | Expansion |
| <b>MA</b> | -1.019          | -1.070         | <b>0.188***</b> | 0.039             | 0.111          | Stable    |

F<sub>s</sub> = Fu's F statistic, D = Tajima's D statistic, R<sub>2</sub> = Ramos-Osisns and Rozas statistic, SSD = sum of standard deviations of mismatch distribution, r = raggedness index of mismatch distribution.  
 \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

| Substitution rate (%) | Node          | TMRCA (MY) | Lower 95% HPD (MY) | Upper 95% HPD (MY) |
|-----------------------|---------------|------------|--------------------|--------------------|
| 0.75                  | ADporh1-ADrh1 | 0.151      | 0.011              | 0.099              |
|                       | AD            | 0.278      | 0.170              | 0.391              |
|                       | ME            | 0.267      | 0.166              | 0.372              |
|                       | MA            | 0.122      | 0.172              | 0.205              |
| 1                     | ADporh1-ADrh1 | 0.120      | 0.016              | 0.086              |
|                       | AD            | 0.212      | 0.129              | 0.298              |
|                       | ME            | 0.191      | 0.122              | 0.265              |
|                       | MA            | 0.117      | 0.051              | 0.193              |

Table S4. Time to the most common ancestor (TMRCA) estimates for the Mediterranean brown trout lineages AD, ME and MA and for the sub-clade ADporh-1, ADrh-1 with 95% highest probability density (HPD) intervals.