Deceiving appearances: anthropogenic introgressive hybridization affects phenotypically-selected hatchery broodstock used in supportive breeding programmes of the critically endangered marble trout *Salmo marmoratus*, Cuvier (Osteichthyes, Salmoniformes, Salmonidae)*.*

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1   Don't tell a book by its cover: anthropogenic introgressive hybridization affects

2   phenotypically-selected hatchery broodstock used in supportive breeding programmes of

3   the critically endangered marble trout *Salmo marmoratus*, Cuvier (Osteichthyes,

4   Salmoniformes, Salmonidae)*.*

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6   Running head: Don't tell a book by its cover: introgression in wild and reared marble trout.

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19

20   **Abstract**

21   Anthropogenic introgressive hybridization is increasingly common in many ecosystems,

22   with inland waters being particularly sensitive to bioinvasions of interfertile non-native taxa.

23   Salmonid native populations are detrimentally affected by such human-induced secondary

24   contacts in several countries promoting sport and commercial salmonid fisheries. In

25   northern Italy, the Critically Endangered marble trout *Salmo marmoratus* Cuvier, 1829, is a

26   species with exceptional cultural, economic, and conservation value. Supporting breeding

27 programs based on phenotypic selection of wild-caught and captive broodstock is a

28 widespread management practice to supplement local marble populations with both

29 declared conservation and exploitation goals. Using mtDNA and nDNA markers (D-loop;

30 15 microsatellites) we compare a hatchery-bred sample of marble trout used as

31 broodstock for support breeding with a smaller sample of wild-caught phenotypically

32 selected individuals collected in the Toce River, a large glacial river within the Lake

33 Maggiore basin (northern Italy and southern Switzerland). We measured genetic integrity

34 and introgression levels with non-native Atlantic *S. trutta*, a non-native species massively

35 introduced in the last two centuries. Consistent levels of introgression were found in these

36 two samples, but asymmetrical introgression with higher mtDNA introgression levels was

37 observed in hatchery-bred individuals. The detected genetic structure of wild-caught and

38 domesticated stocks suggest that in synergy with potential domestication effects,

39 inbreeding and genetic drift, caused by small number of founders and closed reproductive

40 cycle, this practice can have negative effects on the genetic diversity and integrity of the

41 wild population. On the other hand, our preliminary analysis of the Toce River sample

42 suggests that this population may contain genetically pure individuals, thus acting as a key

43 repository of genetic diversity for the long-term conservation of this species. The lack of

44 correspondence between a set of coded coloration traits and genetic traits in both samples

45 highlights the limits of using phenotypic selection during artificial breeding, as also

46 supported by previous studies on this species. Our results strongly suggest to revise

47 current supportive breeding programmes of the marble trout in this system. We finally

48 propose possible future directions for the restoration and sustainable management of the

49 genetic diversity of the Toce River marble trout population.

50

51 Keywords: asymmetrical introgression, conservation genetics, fish conservation, hatchery

52 management, genetic diversity, microsatellites, mtDNA, native fishes.

53

54 **1. Introduction**

55 Fuelled by the ongoing human-induced habitat modifications, translocations, and climate

56 change, anthropogenic introgressive events are increasingly common in many aquatic and

57 terrestrial ecosystems (Ottenburghs 2021). Homoploid introgressive hybridisation is now

58 being acknowledged as a natural evolutionary process promoting change and adaptation

59 in a variety of lineages (Mallet 2007; Meier et al. 2017a, Meier et al. 2017b; Schumer et al.

60 2018; Marques et al. 2019). However, its evolutionary role (e.g., Schumer et al. 2018;

61 Ottenburghs 2021) and underlying mechanisms are still poorly understood (e.g., Gainsford

62 et al. 2020). Adaptive anthropogenic introgression resulting from introductions of non-

63 native species have been documented by genomic studies in a fish (*Fundulus*; Oziolor et

64 al. 2019) and a moth (*Helicoverpa*; Valencia-Montoya et al. 2020), and was also

65 implemented in some carefully planned genetic rescue programmes (Frankham 2015).

66 With these rare exceptions, anthropogenic introgression is typically unintentional and often

67 results in the erosion of native genetic diversity, loss of local genetic adaptations,

68 demographic or genetic swamping, and genetic extinction (Rhymer and Simberloff 1996;

69 Allendorf et al.2001; Todesco et al. 2016). A special case is exemplified by intentional or

70 unintentional introductions of domesticated, inbred, and genetically eroded taxa

71 (Willoughby et al. 2015). Such introductions, whether of native individuals (e.g., in poorly

72 designed reintroduction programmes) or non-native individuals (e.g., in introductions of

73 game birds or fish), can lead to hybridisation and outbreeding depression in wild native

74 populations, lowering their average fitness (Ottenburghs 2021). Therefore, in a

75 conservation perspective, the default strategy remains to implement actions to prevent

76 anthropogenic introgressive hybridisation (ISSG 2000; Todesco et al. 2016; Ottenburghs

77 2021).

78

79   Among aquatic vertebrates, native fish populations are especially sensitive to bioinvasions

80   of non-native fishes, since different taxa are frequently interfertile (Smith 1992; Scribner et

81   al. 2001). In inland waters, the presence of impassable barriers (sea or land) makes

82   freshwater fish populations both naturally fragmented, and geographically and

83   evolutionarily isolated (Oberdorff et al. 1997). Such dispersal barriers make these systems

84   non-equilibrated biogeographic islands that are likely unsaturated with species (Leprieur et

85   al. 2009). Interactions with other widespread anthropogenic stressors, such as habitat

86   alteration and homogenisation, make these systems particularly susceptible to

87   opportunistic invaders (Ricciardi and MacIsaac 2011). Resultantly, native fish populations

88   of intensely managed fisheries can have a low size relative to the number of stocked

89   interfertile non-native individuals, increasing the risk of genetic erosion induced by

90   interbreeding and hybridisation, leading to demographic or genetic swamping, and even

91   driving local populations to genetic extinction (Hansen 2002; Splendiani et al. 2016;

92   Pavlova et al. 2017). Native salmonid populations, fuelling some of the economically most

93   important inland fisheries worldwide (Brown et al. 2019), are heavily affected by

94   interspecific hybridisation with introduced non-native species in several industrialised

95   countries (e.g., Young et al. 2016; Mandeville et al. 2020; Fukui et al. 2021).

96

97   The Italian subendemic marble trout *Salmo marmoratus* Cuvier, 1829 is a species with

98   exceptional cultural, economic, and conservation value (Meraner and Gandolfi 2018a). It is

99   a large salmonid with distinct marbled colouration, and morphological traits (Delling 2002),

100   taxonomically consistent with molecular phylogenetic and phylogeographic reconstructions

101   (reviewed in Polgar et al. 2022a). It is found in lotic and lentic systems of the Adriatic

102   drainage (Meraner and Gandolfi 2018a) and occasionally in seawater (Soldo 2013). In the

103   Northern Adriatic region (Fig. 1; Sommani 1960; Lobón-Cerviá et al. 2019; Splendiani et al.

5

104 2020; Merati et al. 2021), it inhabits the orographic left tributaries of the Po River,

105 characterised by a peculiar geology (Sommani 1960).

106

107 Italian populations of *S. marmoratus* have been increasingly threatened by almost two

108 centuries of massive introductions of domestic strains of non-native Atlantic brown trout

109 *Salmo trutta* Linnaeus, 1758, to support recreational fishing (Sommani 1948; Borroni and

110 Grimaldi 1978; Crivelli 1995; Splendiani et al. 2016; Splendiani et al. 2019). This resulted

111 in well-documented, extensive introgressive hybridisation between the two species

112 (Meraner and Gandolfi 2018a; Giuffra et al. 1996; Lucarda et al. 2000; Meldgaard et al.

113 2007; Meraner et al. 2010; Gibertoni et al. 2014).

114 Although the marble trout is included in the European Union Habitats Directive Annex II

115 (Council Directive 1992), the Least Concern current classification of *S. marmoratus*

116 assigned by the International Union for the Conservation of Nature (IUCN) is not

117 representative of the species. In fact, this is essentially based on few, isolated, and small

118 populations living in Slovenian headwaters (Crivelli 2006), thus being in need of updating.

119 The marble-trout populations of Northern Italy inhabit a much wider variety of

120 environments, including lowlands, and have been much more heavily affected by

121 anthropogenic pressures (Zerunian 2003; Turin et al. 2006; Lucarda 2007). These

122 populations are currently Critically Endangered, due to genetic introgressive hybridisation,

123 habitat destruction, and habitat degradation, that may determine a future 80% decline

124 throughout the whole area (Bianco et al. 2013). Anthropic habitat alterations caused by

125 synergistic changes of land-use, land-cover, and climate (Becciu and Dresti 2015; Saidi et

126 al. 2018) resulted in loss of ecological connectivity, population bottlenecks (Meraner and

127 Gandolfi 2018a), and decreased adaptability to climate warming (Simčič et al. 2015).

128 Natural stochastic events such as floods (Pujolar et al. 2011a) and viral diseases (Pascoli

129 et al. 2015) further impact these vulnerable populations. In the last 5–10 years, the genetic

130   integrity of the marble trout populations of Northern Italy is further threatened by

131   introductions of domesticated, non-native, and potentially interfertile peninsular trout

132   (Gratton et al. 2014; Polgar et al. 2022a, Polgar et al. 2022b).

133   The Toce River basin is located in the subalpine catchment of Lake Maggiore, within the

134   Verbano-Cusio-Ossola (VCO) Province (Fig. 1). Local salmonids include *S. marmoratus*

135   and three non-native taxa: *S. ghigii* Pomini, 1941 (Polgar et al. 2022a; pers. obs.), *S.*

136   *trutta*, and the rainbow trout *Oncorhynchus mykiss* Walbaum, 1792 (Segherloo et al. 2021;

137   Kottelat and Freyhof 2007). The life history traits of the Toce River marble-trout population

138   are currently not known. In the only previous study of this population (Gibertoni et al.

139   2014), the authors assumed that some phenotypic traits indicate the presence of migratory

140   behaviours between the river and lake systems. However, the provided evidence makes it

141   unclear whether these individuals might be native lacustrine-adfluvial or fluvial-adfluvial

142   marble trout populations, straying marble trout stocked in lakes, or hybrids with *S. trutta*

143   with atypical morphology or behaviours.

144

145   Recreational fishing is deeply rooted in the local culture of the VCO Province; from 2014 to

146   2018, a yearly average of ~3,585 resident and non-resident anglers bought a fishing

147   license to fish in VCO waters (F.I.P.S.A.S. VCO, pers. comm.). Trouts stocked yearly into

148   the Toce River have been and are being either translocated from other national and

149   international water bodies, or caught in the wild, phenotypically selected, and restocked

150   using local hatcheries. Currently, the marble-trout fishery is managed implementing

151   supportive breeding, consisting in the release of offspring produced in captivity by wild-

152   caught broodstock in closed or nearly closed reproductive cycles. In the hatcheries active

153   on the Toce River, breeders are routinely selected based on their phenotype, i.e. the lack

154   of black or red spots and parr marks in their adult marbled colouration, considered to be a

155 diagnostic taxonomic trait, and therefore a sign of genetic purity. The progeny of wild-

156 caught founders is used as breeders throughout numerous reproductive cycles, and

157 phenotypically-selected wild-caught individuals (mostly males, more difficult to keep in

158 captivity) are very rarely added to the broodstock (M.I., P.V., pers. obs.). The inefficacy of

159 artificial selection based on phenotypic traits, caused by the scarce correlation between

160 colouration patterns and genotype in this species (Chiesa et al. 2016; Meraner and

161 Gandolfi 2018a), the high level of inbreeding caused by the closed reproductive cycle, and

162 the risk of introducing non-native genes in the restocked wild population, posed by the lack

163 of genetic screening and monitoring and already documented in other molecular

164 investigations of Italian hatchery broodstocks (Chiesa et al. 2016; Splendiani et al. 2019),

165 clearly imply significant conservation risks.

166

167 With the aim to describe the genetic makeup of phenotypically-selected marble-trout

168 breeders and their progeny used for supportive breeding and restocking in the Toce River

169 system, we analysed a sample of marble-trout breeders from the main local hatchery,

170 measuring both introgression rates with non-native *S. trutta* and their sibship. We also

171 gathered baseline genetic data on a smaller sample from the Toce River, preliminarily

172 comparing these two samples. In order to observe whether the measured levels of

173 introgression are associated with different levels of phenotypic purity, we also tested the

174 correspondence between a set of coded colouration traits and the genetic traits used to

175 estimate introgression levels. Based on our results, we highlight the risks of the current

176 hatchery practices and discuss possible directions for future long-term hatchery

177 management and research to restore the genetic diversity of the Toce River marble trout

178 population.

179

180 **2. Methods** (dx.doi.org/10.17504/protocols.io.bp2l692mdlqe/v1)

181   *2.1. Study area, fish sampling, phenotypic selection, age determination*

182   The Toce River (length 83.6 km; catchment area ~1,780 km^2, average slope 2.4%;

183   Regione Piemonte 2004) is one of the main tributaries of Lake Maggiore (Fig. 1), rising

184   from glacier valleys at ~1,720 m above sea level (a.s.l.; Geoportale Piemonte 2021). It is

185   located in the Italian North-western Alps (Marazzi 2005), in the Padano-Venetian

186   ichthyogeographic region (Bianco 1998).

187   Fish samples were collected from May 2016 to November 2020. The sample obtained

188   from the main hatchery of the VCO F.I.P.S.A.S., located in Caddo (Caddo hatchery) (A, n=

189   72) was collected by netting haphazardly with a circular dip net (60 cm in diameter) in a

190   1.5 m (depth) x 5 m (diameter) tank containing >500 hatchery-bred mature and immature

191   marble trouts. The Caddo hatchery broodstock derives from <50 phenotypically-selected

192   individuals collected in the Toce River in the late 1990s, with some individuals also

193   collected from other basins, such as the Stura di Lanzo River in the early 2000s; no wild-

194   caught broodstock were used for at least one decade, managing the stock as a closed

195   reproductive cycle (M.I., pers. obs.). A smaller sample of wild-caught fish (B, n= 27; Tables

196   S1, S2; Fig. 1) was also collected in the middle and lower tracts of the Toce River, using

197   electrofishing and rod-and-line techniques. For electrofishing, we used a built-in-frame

198   EL64GII electrofishing device (Scubla aquaculture, 3.5 KW, 600 V, DC current) with a

199   copper cathode (width 2 cm, length 300 cm) and a steel ring anode (thickness 0.8 cm,

200   diameter 50 cm). Wild-caught fishes were phenotypically selected based on the presence

201   of marbled spots in their colouration pattern (Table 1; Fig. 2), thus simulating the artificial

202   selective process occurring in the hatchery. All fish were mildly anaesthetised after capture

203   (eugenol, i.e. a 1:5 solution of clove oil in ethanol, then adding 2 ml of this solution to 10 l

204   of water, in accordance with relevant guidelines and regulations), measured (total length:

205   *TL*, in cm, to the nearest mm; wet body mass: *W*, in g, to the nearest g), and photographed

206   in lateral view (Figs. S1–S3; Tables S1; S2).

207

208   *2.2. Genetic analyses*

209   Anal fin clips were stored in 96% ethanol at 4°C. Whole genomic DNA was extracted and

210   purified from the fin clips using a KingFisher Cell and Tissue DNA Kit (Thermo Fisher

211   Scientific Inc., Fremont, CA, USA), according to manufacturer protocols. The mitochondrial

212   control region (D-loop) was amplified using LN20 e HN20 primers (Bernatchez and

213   Danzmann 1993). Sanger sequencing was performed using LN20 primer on a 3130XL

214   sequencer (Applied Biosystems). Partial d-loop sequences (531 bp) were aligned with

215   GenBank references and assigned to one of the five major *S. trutta* complex mtDNA

216   lineages (Bernatchez 2001) using BLASTN (Altschul et al. 1990; BLASTN 2018).

217   Individuals were also genotyped at 15 nuclear microsatellite loci amplified with 14 primer

218   pairs (Meraner and Gandolfi 2018b). The loci were genotyped using a 3130XL sequencer

219   (Applied Biosystems) and scored using GeneMapper v.4.0 (Applied Biosystems). The

220   analysis included five reference samples: domesticated Atlantic *S. trutta* (n= 40; *TRUTg*);

221   wild-caught *S. marmoratus* from the Adda River (n= 30; *Adda*), Adige River (n= 30; *Adige*)

222   and Isonzo River (n= 15; *SR*; Meraner and Gandolfi 2018b); and a completely introgressed

223   sample that originated from hybrid Atlantic *S. trutta* x *S. marmoratus* founders collected in

224   the Adige River and reared in a hatchery for several generations (n= 27; *MARMxTRUT*;

225   this study).

226

227   The genetic relationships among samples were investigated with factorial correspondence

228   analysis (FCA) using GENETIX v.4.0 (Belkhir et al. 1999). Population genetic structure

229   and introgression patterns were estimated using STRUCTURE v.2.3.3 (Pritchard et al.

230   2000), which implements a Bayesian clustering algorithm that minimises both linkage and

231 Hardy-Weinberg disequilibria within inferred clusters (*K*). Twenty runs were performed for

232 each value of the genetic clusters from 1 to 10 (100,000 burn-in, 200,000 Markov chain

233 steps, and admixture model with independent allele frequencies). The most likely number

234 of genetic clusters was estimated with the *ΔK* method to describe the uppermost genetic

235 structure (Evanno et al. 2005); and with MedMed K, MedMean K, Max-Med K, and

236 MaxMean K statistics, to describe fine-scale genetic structure (Puechmaille 2016), using

237 StructureSelector (Li and Liu 2018). The individual admixture proportions, i.e., the

238 proportions of membership of each individual to each of the *K* genetic clusters (q values)

239 and their 90% Bayesian credible intervals (BCI) were obtained from a single replicate

240 representative of the mode having the highest mean posterior probability, as estimated by

241 CLUMPAK (Kopelman et al. 2015). The same analysis (*K*= 1–6) was performed on a

242 reduced dataset including only *TRUTg*, *Caddo*, and *Toce* samples. Neighbour-Joining (NJ)

243 trees reconstructing relationships among the detected genetic clusters were built in

244 STRUCTURE v.2.3.3, using the estimated genetic distance among the clusters (matrix of

245 allele-frequency divergence).

246

247 Full-sibship (*FS*, sharing both parents) or half-sibship (*HS*, sharing one parent)

248 relationships and the number of families within and between the *Caddo* and *Toce* samples

249 were estimated with a pairwise- and full-likelihood sibship reconstruction method,

250 respectively, in COLONY v.2.0.6.6 (Jones and Wang 2010), since family relationships

251 could affect the structure analysis (Anderson and Dunham 2008). A polygamous mating

252 scheme was assumed for both sexes (allelic dropout rate= 0.0000, other error rate=

253 0.0001), excluding full-sibship relationships for pairs of individuals not sharing the same

254 mtDNA haplotype (Excluded Maternal Sibship prior).

255

256 *2.3. Correlation between phenotype and genotype*

257 Individual colouration patterns of the head and body (Fig. 2; Figs. S1–S3, S5; Table S1)

258 were observed in digital photos of all individuals (excluding 6 specimens for which photos

259 were unavailable; n= 93; Table S1), transformed into a numerical score, and correlated to

260 a numerical score of their measured genetic makeup. Immature individuals of *S.*

261 *marmoratus* exhibit red and black dots, a preopercular blotch, and parr marks, i.e. non-

262 species-specific colouration traits (Delling et al. 2000; Polgar et al. 2022a, Polgar et al.

263 2022b; Fig. S4). However, sexual maturity could not be observed in 15 wild-caught and 16

264 hatchery-bred individuals (Table S1), likely due to the timing of the sampling sessions

265 relative to the reproductive season. Therefore, assuming correlation between size and

266 sexual maturity, we included in the analysis only individuals with size equal to or larger

267 than that of the smallest sexually mature individual (Caddo: 23.0 cm *TL*, n= 69; Toce: 25.4

268 cm *TL*, n= 14; Table S1).

269

270 Observed colouration elements include six types of "spots" (Fig. 2; Table 1), i.e. round or

271 irregular areas larger than one scale, darker than background, and with distinct margins;

272 one preopercular "blotch", i.e. a round area larger than a spot, darker than background,

273 and with diffused and indistinct margins, typically overlapped with darker spots; and parr

274 "marks", i.e. vertical areas larger than spots and blotches, slightly darker than background,

275 and with diffused and indistinct margins, typically overlapped with darker spots. In order to

276 summarise individual colouration patterns, sets of elements are represented by lowercase

277 italicised letters separated by "/". Each pattern includes (i) three elements (*m*, *f*, *d*) on the

278 scaleless area including the visible portion of the preopeculum, operculum, and cleithrum;

279 (ii) five elements on the body in lateral view, except dorsal and ventral areas (*m*, *f*, *d*, *r*, *p*);

280 (iii) ocellated spots (*c*); (iv) a preopercular blotch (*b*); and (v) parr marks (*k*) (Fig. 2; Table

281 1). In each set, the absence of an element is coded as *0*. The reference phenotype of a

282 sexually mature individual of *S. marmoratus* is defined by the exclusive presence of

12

283   marbled spots, i.e., as *m00/m0000/0/0/0* (Fig. S5; Table S1). The *S. marmoratus* x *S.*

284   *trutta* hybrid phenotype of a sexually mature individual is defined as either *m**/m****/0/0/0*,

285   or ****/m****/0/0/0*, or *m**/*****/0/0/0*, (where *= at least one of any element different than

286   *m*). In order to minimise researcher effects, three different researchers examined the fish

287   sample and coded the colouration patterns, and fish were re-examined to eliminate

288   reading mismatches. Individual phenotypic and genotypic scores (values 0–1) were

289   obtained from coded colouration patterns and genetic data (Table S3; Note S1). A t-test

290   was done, for testing whether the sample Pearson correlation between the phenotypic and

291   genotypic scores differed significantly from 0.

292

293   **3. Results**

294   Native mtDNA haplotypes (MA haplogroup) dominate the Toce River sample (Toce,

295   77.8%), while non-native haplotypes (AT haplogroup) dominate the captive-bred hatchery

296   sample (*Caddo*, 65.3%) (Figs. 3, a_2; 4; Bernatchez et al. 1992; Meraner and Gandolfi

297   2018a; Sanz 2018). Two AT and three MA haplotypes were found. For the covered

298   sequence length, the first AT haplotype is identical to At1e (GenBank Acc. N. DQ841192

299   DQ841192; Meraner et al. 2007), largely diffused in non-native domestic Atlantic lineages

300   [Caddo (n_1= 47); Toce (n_2= 5)]; the second AT haplotype is first described in this study

301   (AT-toce1, GenBank Acc. N. OL504771; n_2= 1). The three MA haplotypes correspond to

302   Ma1a (GenBank Acc. N. DQ841191; Meraner et al. 2007; n_1= 4, n_2= 9); a sequence

303   shared between Ma2a and Ma2b (DQ841189, and DQ841190, respectively; Meraner et al.

304   2007; henceforth in this study: Ma2; n_1= 19, n_2= 11); and MAsl1 (MK948036; Splendiani et

305   al. 2020; n_1= 2, n_2= 1). As with previous studies in this region, we did not find Adriatic (AD)

306   and Mediterranean (ME) mitochondrial haplotypes (Baraldi et al. 2010; Pujolar et al.

307   2011b; Chiesa et al. 2016); however, we also did not find Danubian (DA) haplotypes,

308   which were found in the neighbouring Ticino and Adda basins (Pujolar et al. 2011b).

13

309

310   Along the FCA factor 1, explaining ~5% of total inertia, *S. trutta* (*TRUTg*) is separated from

311   the *S. marmoratus* reference samples and the hybrid reference (*MARMxTRUT*); along this

312   axis, *MARMxTRUT* is overlapped with *Caddo*, *Toce*, and *Adige*, but separated from *Adda*

313   and *SR*. Along factor 2 (~3% of total inertia), *SR* is separated from the other marble trout

314   references. *Adige* plus *MARMxTRUT* are separated from other references along factor 3

315   (~3% of total inertia), and are separated from each other along factor 4 (~2% of total

316   inertia; Fig. 5).

317

318   The analysis of the uppermost genetic structure of the entire dataset identifies cluster A

319   (sky blue) and B (dark green) (Fig. 3a$_1$, 3a$_2$). *TRUTg* has individual q values of cluster A

320   (q_A)~ 1.000 (average q_A or Q_A= 0.997; Fig. 3a$_2$). *Adige*, *Adda*, and *SR* have individual q

321   values of cluster B (q_B)> 0.985 (Fig. 3a$_2$). *MARMxTRUT* individuals have variable

322   assignment proportions for the two clusters (Q_B= 0.633). *Caddo* has a high admixture

323   proportion of cluster B (Q_B= 0.987), with only three individuals having q_B< 0.950. *Toce* has

324   a lower Q_B= 0.858, with 16 individuals having q_B> 0.950, and 11 being variably admixed

325   (q_B 0.424–0.894; Figs. 3 a$_2$; 4 a).

326

327   The analysis of the fine-scale genetic structure of the entire dataset identifies six clusters

328   (C–H; Fig. 3b$_1$, 3b$_2$). *TRUTg*, *Adige*, *Adda*, and *SR* have individual admixture proportions

329   of clusters C, E, F, and G (i.e. q_C, q_E, q_F, q_G), larger than 0.950, respectively.

330   *MARMxTRUT* has high individual admixture proportions of cluster D (Q_D= 0.962); the

331   detection of this cluster is likely the result of the peculiar genetic composition of

332   *MARMxTRUT*, that comes from a captive-bred population that was virtually isolated from

333   the parental species for several generations, thus likely having highly admixed individual

334   genomes (Anderson and Dunham 2008). Most individuals of *Caddo* and *Toce* have

14

335   substantial proportions of cluster H (*Caddo*: q_H 0.809–0.991, Q_H= 0.977; *Toce*: q_H 0.080–

336   0.991, Q_H= 0.834), mirroring the uppermost genetic structure observed in the K= 2

337   solution. *Toce* also has several individuals with substantial proportions of cluster C and D

338   (q_C 0.063–0.500, n= 9; q_D 0.076–0.811, n= 4; Fig. 3b$_2$). The NJ tree shows that clusters E

339   to H are more closely related to each other than to cluster C, while cluster D has an

340   intermediate position. Cluster H, that characterises *Caddo* and *Toce*, is sister to cluster F,

341   which characterises *Adda* (Fig. 3b$_3$). Consistently, *Adda* is the marble trout population

342   geographically closest to *Toce*.

343

344   The analysis of the uppermost genetic structure of the reduced dataset identifies two

345   clusters, as the analysis of the entire dataset, with similar individual admixture proportions

346   (Figs. 3 c$_1$, c$_2$; 4 b). The analysis of the fine-scale genetic structure of the reduced dataset

347   identifies three clusters (K–M, Fig. 3d$_1$). *TRUTg* has individual admixture proportions of

348   cluster K (q_K) ~1.000. *Caddo* has individual values of q_K< 0.05, while eight *Toce* individuals

349   have q_K 0.085–0.472. Both *Caddo* and *Toce* individuals are variably admixed with clusters

350   L (*Caddo*: q_L 0.003–0.985, Q_L= 0.285; *Toce*: q_L 0.004–0.995, Q_L= 0.769) and M (*Caddo*:

351   q_M 0.012–0.995, Q_M= 0.712; *Toce*: q_M 0.003–0.994, Q_M= 0.145), with the two samples

352   showing opposite admixture patterns (Fig. 3d$_2$). In the NJ tree, clusters L and M are

353   closely related, both being distantly related to cluster K (Fig. 3d$_3$).

354

355   Fifteen and 26 full-sibship (*FS*) relationships were estimated within *Caddo*, 1 and 1 within

356   *Toce*, 0 and 2 between them (majority thresholds p> 0.90 and p> 0.50, respectively). Four

357   and 117 half-sibship (*HS*) relationships were estimated within *Caddo*, 4 and 16 within

358   *Toce*, 0 and 13 between them (p> 0.90 and p> 0.50, respectively; Table 2; Fig. 6a).

359   Overall, 79 *FS* families were reconstructed, originated by 29 males and 50 females: 53

360   within *Caddo*, 11 of which represented by more than a single offspring; 25 within *Toce*,

361   with one having 2 offspring; and one more reconstructed family having 2 offspring, each

362   from one of the two samples (Fig. 6b).

363

364   In the Caddo hatchery sample, the most frequent head colouration pattern is *mfd* (65% of

365   the sample) and the most frequent body patterns are *m000* (38%) and *0/0/0* (96%); in the

366   Toce River sample, the most frequent head pattern is still *mfd* (71%) and the most

367   frequent ones on the body are *mfd00/* (57%) and *0/0/0* (79%) (Table 3). Only 3 individuals

368   (Caddo hatchery sample) had pure marbled patterns on both head and body

369   (*m00/m0000/0/0/0*), and no individuals had pure marbled patterns on head and marbled

370   patterns with only red or ocellated spots on body (Table S1). No examined individuals

371   (Caddo hatchery: average size ~38 cm TL; Toce River: ~32 cm TL; Tables 3; S1) showed

372   parr marks, supporting the assumed sexual maturity (Polgar et al. 2022a). Our data don't

373   support any statistically significant correlation between genotype and phenotype scores.

374   The estimated Pearson correlation coefficient is 0.125, and is not statistically significant (p-

375   value ~0.3; Fig. 7).

376

377   **4. Discussion and conclusion**

378   The objective of this study is to investigate the genetic makeup of phenotypically-selected

379   marble-trout breeders and their progeny, used for supportive breeding and restocking of

380   the wild Toce population. For this reason, we only examined trouts with marbled elements

381   in their colouration pattern. In the Caddo hatchery sample, we detected high non-native (*S.*

382   *trutta*) mitochondrial introgression demonstrated by the prevalence of Atlantic haplotypes,

383   as already observed in different marble-trout breeding stocks from three other hatcheries

384   of Northern Italy (Chiesa et al. 2016), and consistent with the past and ongoing massive

385   introductions of non-native *S. trutta* (section 1). While FCA showed an overlap with the

386   hybrid reference along FCA factor 1, in line with the high frequency of Atlantic haplotypes,

387   the Bayesian analysis detected a relatively small signal of non-native ancestry, showing a

388   small genetic distance between Caddo and the marble-trout reference groups. This

389   asymmetric introgression may be related to sex-biased breeders' selection (hybrid mothers

390   and less introgressed fathers; Meraner and Gandolfi 2018a; Lucarda 2007), random drift

391   (Sušnik Bajec et al. 2015), backcrossing (Ostberg et al. 2004), artificial selection of

392   marbled colouration traits, or a combination of these factors.

393

394   Due to the lack of correspondence between colouration traits and genetic makeup,

395   consistent with several other studies (e.g., Delling et al. 2000; Djurdjevič et al. 2019), the

396   use of few phenotypically-selected breeders in a virtually closed reproductive cycle likely

397   increased the frequency of backcrossing or mating between hybrids and the parental

398   species, thus diluting rare genetic traits of the parental species after a few generations

399   (Vrijenhoek 1998; Meraner and Gandolfi 2018b). This is also consistent with the relatively

400   high proportion of full-sibship relationship detected among hatchery-reared individuals,

401   which may have inflated the measured genetic structure (Meraner and Gandolfi 2018b;

402   Anderson and Dunham 2008; Vähä et al. 2006). The possible presence of two slightly

403   different genetic clusters in the hatchery sample, described by the admixture analysis

404   conducted on the reduced dataset, is consistent both with the mentioned translocation of

405   breeders from the Stura di Lanzo River, or with the presence of two distinct groups of more

406   closely related individuals (Anderson and Dunham 2008).

407

408   In the Toce River sample, our preliminary analysis of selected wild-caught individuals may

409   underestimate the actual introgression levels of the population (e.g., Meraner and Gandolfi

410   2018b), since the presence of wild and introgressed marble trout individuals without

411   marbled colouration elements, not included in our sample, cannot be ruled out. The

412   mitochondrial and nuclear introgression levels of phenotypically-determined marble trouts

17

413 of the Toce River are consistent. Twelve out of 27 Toce River samples show both MA

414 haplotype and $q_2 \geq 0.99$; eight of these also have a lower 90% BCI limit that is at least 0.99,

415 thus very likely being purebred marble trouts (Meraner and Gandolfi 2018a, Meraner and

416 Gandolfi 2018b; Fig. 4b). The measured introgression is consistent with previous accounts

417 of *S. marmoratus* x Atlantic *S. trutta* introgressive hybridisation in wild marble trout

418 populations of northern Italy (Meraner and Gandolfi 2018a), including an account from our

419 study area (Gibertoni et al. 2014). This scenario is in line with recent and ongoing

420 anthropogenic hybridisation between the two species in the wild, induced by the ongoing

421 stocking of Atlantic domesticated trout in these waters.

422

423 Although diagnostic elements in the marble trout colouration patterns can be identified

424 (Delling 2002), colouration patterns are influenced by numerous factors in salmonids and

425 in teleosts in general. These include social interactions (Griffith et al. 2006; Watt et al.

426 2017), reproductive status (Mobley et al. 2021), ontogenetic stages (Delling 2002; Polgar

427 et al. 2022a, Polgar et al. 2022b), environmental conditions, including domestication, and

428 genetic makeup (Westley et al. 2013; Jørgensen et al. 2018). Although we did not control

429 for several of these possibly confounding factors, the observed absence of correlation

430 between colouration patterns and mitochondrial plus nuclear genes strongly supports the

431 inefficacy of phenotype-based artificial selection in supportive breeding programmes of *S.*

432 *marmoratus*. Perhaps more importantly, virtually all the examined domesticated marble

433 trout had colouration elements that are typically found in non-native *Salmo* species,

434 demonstrating either a high level of subjectivity in the artificial selection, or relatively loose

435 selective criteria, or both. This also suggests limited ongoing intentional selection on

436 broodstock colouration patterns, implying that phenotypic selection essentially occurred

437 during the early establishment of the breeding stock, more than a decade ago. On the

438 other hand, domestication can also effectively and unintentionally select a variety of

439   biological traits, including wound healing, immune responses, metabolism and even anti-

440   predatory behaviours, even after a single generation (Tymchuk et al. 2009; Christie et al.

441   2015).

442

443   Supportive breeding is a widely applied practice since the '90s (e.g., Ryman and Laikre

444   1991), with both the declared conservation goal of preventing the introduction of non-

445   native genes into the managed system, and of increasing the wild population size, thus

446   sustaining the local recreational fishery. However, domestication effects and typically

447   smaller size and higher reproductive output of the hatchery population relative to the wild

448   population entail risks of (i) inbreeding and drift-mediated loss of genetic variability

449   (Hansen et al. 2000; Wang and Ryman 2001), and (ii) carry-over of genetic domestication

450   effects in wild-born descendants of captive-bred parents (Araki et al. 2009). Such risks are

451   exacerbated in closed reproductive cycles, since no new native genes are periodically

452   introduced from selected wild breeders, thus facilitating genome-wide or region-specific

453   inbreeding depression (Paul et al. 2022) and possibly loss of wild, locally adaptive genetic

454   variants (Garcia de Leaniz et al. 2007). Such effects can be forestalled through specifically

455   designed breeding designs, pedigree analyses, and open reproductive cycles (Vrijenhoek

456   1998; Giles et al. 2004; Anderson et al. 2020; Gandolfi et al. 2020).

457

458   The presence of genetic introgression within the hatchery system, likely a consequence of

459   hatchery protocols and phenotypic selection of a limited number of founders, is at odds

460   with the attempt to conserve and support the natural breeding of this endangered species

461   (Meraner and Gandolfi 2018a, Meraner and Gandolfi 2018b). Even low admixture levels

462   may induce outbreeding depression (Muhlfeld et al. 2009) and eventually fuel the

463   formation of hybrid swarms in the wild population, a critical threat to its long-term survival

464   (Splendiani et al. 2019). Further, the introduction of captive-bred hybrids into the wild may

465   act as a genetic bridge, promoting hybridisation and introgression between completely or

466   partially reproductively isolated native or non-native species (Largiadèr and Scholl 1995;

467   McDonald et al. 2008). In the last 10–15 years, the introduction in a vast area from the

468   South-western Alps to the Lake of Garda of non-native, variably introgressed, and

469   domesticated "Mediterranean brown trout", collected from a variety of trout populations

470   throughout Italy, well exemplifies this risk (Polgar et al. 2022a, Polgar et al. 2022b).

471

472   Conservation genetics is a modern fundamental tool in the management of salmonid

473   inland sport fisheries (Meraner and Gandolfi 2018a; Ayllón et al. 2019). The marble trout is

474   a precious subendemism of northern Italy and the risks of genetic erosion and

475   introgressive hybridisation when introducing non-native salmonids has been repeatedly

476   demonstrated (e.g., Meraner and Gandolfi 2018a; Bianco et al. 2013). On the other hand,

477   the moderate levels of introgression in the selected individuals from the Toce River

478   observed in our preliminary analysis suggest that this population might be a potentially key

479   repository of genetic diversity for the long-term conservation of this species.

480

481   With the overarching goal of increasing the presence of the native marble trout genome in

482   this system and considering the measured threats, our results strongly suggest to (i) stop

483   introductions of non-native trout species and populations (MATTM 2020; Polgar et al.

484   2022a, Polgar et al. 2022b); (ii) describe the genetic structure and possible

485   microgeographic patterns of marble trout subpopulations, thus identifying evolutionary

486   significant units for conservation (ESUs; Meraner and Gandolfi 2018a; Splendiani et al.

487   2020; Moritz 1994); (iii) implement ESU-specific supportive breeding programmes,

488   genetically testing and selecting breeders without significant introgression signatures from

489   the same populations; (iv) implement modern practices in hatchery management, such as

490   routine genetic screening and selection of breeders, using them for only one reproductive

491 cycle (Ferguson 2007; Araki et al. 2007; Rodriguez Barreto et al. 2019; Gandolfi et al.

492 2020); (v) monitor temporal dynamics of spatial patterns of genetic diversity; and (vi)

493 evaluate the potential for site-specific genetic conservation and restoration actions.

494

495 The long-term sustainability of inland recreational fisheries is a priority in industrialised

496 countries, in dire need of shared economic, research, and conservation-oriented goals

497 (Cooke et al. 2015). Management strategies of such socio-ecological systems must merge

498 the priorities and needs of all the stakeholders, from conservation scientists to anglers, if

499 the common goal of bequeathing the structure and function of these valuable ecosystems

500 to future generations is to be achieved.

501

502 **Acknowledgements**

510

511 **Funding**
515

516 **Competing interests**
517 The authors declare no competing interests. The funders had no role in the design of the
518 study, in the collection, analyses, or interpretation of data, in the writing of the manuscript,
519 or in the decision to publish the results.
520

521 **Data availability statement**
522 The datasets generated during and/or analysed during the current study are available from
523 the corresponding author on reasonable request.
524

References

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16: 613-622. https://doi.org/10.1016/S0169-5347(01)02290-X.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anderson EC, Dunham KK (2008) The influence of family groups on inferences made with the program STRUCTURE. Molecular Ecology 8: 1219-1229. https://doi.org/10.1111/j.1755-0998.2008.02355.x.

Anderson JH, Warheit KI, Craig BE, Seamons TR, Haukenes AH (2020) A review of hatchery reform science in Washington State. Washington Department of Fish and Wildlife. Final report to the Washington Fish and Wildlife Commission, 23/1/2020: 1-168.

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318(5847):100-103. https://doi.org/10.1126/science.1145621.

Araki H, Cooper B, Blouin MS (2009) Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. Biology Letters 5: 723-725. https://doi.org/10.1098/rsbl.2009.0315.

Ayllón D, Nicola GG, Elvira B, Almodóvar A (2019) Optimal harvest regulations under conflicting tradeoffs between conservation and recreational fishery objectives. Fisheries Research 216: 47-58. https://doi.org/10.1016/j.fishres.2019.03.021.

Baraldi F, Lunelli F, Gandolfi A (2010) Stima dell'introgressione tra linee autoctone di trota marmorata del bacino del fiume Adige e ceppi introdotti di trota fario mediante marcatori molecolari nucleari e mitocondriali. Studi Trentini di Scienze Naturali 87: 53-59.

Barbanti L (1994) Osservazioni sul Lago Maggiore. Lineamenti del Territorio del Verbano. Circolo del Pallanzotto (Verbania): 1-113.

Becciu G, Dresti C (2015). Uncertainties in flow duration curves in anthropized catchments. In: Brebbia, CA (Ed) Management of natural resources, sustainable development and ecological hazards IV. WIT Transactions on Ecology and the Environment 199: 123-134. https://doi.org/10.2495/RAV150111.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1999) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. https://kimura.univ-montp2.fr/genetix/.

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. https://doi.org/10.1111/j.0014-3820.2001.tb01300.x.

23

574   Bernatchez L, Danzmann RG (1993) Congruence in control-region sequence and
575   restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill).
576   Molecular Biology and Evolution 10: 1002-1014.
577   https://doi.org/10.1093/oxfordjournals.molbev.a040062.

579   Bernatchez L, Guyomard R, Bonhomme F (1992) DNA sequence variation of the
580   mitochondrial control region among geographically and morphologically remote European
581   brown trout *Salmo trutta* populations. Molecular Ecology 1: 161-173.
582   https://doi.org/10.1111/j.1365-294x.1992.tb00172.x.

584   Bianco PG (1998) Freshwater fish transfers in Italy: history, local modification of fish
585   composition, and a prediction on the future of native populations. In: Cowx IG (Ed)
586   Stocking and introductions of fishes. Blackwell Science (Oxford): 167-185.

588   Bianco PG, Caputo V, Ferrito V, Lorenzoni M, Nonnis Marzano F, Stefani F, Sabatini A,
589   Tancioni L (2013) *Salmo marmoratus*. The IUCN Italian Red List of Threatened Species.
590   http://www.iucn.it/scheda.php?id=-788860032.

592   BLASTN (2018) Standard Nucleotide Basic Local Alignment Search Tool.
593   https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandPAGE_TYPE=BlastSearcha
594   ndLINK_LOC=blasthome.

596   Borroni I, Grimaldi E (1978) Fattori e tendenze di modificazione dell'ittiofauna italiana
597   d'acqua dolce. Italian Journal of Zoology 45: 63-73.
598   https://doi.org/10.1080/11250007809440267.

600   Brown JC, Lokensgard KH, Snyder S, Draper M (2019) The cultural currents and social
601   values of trout. In: Kershner JL, Williams JE, Gresswell RE, Lobón-Cerviá J (Eds) Trout
602   and char of the world. American Fisheries Society (Bethesda): 65-93.

604   Chiesa S, Filonzi L, Ferrari C, Vaghi M, Bilò F, Piccinini A, Zuccon G, Wilson RC, Ulheim
605   J, Nonnis Marzano F (2016) Combinations of distinct molecular markers allow to
606   genetically characterize marble trout (*Salmo marmoratus*) breeders and stocks suitable for
607   reintroduction plans. Fisheries Research 176: 55-64.
608   https://doi.org/10.1016/j.fishres.2015.12.009.

610   Christie MR, Marine ML, Fox SE, French RA, Blouin MS (2015) A single generation of
611   domestication heritably alters the expression of hundreds of genes. Nature
612   Communications 7, 10676. https://doi.org/10.1038/ncomms10676.

614   Cooke S, Arlinghaus R, Johnson BM, Cowx IG (2015) Recreational fisheries in inland
615   waters. In: Craig JF (Ed) Freshwater fisheries ecology. John Wiley & Sons, Ltd
616   (Chichester): 449-465. https://doi.org/10.1002/9781118394380.ch36.

618   Council Directive (1992) On the Conservation of Natural Habitats and of Wild Fauna and
619   Flora. 92/43/EEC 21/5/1992: 7-50.

621   Crivelli AJ (1995) Are fish introductions a threat to endemic freshwater fishes in the
622   northern Mediterranean region? Biological Conservation 72: 311-319.
623   https://doi.org/10.1016/0006-3207(94)00092-5.

625 Crivelli AJ (2006) *Salmo marmoratus*. The IUCN Red List of Threatened Species 2021 v.
626 3.1. https://www.iucnredlist.org/species/19859/9043279.

628 Delling B (2002) Morphological distinction of the marble trout, *Salmo marmoratus*, in
629 comparison to marbled *Salmo trutta* from River Otra, Norway. Cybium 26: 283-300.
630 https://doi.org/10.26028/cybium/2002-264-004.

632 Delling B, Crivelli AJ, Rubin J-F, Berrebi P (2000) Morphological variation in hybrids
633 between *Salmo marmoratus* and alien *Salmo* species in the Volarja stream, Soca River
634 basin, Slovenia. Journal of Fish Biology 57: 1199-1212.
635 https://doi.org/10.1006/jfbi.2000.1383.

637 Djurdjevič I, Furmanek T, Miyazawa S, Sušnik Bajec S (2019) Comparative transcriptome
638 analysis of trout skin pigment cells. BMC Genomics 20, 359.
639 https://doi.org/10.1186/s12864-019-5714-1.

641 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals
642 using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.
643 https://doi.org/10.1111/j.1365-294X.2005.02553.x.

645 Ferguson A (2007) Genetic impacts of stocking on indigenous brown trout populations.
646 Science Report: SC040071/SR. Environment Agency (Bristol): 1-81.

648 Frankham R (2015) Genetic rescue of small inbred populations: Metaanalysis reveals
649 large and consistent benefits of gene flow. Molecular Ecology 24(11): 2610-2618.
650 https://doi.org/10.1111/mec.13139.

652 Fukui S, Kasugai K, Sawada A, Koizumi I (2021) Evidence for Introgressive Hybridization
653 between Native Dolly Varden (*Salvelinus curilus* (syn. *Salvelinus malma*)) and Introduced
654 Brook Trout (*Salvelinus fontinalis*) in the Nishibetsu River of Hokkaido, Japan. Zoological
655 Science 38(3): 247-251. https://doi.org/10.2108/zs200041.

657 Gainsford A, Jones GP, Hobbs J-PA, Heindler FM, van Herwerden L (2020) Species
658 integrity, introgression, and genetic variation across a coral reef fish hybrid zone. Ecology
659 and Evolution 10(21): 11998-12014. https://doi.org/10.1002/ece3.6769.

661 Gandolfi A, Wieser J, Eisendle D, Meraner A (2020) Progetto MarmoGen, relazione finale
662 e sintesi tecnica di progetto. Fondazione Edmund Mach (Provincia Autonoma di Bolzano):
663 1-54.

665 Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-
666 Horth N, Lajus D, Letcher BH, Youngson AF, Webb JH, Vøllestad LA, Villanueva B,
667 Ferguson A, Quinn TP (2007). A critical review of adaptive genetic variation in Atlantic
668 salmon: implications for conservation. Biological Reviews 82(2): 173-211.
669 https://doi.org/10.1111/j.1469-185X.2006.00004.x.

671 Geoportale Piemonte (2021) Integrated infrastructure for the geographic information of
672 Piemonte, Italy. http://www.geoportale.piemonte.it/geocatalogorp/?sezione=mappa.

674 Gibertoni P, Penserini M, Esposito S, Foglia A, Dagani D, Bazzoni P, Rubin J-F, Fumagalli
675 L (2014) Presence of a migratory lacustrine life-history strategy in the marble trout (*Salmo*

676 *marmoratus*): The case of the native trout population of Lake Maggiore spawning in the
677 Toce River (Italy). Italian Journal of Freshwater Ichthyology 1: 25-37.
678
679 Giles N, Westgarth J, Hewlett N (2004) Management advice for trout, grayling and Arctic
680 char fisheries. Fisheries Technical Manual No. 7. R&D Technical Report W2-045/TR, v.
681 1.0-02/04. Environment Agency (Bristol) 1-294.
682
683 Giuffra E, Guyomard R, Forneris G (1996) Phylogenetic relationships and introgression
684 patterns between incipient parapatric species of Italian brown trout (*Salmo trutta* L.
685 complex). Molecular Ecology 5: 207-220. https://doi.org/10.1046/j.1365-
686 294X.1996.00074.x.
687
688 Gratton P, Allegrucci G, Sbordoni V, Gandolfi A (2014) The evolutionary jigsaw puzzle of
689 the surviving trout (*Salmo trutta* L. complex) diversity in the Italian region. A multilocus
690 Bayesian approach. Molecular Phylogenetics and Evolution 79: 292-304.
691 https://doi.org/10.1016/j.ympev.2014.06.022.
692
693 Griffith SC, Parker TH, Olson VA (2006) Melanin- versus carotenoid-based sexual signals:
694 is the difference really so black and red? Animal Behaviour 71: 749-763.
695 https://doi.org/10.1016/j.anbehav.2005.07.016.
696
697 Hansen MM (2002) Estimating the long-term effects of stocking domesticated trout into
698 wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis
699 of historical and contemporary samples. Molecular Ecology 11(6): 1003-1015.
700 https://doi.org/10.1046/j.1365-294x.2002.01495.x.
701
702 Hansen MM, Nielsen EE, Ruzzante DE, Bouza C, Mensberg K-LD (2000) Genetic
703 monitoring of supportive breeding in brown trout (*Salmo trutta* L.), using microsatellite DNA
704 markers. Canadian Journal of Fisheries and Aquatic Sciences 57: 2130-2139.
705 https://doi.org/10.1139/cjfas-57-10-2130.
706
707 ISSG (Invasive Species Specialist Group, Species Survival Commission, International
708 Union for the Conservation of Nature) (2000) IUCN Guidelines for the Prevention of
709 Biodiversity Loss Caused by Alien Invasive Species (Gland): 1-25.
710
711 Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from
712 multilocus genotype data. Molecular Ecology Resources 10: 551-555.
713 https://doi.org/10.1111/j.1755-0998.2009.02787.x.
714
715 Jørgensen KM, Solberg MF, Besnier F, Thorsen A, Fjelldal PG, Skaala Ø, Malde K, Glover
716 KA (2018) Judging a salmon by its spots: environmental variation is the primary
717 determinant of spot patterns in *Salmo salar*. BMC Ecology 14(14).
718 https://doi.org/10.1186/s12898-018-0170-3.
719
720 Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) CLUMPAK: a
721 program for identifying clustering modes and packaging population structure inferences
722 across K. Molecular Ecology Resources 15: 1179-1191. https://doi.org/10.1111/1755-
723 0998.12387.
724
725 Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Kottelat M (Cornol),
726 Freyhof J (Berlin): 1-646.

727

728 Largiadèr CR, Scholl A (1995) Effects of stocking on the genetic diversity of brown trout
729 populations of the Adriatic and Danubian drainages in Switzerland. Journal of Fish Biology
730 47: 209-255. https://doi.org/10.1111/j.1095-8649.1995.tb06057.x.

731

732 Leprieur F, Brosse S, Garcıá-Berthou E, Oberdorff T, Olden JD, Townsend CR (2009)
733 Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes.
734 Fish and Fisheries 10: 88-97. https://doi.org/10.1111/j.1467-2979.2008.00314.x.

735

736 Li Y-L, Liu J-X (2018) StructureSelector: A web-based software to select and visualize the
737 optimal number of clusters using multiple methods. Molecular Ecology Resources 18: 176-
738 177. https://doi.org/10.1111/1755-0998.12719.

739

740 Lobón-Cerviá J, Esteve M, Berrebi P, Duchi A, Lorenzoni M, Young KA (2019) Trout and
741 char of central and southern Europe and northern Africa. In: Kershner JL, Williams JE,
742 Gresswell RE, Lobón-Cerviá J (Eds) Trout and char of the world. American Fisheries
743 Society (Bethesda): 379-410.

744

745 Lucarda AN (2007) Metodi di ricerca e di gestione finalizzati alla conservazione della trota
746 marmorata. Biologia Ambientale 21: 75-91.

747

748 Lucarda AN, Patarnello T, Forneris G, Pascale M (2000) Population genetics of *S. trutta*
749 *marmoratus*, *S. trutta trutta* and their hybrid of the Pellice River basin (Piemonte, Italy).
750 Quaderni ETP 29: 1-5.

751

752 Mallet J (2007) Hybrid speciation. Nature 446(7133): 279-283.
753 https://doi.org/10.1038/nature05706.

754

755 Mandeville EG, Walters AW, Nordberg BJ, Higgins KH, Burckhardt JC, Wagner CE (2019)
756 Variable hybridization outcomes in trout are predicted by historical fish stocking and
757 environmental context. Molecular Ecology 28(16): 3738-3755.
758 https://doi.org/10.1111/mec.15175.

759

760 Marazzi S (2005) Atlante orografico delle Alpi. SOIUSA. Suddivisione orografica
761 internazionale unificata del sistema alpino. Priuli & Verlucca Editori (Torino): 1-416.

762

763 Marques DA, Meier JI, Seehausen O (2019) A combinatorial view on speciation and
764 adaptive radiation. Trends in Ecology & Evolution 34(6): 531-544.
765 https://doi.org/10.1016/j.tree.2019.02.008.

766

767 MATTM – Ministero dell'Ambiente e della Tutela del Territorio e del Mare (2020) Criteri per
768 la reintroduzione e il ripopolamento delle specie autoctone di cui all'allegato D del decreto
769 del Presidente della Repubblica 8 settembre 1997, n. 357, e per l'immissione di specie e di
770 popolazioni non autoctone (20A02112). Gazzetta Ufficiale della Repubblica Italiana 161: 2-
771 6. https://www.gazzettaufficiale.it/eli/id/2020/04/14/20A02112/sg.

772

773 McDonald DB, Parchman TL, Bower MR, Hubert WA, Rahel FJ (2008) An introduced and
774 a native vertebrate hybridize to form a genetic bridge to a second native species.
775 Proceedings of the National Academy of Sciences 105: 10837-10842.
776 https://doi.org/10.1073/pnas.0712002105.

777

778 Meier JI, Marques DA, Mwaiko S, Wagner CE, Excoffier L, Seehausen O (2017a) Ancient
779 hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications 8: 14363.
780 https://doi.org/10.1038/ncomms14363.
781
782 Meier JI, Sousa VC, Marques DA, Selz OM, Wagner CE, Excoffier L, Seehausen O
783 (2017b) Demographic modelling with whole-genome data reveals parallel origin of similar
784 *Pundamilia* cichlid species after hybridization. Molecular Ecology 26: 123-141.
785 https://doi.org/10.1111/mec.13838.
786
787 Meldgaard T, Crivelli AJ, Jesensek D, Poizat G, Rubin J-F, Berrebi P (2007) Hybridization
788 mechanisms between the endangered marble trout (*Salmo marmoratus*) and the brown
789 trout (*Salmo trutta*) as revealed by in-stream experiments. Biological Conservation 136:
790 602-611. https://doi.org/10.1016/j.biocon.2007.01.004.
791
792 Meraner A, Gandolfi A (2018a) Genetics of the genus *Salmo* in Italy: Evolutionary history,
793 population structure, molecular ecology and conservation. In: Lobón-Cerviá J, Sanz N
794 (Eds) Brown trout: Biology, ecology and management. John Wiley & Sons Ltd
795 (Hoboken/Chichester): 65-102. https://doi.org/10.1002/9781119268352.ch3.
796
797 Meraner A, Gandolfi A (2018b) Anwendung der Genetik im aquatischen Artenschutz:
798 Fokus Marmorierte Forelle. Wasserwirtschaft 108: 35-40. https://doi.org/10.1007/s35147-
799 018-0019-x.
800
801 Meraner A, Baric S, Pelster B, Dalla Via J (2007) Trout (*Salmo trutta*) mitochondrial DNA
802 polymorphism in the centre of the marble trout distribution area. Hydrobiologia 579: 337-
803 349. https://doi.org/10.1007/s10750-006-0479-3.
804
805 Meraner A, Baric S, Pelster B, Dalla Via J (2010) Microsatellite DNA data point to
806 extensive but incomplete admixture in a marble and brown trout hybridization zone.
807 Conservation Genetics 11: 985-998. https://doi.org/10.1007/s10592-009-9942-9.
808
809 Merati F, Pascale M, Perosino GC (2021) Storia della trota mediterranea in Piemonte.
810 Centro Ricerche in Ecologia e Scienze del Territorio (CREST). https://green-
811 crest.blog/area-tecnico-scientifica/.
812
813 Mobley KB, Aykanat T, Czorlich Y, House A, Kurko J, Miettinen A, Moustakas-Verho J,
814 Salgado A, Sinclair-Waters M, Verta J-P, Primmer CR (2021) Maturation in Atlantic salmon
815 (*Salmo salar*, Salmonidae): a synthesis of ecological, genetic, and molecular processes.
816 Reviews in Fish Biology and Fisheries 31: 523-571. https://doi.org/10.1007/s11160-021-
817 09656-w.
818
819 Moritz C (1994) Defining 'evolutionary significant units' for conservation. Trends in Ecology
820 and Evolution 9: 373-375. https://doi.org/10.1016/0169-5347(94)90057-4.
821
822 Muhlfeld CC, Kalinowski ST, McMahon TE, Taper ML, Painter S, Leary RF, Allendorf FW
823 (2009) Hybridization rapidly reduces fitness of a native trout in the wild. Biology Letters 5:
824 328-331. https://doi.org/10.1098/rsbl.2009.0033.
825
826 Oberdorff T, Hugueny B, Guégan J-F (1997) Is there an influence of historical events on
827 contemporary fish species richness in rivers? Comparisons between Western Europe and

North America. Journal of Biogeography 24: 461-467. https://doi.org/10.1111/j.1365-2699.1997.00113.x.

Ostberg CO, Slatton SL, Rodriguez RJ (2004) Spatial partitioning and asymmetric hybridization among sympatric coastal steelhead trout (*Oncorhynchus mykiss irideus*), coastal cutthroat trout (*O. clarki clarki*) and interspecific hybrids. Molecular Ecology 13: 2773-2788. https://doi.org/10.1111/j.1365-294X.2004.02268.x.

Ottenburghs J (2021) The genic view of hybridization in the Anthropocene. Evolutionary Applications 14: 2342-2360. https://doi.org/10.1111/eva.13223.

Oziolor EM, Reid NM, Yair S, Lee KM, Guberman VerPloeg S, Bruns PC, Shaw JR, Whitehead A, Matson CW (2019) Adaptive introgression enables evolutionary rescue from extreme environmental pollution. Science 364(6439): 455-457. https://doi.org/10.1126/science.aav4155.

Pascoli F, Bilò F, Nonnis Marzano F, Borghesan F, Mancin M, Manfrin A, Toffan A (2015) Susceptibility of genotyped marble trout *Salmo marmoratus* (Cuvier, 1829) strains to experimental challenge with European viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV). Aquaculture 435: 152-156. https://doi.org/10.1016/j.aquaculture.2014.09.038.

Paul K, D'Ambrosio J, Phocas F (2022) Temporal and region-specific variations in genome-wide inbreeding effects on female size and reproduction traits of rainbow trout. Evolutionary Applications 15(4): 645-662. https://doi.org/10.1111/eva.13308.

Pavlova A, Beheregaray LB, Coleman R, Gilligan D, Harrisson KA, Ingram BA, Kearns J, Lamb AM, Lintermans M, Lyon J, Nguyen TTT, Sasaki M, Tonkin Z, Yen JDL, Sunnucks P (2017) Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. Evolutionary Applications 10(6), 531-550. https://doi.org/10.1111/eva.12484.

Polgar G, Iaia M, Volta P (2022b) Autoctonia della trota peninsulare *Salmo ghigii* nell'arco alpino: stato attuale delle conoscenze e rischi associati alle semine di "trota fario mediterranea" in Nord Italia. Biologia Ambientale 36: 24-44. https://doi.org/10.30463/es221.003.

Polgar G, Iaia M, Righi T, Volta P (2022a) The Italian Alpine and subalpine trouts. Taxonomy, evolution, and conservation. Biology 11(4), 576. https://doi.org/10.3390/biology11040576.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959. https://doi.org/10.1093/genetics/155.2.945.

Puechmaille SJ (2016) The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Molecular Ecology Resources 16: 608-627. https://doi.org/10.1111/1755-0998.12512.

878 Pujolar JM, Lucarda AN, Simonato M, Patarnello T (2011b) Restricted gene flow at the
879 micro- and macro-geographical scale in marble trout based on mtDNA and microsatellite
880 polymorphism. Frontiers in Zoology 8, 7. https://doi.org/10.1186/1742-9994-8-7.
881
882 Pujolar JM, Vincenzi S, Zane L, Jesensek D, De Leo GA, Crivelli AJ (2011a) The effect of
883 recurrent floods on genetic composition of marble trout populations. PLoS ONE 6. e23822.
884 https://doi.org/10.1371/journal.pone.0023822.
885
886 Regione Piemonte (2004) Indagini e Studi Finalizzati alla Predisposizione del Piano di
887 Tutela delle Acque (D.Lgs. 152/99). Caratterizzazione bacini Idrografici (elab. I.a/5; I.c/7):
888 1-29.
889
890 Ricciardi A, MacIsaac HJ (2011) Impacts of biological invasions on freshwater
891 ecosystems. In: Richardson DM (Ed) Fifty years of invasion ecology: the legacy of Charles
892 Elton. John Wiley & Sons, Ltd. (Chichester): 211-224.
893
894 Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual*
895 Review of Ecology and Systematics 27: 83-109.
896 https://doi.org/10.1146/annurev.ecolsys.27.1.83.
897
898 Rodriguez Barreto D, Garcia de Leaniz C, Verspoor E, Sobolewska H, Coulson M,
899 Consuegra S (2019). DNA methylation changes in the sperm of captive-reared fish: A
900 route to epigenetic introgression in wild populations. Molecular Biology and Evolution
901 36(10): 2205-2211. https://doi.org/10.1093/molbev/msz135.
902
903 Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective
904 population size. Conservation Biology 5: 325-329. https://doi.org/10.1111/j.1523-
905 1739.1991.tb00144.x.
906
907 Saidi H, Dresti C, Manca D, Ciampittiello M (2018) Quantifying impacts of climate
908 variability and human activities on the streamflow of an Alpine river. Environmental Earth
909 Sciences 77, 690. https://doi.org/10.1007/s12665-018-7870-z.
910
911 Sanz N (2018) Phylogeographic history of brown trout: A review. In: Lobón-Cerviá J, Sanz
912 N (Eds) Brown trout: Biology, ecology and management. John Wiley & Sons Ltd
913 (Hoboken/Chichester): 17-64.
914
915 Schumer M, Rosenthal GG, Andolfatto P (2018) What do we mean when we talk about
916 hybrid speciation? Heredity 120: 379-382. https://doi.org/10.1038/s41437-017-0036-z.
917
918 Scribner KT, Page, KS, Bartron ML (2001) Hybridization in freshwater fishes: a review of
919 case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and
920 Fisheries 10: 293-323. https://doi.org/10.1023/A:1016642723238.
921
922 Segherloo IH, Freyhof J, Berrebi P, Ferchaud A-L, Geiger M, Laroche J, Levin BA,
923 Normandeau E, Bernatchez L (2021) A genomic perspective on an old question: *Salmo*
924 trouts or *Salmo trutta* (Teleostei: Salmonidae)? Molecular Phylogenetics and Evolution
925 162, 107204. https://doi.org/10.1016/j.ympev.2021.107204.
926

927 Simčič T, Jesenšec D, Brancelj A (2015) Effect of increased temperature on metabolic
928 activity and oxidative stress in the first life stages of marble trout (*Salmo marmoratus*). Fish
929 Physiology and Biochemistry 41: 1005-1514. https://doi.org/10.1007/s10695-015-0065-6.
930
931 Smith GR (1992) Introgression in fishes: significance for paleontology, cladistics, and
932 evolutionary rates. Systematic Biology 41(1): 41-57. https://doi.org/10.1093/sysbio/41.1.41.
933
934 Soldo A (2013) First marine record of marble trout *Salmo marmoratus*. Journal of Fish
935 Biology 82: 700-702. https://doi.org/10.1111/jfb.12036.
936
937 Sommani E (1948) Sulla presenza del *Salmo fario* L. e del *Salmo marmoratus* Cuv.
938 nell'Italia settentrionale: loro caratteristiche ecologiche e considerazioni relative ai
939 ripopolamenti. Bollettino di Pesca Piscicoltura e Idrobiologia 3: 136-145.
940
941 Sommani E (1960) Il *Salmo marmoratus* Cuv.: sua origine e distribuzione nell'Italia
942 settentrionale. Bollettino di Pesca Piscicoltura e Idrobiologia 15: 40-47.
943
944 Splendiani A, Ruggieri P, Giovannotti M, Pesaresi S, Occhipinti G, Fioravanti T, Lorenzoni
945 M, Nisi Cerioni P, Caputo Barucchi V (2016) Alien brown trout invasion of the Italian
946 peninsula: the role of geological, climate and anthropogenic factors. Biological Invasions
947 18: 2029-2044. https://doi.org/10.1007/s10530-016-1149-7.
948
949 Splendiani A, Giovannotti M, Righi T, Fioravanti T, Nisi Cerioni P, Lorenzoni M, Carosi A,
950 La Porta G, Caputo Barucchi V (2019) Introgression despite protection: the case of native
951 brown trout in Natura 2000 network in Italy. Conservation Genetics 65: 460-473.
952 https://doi.org/10.1007/s10592-018-1135-y.
953
954 Splendiani A, Berrebi P, Tougard C, Righi T, Reynaud N, Fioravanti T, Lo Conte P,
955 Delmastro GB, Baltieri M, Ciuffardi L, Candiotto A, Sabatini A, Caputo Barucchi V (2020)
956 The role of the south-western Alps as a unidirectional corridor for Mediterranean brown
957 trout (*Salmo trutta* complex) lineages. Biological Journal of the Linnean Society 20: 1-18.
958 https://doi.org/10.1093/biolinnean/blaa125.
959
960 Sušnik Bajec S, Pustovrh G, Jesenšek D, Snoj A (2015) Population genetic SNP analysis
961 of marble and brown trout in a hybridization zone of the Adriatic watershed in Slovenia.
962 Biological Conservation 184: 239-250. https://doi.org/10.1016/j.biocon.2015.01.033.
963
964 Todesco M, Pascual MA, Owens GL, Ostevik KL, Moyers BT, Hübner S, Heredia SM,
965 Hahn MA, Caseys C, Bock DG, Rieseberg LH (2016) Hybridization and extinction.
966 Evolutionary Applications 9(7): 892-908. https://doi.org/10.1111/eva.12367.
967
968 Turin P, Zanetti M, Bilò MF (2006) Distribuzione e stato delle popolazioni di trota
969 marmorata nelle acque del bacino dell'Alto Adriatico. Biologia Ambientale 20: 39-44.
970
971 Tymchuk W, Sakhrani D, Devlin R (2009) Domestication causes large-scale effects on
972 gene expression in rainbow trout: Analysis of muscle, liver and brain transcriptomes.
973 General and Comparative Endocrinology 164: 175-183.
974
975 Vähä J-P, Primmer CR (2006) Efficiency of model-based Bayesian methods for detecting
976 hybrid individuals under different hybridization scenarios and with different numbers of loci.
977 Molecular Ecology 15: 63-72. https://doi.org/10.1111/j.1365-294X.2005.02773.x.

978

979 Valencia-Montoya WA, Elfekih S, North HL, Meier JI, Warren IA, Tay WT, Gordon KHJ,
980 Specht A, Paula-Moraes SV, Rane R, Walsh TK, Jiggins CD (2020) Adaptive introgression
981 across semipermeable species boundaries between local *Helicoverpa zea* and invasive
982 *Helicoverpa armigera* moths. Molecular Biology and Evolution 37(9): 2568-2583.
983 https://doi.org/10.1093/molbev/msaa108.
984

985 Vrijenhoek RC (1998) Conservation genetics of freshwater fish. Journal of Fish Biology 53
986 (Suppl. A): 394-412. https://doi.org/10.1111/j.1095-8649.1998.tb01039.x.
987

988 Wang JL, Ryman N (2001) Genetic effects of multiple generations of supportive breeding.
989 Conservation Biology 15: 1619-1631. https://doi.org/10.1046/j.1523-1739.2001.00173.x.
990

991 Watt C, Swanson C, Miller D, Chen L, May C (2017) Social hierarchies override
992 environmental conditions in determining body coloration of brook trout. Journal of
993 Freshwater Ecology 32(1): 575-580. https://doi.org/10.1080/02705060.2017.1360218.
994

995 Westley PAH, Stanley R, Fleming IA (2013) Experimental tests for heritable morphological
996 color plasticity in non-native brown trout (*Salmo trutta*) populations. PLoS ONE 8(11),
997 e80401. https://doi.org/10.1371/journal.pone.0080401.
998

999 Willoughby JR, Fernandez NB, Lamb MC, Ivy JA, Lacy RC, DeWoody JA (2015) The
1000 impacts of inbreeding, drift and selection on genetic diversity in captive breeding
1001 populations. Molecular Ecology 24(1): 98-110. https://doi.org/10.1111/mec.13020.
1002

1003 Young MK, Isaak DJ, McKelvey KS, Wilcox TM, Pilgrim KL, Carim KJ, Campbell MR, Corsi
1004 MP, Horan DL, Nagel DE, Schwartz MK (2016) Climate, demography, and zoogeography
1005 predict introgression thresholds in salmonid hybrid zones in Rocky Mountain streams.
1006 PLoS ONE 11 (11), e0163563. https://doi.org/10.1371/journal. pone.0163563.
1007

1008 Zerunian S (2003) Piano d'azione generale per la conservazione dei pesci d'acqua dolce
1009 in Italia. Quaderni di Conservazione della Natura17. Ministero dell'Ambiente e della Tutela
1010 Territorio – Direzione per la Protezione della Natura, Istituto Nazionale per la Fauna
1011 Selvatica Alessandro Ghigi (Modena): 1-123.
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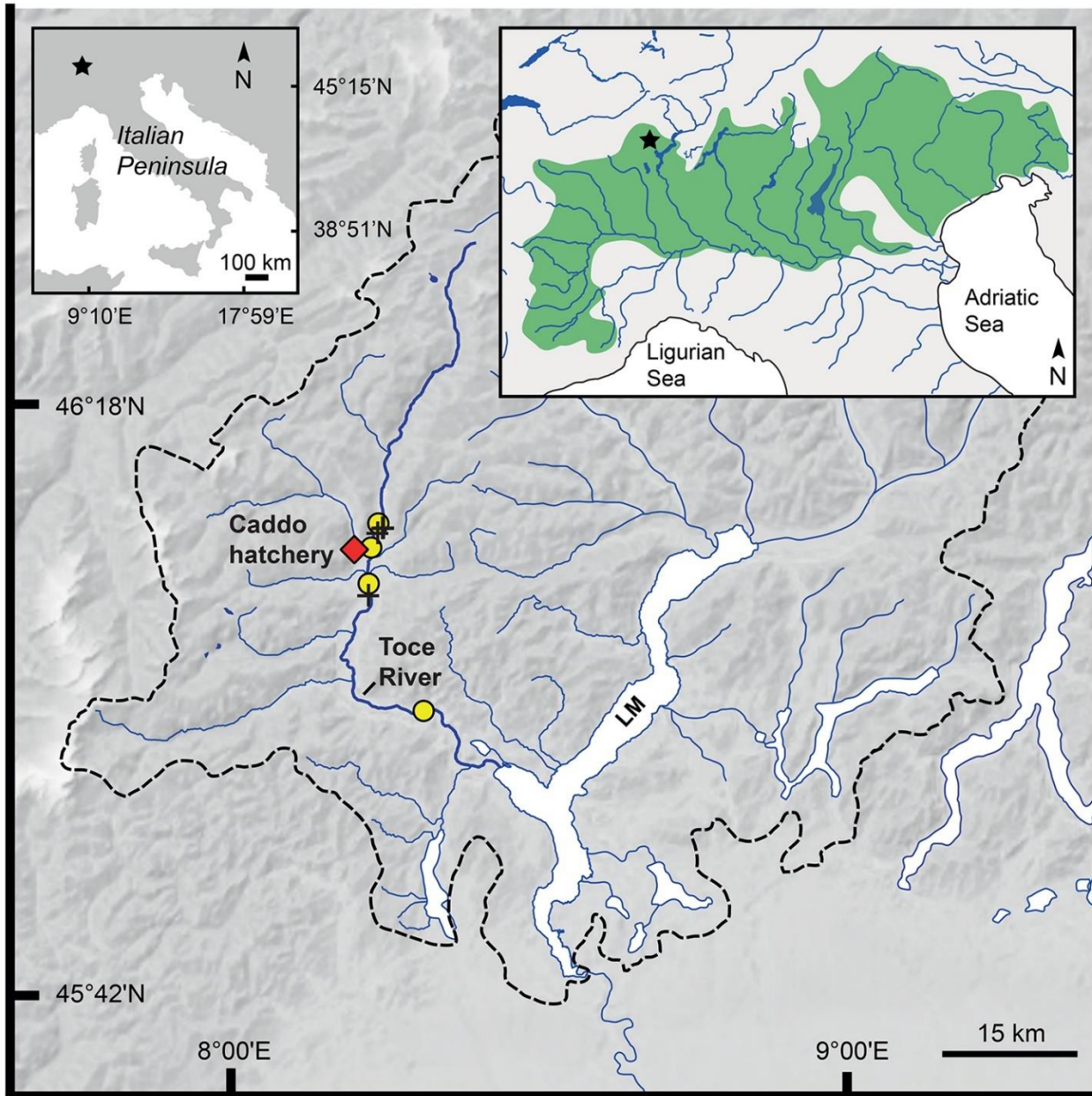
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Figure 1. Study site: Caddo hatchery and Toce River. Drainage divide: *dashed black line* (Barbanti 1994). Left inset: position of the study sites (*star symbol*) in the Italian Peninsula. Right inset: geographical distribution of Italian Northern Adriatic populations of *S. marmoratus* (*green area*), and position of the study sites (*star symbol*). *LM*: Lake Maggiore; *yellow dots*: electrofishing sample (A); *black crosses*: rod-and-line sample (B).

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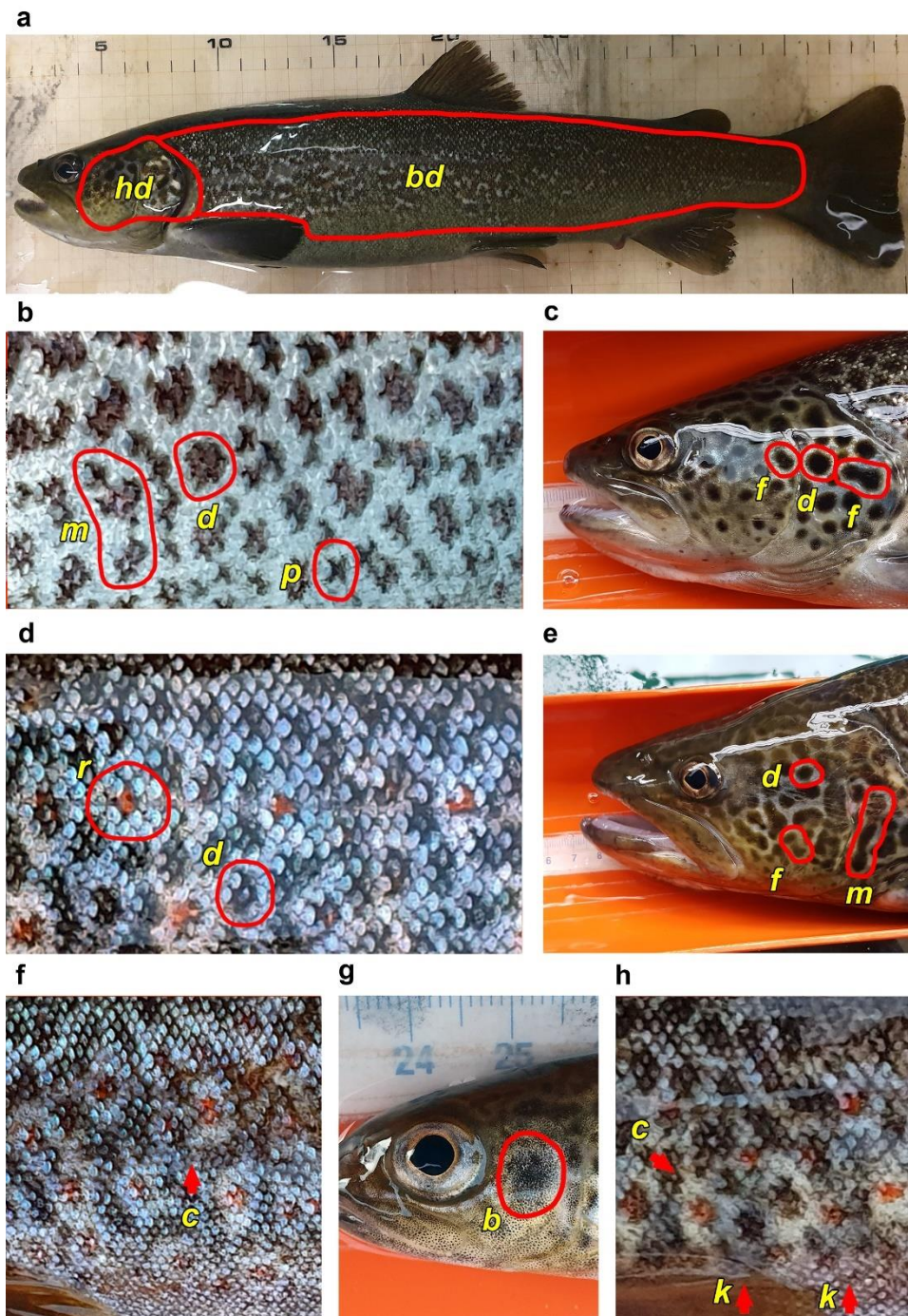
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1024 **Figure 2. Examples of colouration traits in variably introgressed marble trout. a**,
1025 areas examined for the presence of colouration traits; *hd*= scaleless area including the
1026 visible portion of the preopeculum, operculum, and cleithrum areas; *bd*= scaled area
1027 including the flanks, except the dorsal and ventral region, where perspective can alter the
1028 perception of the shape of the colouration traits; **b**, **c**, examples of colouration traits on the
1029 flanks of two trout; *d*= regular dark spot, *f*= fused spot, *m*= marbled spot, *p*= polygonal
1030 spot; **d**, **e**, examples of the same traits on the head of two other specimens; **f**, examples of
1031 *c*= ocellated spot; **g**, examples of *b*= preopercular blotch; **h**, examples of *k*= parr mark and
1032 *r*= regular red spot (Table 1).
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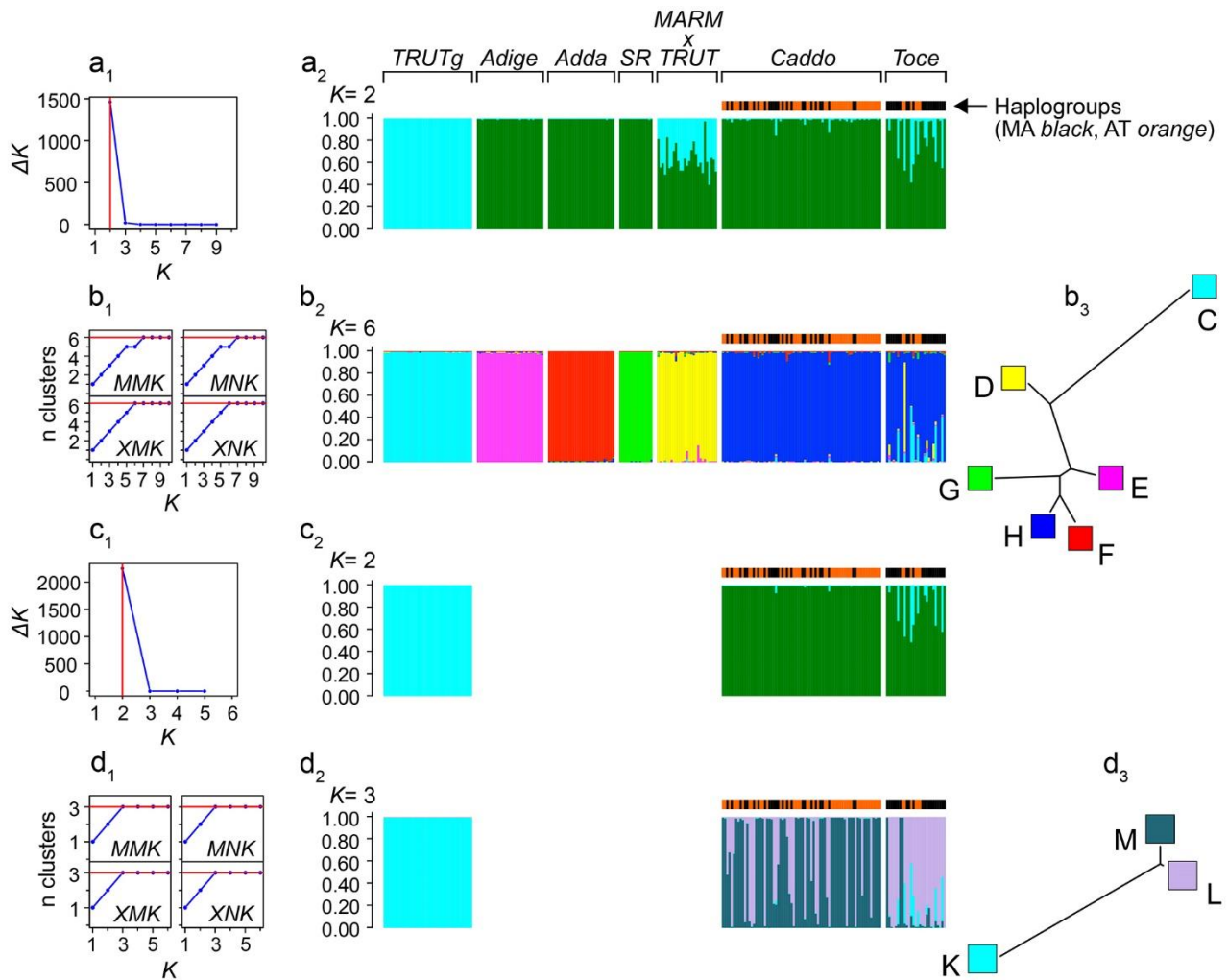
Figure 3. Bayesian clustering analysis (STRUCTURE), based on data from 15 polymorphic microsatellite markers, and haplogroup distribution. The entire dataset (**a**, **b**) includes the two study samples from the captive-bred Caddo hatchery (*Caddo*) plus the Toce River (*Toce*), and the 5 reference samples Atlantic *S. trutta* (*TRUTg*), *S. marmoratus* from the Adige River (*Adige*), *S. marmoratus* from the Adda River (*Adda*), *S. marmoratus* from the Isonzo River (*SR*), and captive-bred *S. marmoratus* x *S. trutta* hybrids (*MARMxTRUT*). The reduced dataset (**c**, **d**) includes *Caddo*, *Toce*, and *TRUTg*. The uppermost genetic structure (**a₁**, **c₁**) is described by ΔK statistics as a function of two genetic clusters ($K= 2$). The fine-scale genetic structure (**b₁**, **d₁**) is described by MedMed K (*MMK*), MedMean K (*MNK*), Max-Med K (*XMK*), and MaxMean K (*XNK*) statistics as a function of six and three genetic clusters ($K= 6$ and 3, respectively). Barplots (**a₂**, **b₂**, **c₂**, **d₂**) illustrate the individual admixture proportions of the identified clusters. For *Caddo* and *Toce*, the mtDNA D-loop haplogroup (MA, AT) is indicated on top of the admixture barplots. NJ distance trees are illustrated (**b₃**, **d₃**) for the entire ($K= 6$) and simplified ($K= 3$) datasets, respectively.

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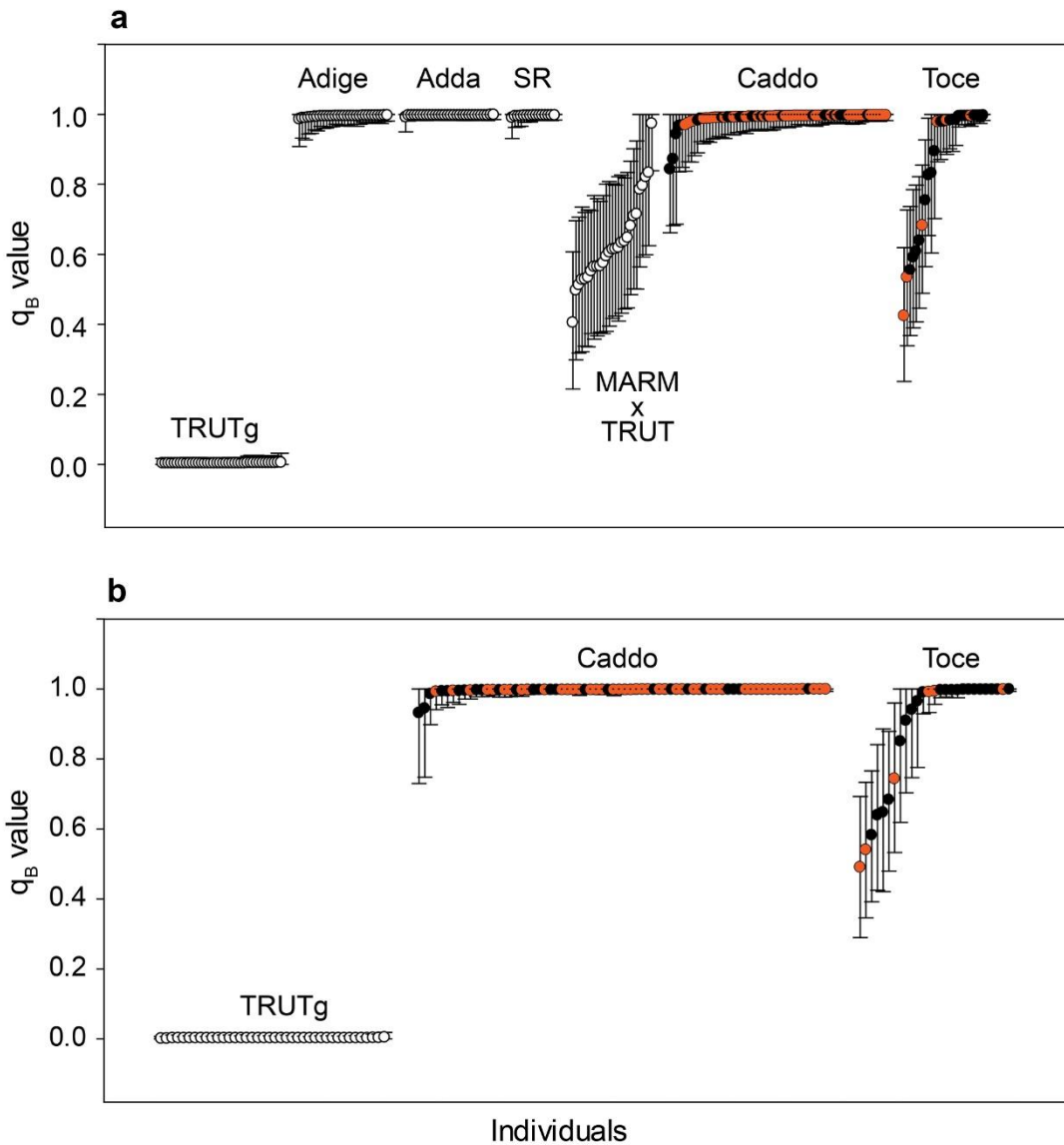
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Figure 4. Individual admixture proportions at the uppermost genetic structure level. **a** entire dataset; **b** reduced dataset; *whiskers*: 90% BCIs. In both graphs, individuals are in ascending order of q_B value along the x-axis. *Black* and *orange* colours in the *Caddo* and *Toce* samples indicate MA and AT haplogroups, respectively. Other abbreviations and details in Fig. $3a_2$, $3c_2$.

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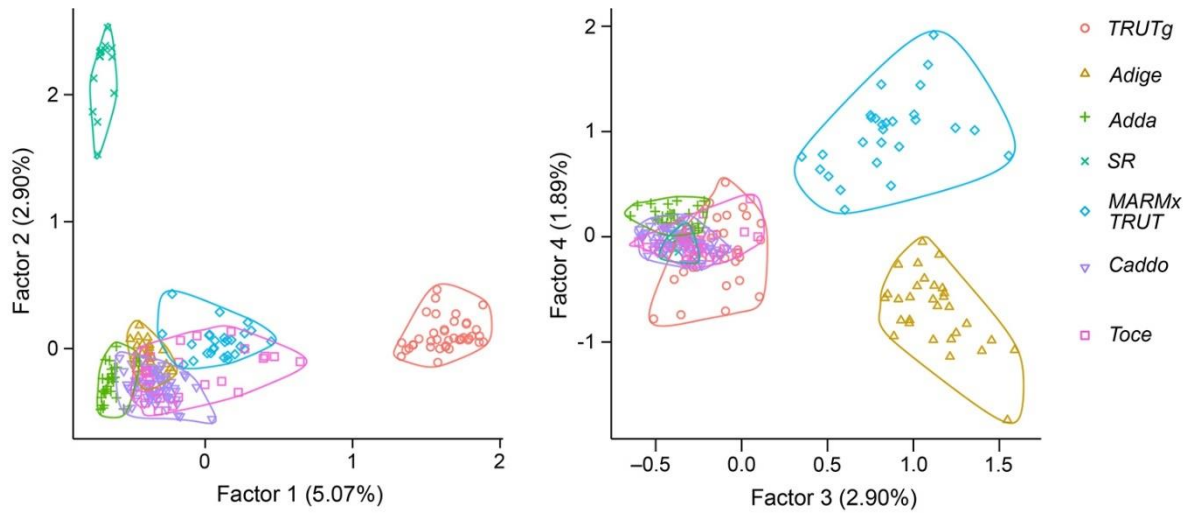
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Figure 5. Factorial Correspondence Analysis (FCA). Genetic data from 15 polymorphic microsatellite markers, including hatchery captive-bred (*Caddo*) and Toce River (*Toce*) phenotypically-determined *S. marmoratus*, and a set of 5 reference samples including *S. marmoratus* from the Adda River (*Adda*), Adige River (*Adige*), and Isonzo River (*SR*), a reference sample of captive-bred *S. marmoratus* x Atlantic *S. trutta* hybrids (*MARMxTRUT*), and a sample of domesticated Atlantic *S. trutta* (*TRUTg*). Percentages of total inertia of the FCA factors 1–4 are *in parentheses*. Individuals from each sample are indicated with different combinations of symbols and colours.
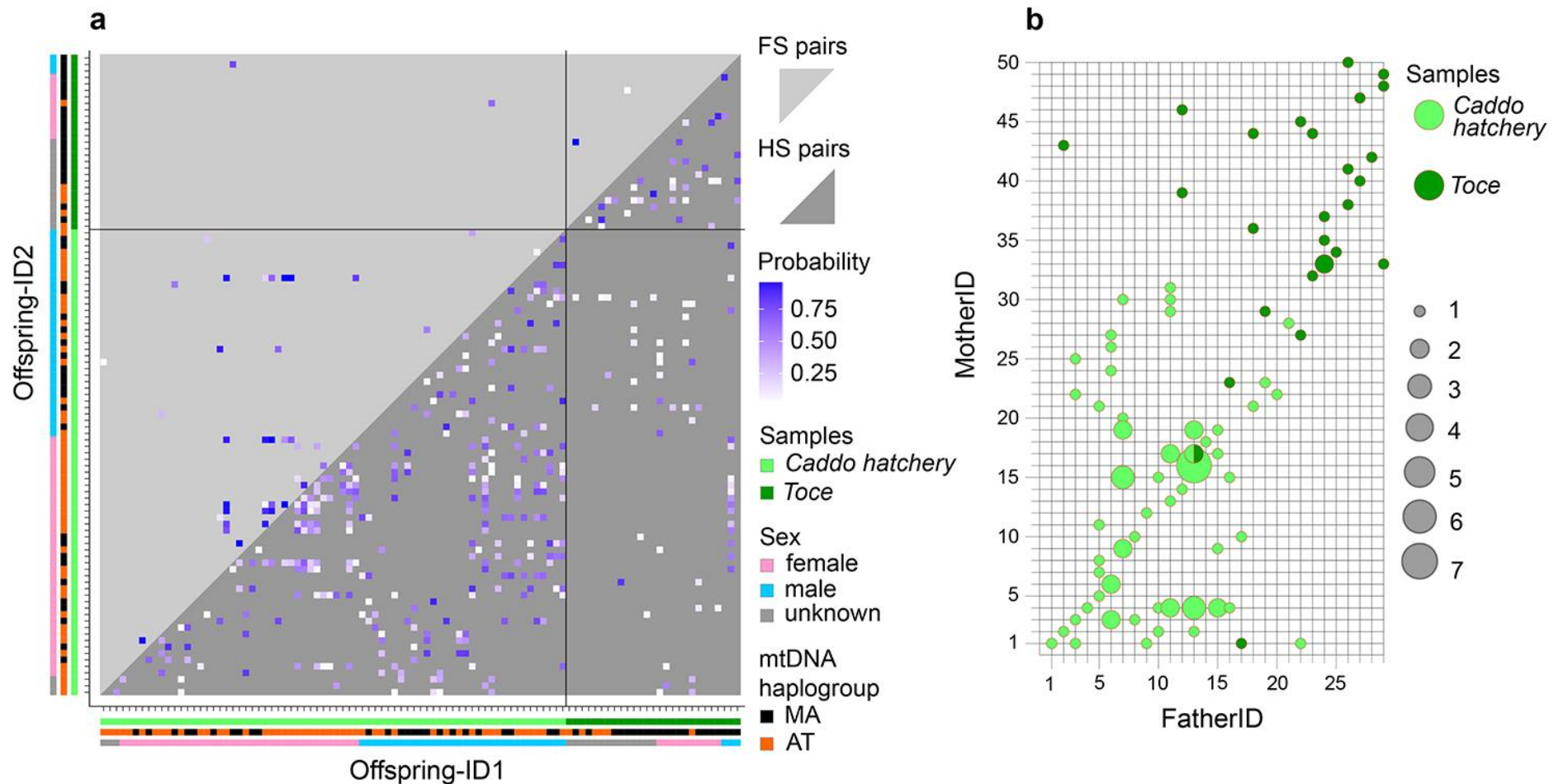
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Figure 6. Sibship and reconstructed families. **a** pairwise-likelihood sibship arrangement of the hatchery (*Caddo*) and Toce River (*Toce*) marble-trout samples; full- (*FS*) or half-sibship (*HS*) probabilities were estimated for each pair of individuals using multilocus genotypic data; **b** full-likelihood reconstructed *FS* families.
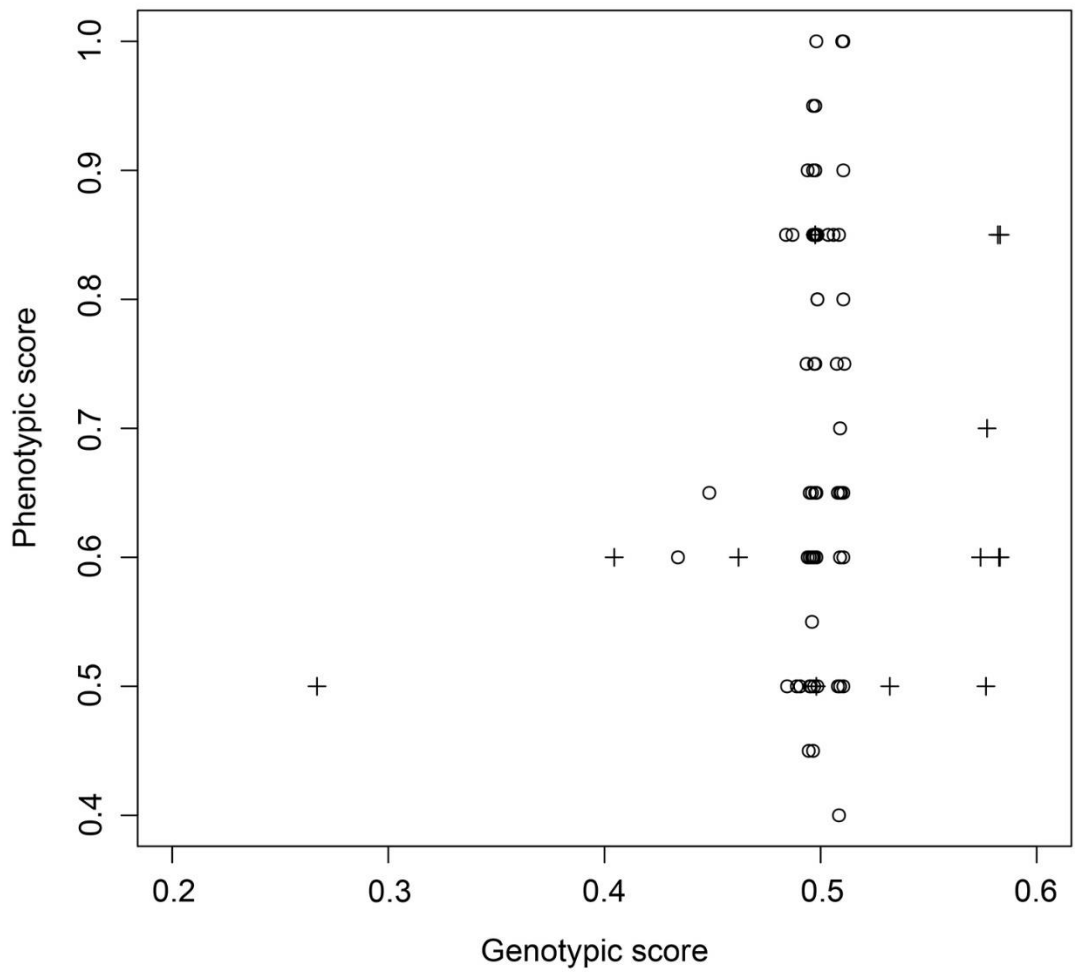
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Figure 7. Correlation between phenotype and genotype. Plotted phenotypic and genotypic scores of *S. marmoratus*; *crosses*: Toce River sample, *empty circles*: Caddo hatchery sample. There is no significant correlation between the scores (Pearson's r 0.125, t-test p value 0.26, 95% CI –0.09, 0.33).

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1077 **Table 1.** Live colouration traits in lateral view, used to describe different trout phenotypes.
1078 *Codes*: letters used to describe the coded colouration pattern.

Colouration traits: definitions	Codes
Marbled spot: spot with amoeboid shape	*m*
Fused spot: two regular spots overlapped or connected by a streak of pigment	*f*
Regular dark spot: dark brown or black spot with round or elliptical shape	*d*
Regular red spot: red spot of round or elliptical shape, also overlapped with other spots	*r*
Polygonal spot: dark brown or black spot of polygonal or stellate shape	*p*
Ocellated spot: regular or irregular spot surrounded by an areola paler than both spot and background colour	*c*
Preopercular blotch: oval blotch in preopercular area	*b*
Parr marks: large marks along flanks	*k*

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Table 2. Pairs and sibship relationships within and between the sequenced and genotyped Caddo hatchery and Toce marble-trout samples. *FS*: full-sibship; *HS*: half-sibship (Fig. 6); *n*: number of individuals; *n pairs*: number of pairs.

Pairs/ relationships	n	n pairs
Caddo	72	2,556
Toce	27	351
between	0	1,944
overall	99	4,851
FS	p> 0.90	p> 0.50
Caddo	15	26
Toce	1	1
between	0	2
overall	16	29
HS	p> 0.90	p> 0.50
Caddo	4	117
Toce	4	16
between	0	13
overall	8	146

084 **Table 3.** Frequency of colouration patterns of *S. marmoratus* in the Caddo hatchery and in Toce
085 samples. *Caddo hatchery*: Caddo hatchery sample (n= 69); *Toce River*: Toce River sample (n=
086 14); *n*: number of individuals. *M*: male, *F*: female, *Head*: coded head colouration patterns, *Body*:
087 coded body colouration patterns, *TL*: total length (cm), *Age*: age in months, *ave*: average, SD:
088 standard deviation. In the Caddo hatchery Toce River samples, three and two individuals have
089 unknown age, respectively.

	Caddo hatchery	Toce River
	n	n
M	33	4
F	23	7
us	13	3
Head		
m0d/	8	0
mfd/	45	10
mf0/	4	1
0fd/	7	2
m00/	3	0
00d/	2	1
Body		
m0000/	26	4
m0d00/	10	1
m00r0/	6	0
mfd00/	17	8
mfdr0/	4	1
mf000/	1	0
m0dr0/	3	0
mfdrp/	1	0
mfd0p/	1	0
0/0/0	66	11
c/0/0	1	0
0/b/0	2	3
Size, Age		
TL range	23.0–80.0	25.4–45.0
TL ave±SD	37.9±8.8	32.2±7.1
Age range	20–55	32–80
Age ave±SD	34±8	43±15

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