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Morphologic and genetic characterisation of Corsican and Sardinian trout with comments on *Salmo* taxonomy

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Short title: Morphology and genetics of Tyrrhenian trout

19 **Abstract** - Both morphological and molecular data are presented and discussed for
20 indigenous *Salmo* sp. from Corsica and Sardinia, here called Tyrrhenian trout. For
21 comparison, morphological data obtained from museum specimens, including the Algerian *S.*
22 *macrostigma*, are discussed in the light of recent and new molecular findings. In total, 29
23 measurements and 20 meristic characters were taken from each specimen. Out of the meristic
24 characters, 12 were obtained by means of X-ray. One important morphometric character in the
25 present study is the size of the head measured from premaxilla to posterior margin of
26 preoperculum. This character was particularly stable in all Tyrrhenian trout, showing
27 relatively large head compared to Atlantic trout and to *S. macrostigma*. On the contrary, other
28 characters like body punctuations, black and white edges of fins, body depth or number of
29 epurals in the caudal skeleton are quite polymorphic. In certain meristic characters, range of
30 variation of Tyrrhenian trout even exceeds that of the extensive comparative material. Each
31 trout has been genetically characterized. New haplotypes from Tyrrhenian trout were
32 discovered, belonging to three mitochondrial lineages viz. Adriatic, marble and
33 Mediterranean, however, Adriatic haplotypes are dominant. Mixing morphological and
34 genetic data, observed morphology lacks any obvious correlation to mitochondrial lineages
35 and it is concluded that Tyrrhenian trout show no particular affinity to *S. macrostigma* from
36 Algeria.

37

38 **Keywords:** brown trout / Tyrrhenian Sea / morphology / meristics / mtDNA

39

40 **Caractérisation morphologique et génétique de la truite corse et sarde avec**
41 **commentaires sur la taxonomie du genre *Salmo*.**

42 **Bo Delling, Andrea Sabatini, Stéphane Muracciole, Christelle Tougard, Patrick Berrebi.**

43 **Résumé** - La présente étude détaille et discute les données morphologiques et moléculaires
44 des truites indigènes, *Salmo* sp. de Corse et de Sardaigne, ici appelée truites tyrrhéniennes. À
45 titre de comparaison, les données morphologiques obtenues à partir de spécimens de musée, y
46 compris *S. macrostigma* d'Algérie, sont discutées à la lumière des découvertes moléculaires
47 récentes et nouvelles. Au total, 29 mesures et 20 caractères méristiques ont été considérés
48 pour chaque spécimen. Parmi ces caractères méristiques, 12 ont été obtenus au moyen de
49 rayons X. Un caractère morphométrique important dans la présente étude est la taille de la tête
50 mesurée du prémaxillaire à la marge postérieure du préopercule. Ce caractère est
51 particulièrement stable chez toutes les truites tyrrhéniennes, qui ont montré une tête
52 relativement grande par rapport celle de la truite de l'Atlantique et de *S. macrostigma*. Au
53 contraire, d'autres caractères comme les ponctuations du corps, les franges noires et blanches
54 des nageoires, la profondeur du corps ou le nombre d'hypuraux dans le squelette caudal sont
55 assez polymorphes. Pour certains caractères méristiques, la gamme de variation de la truite
56 tyrrhénienne dépasse celle de tous les taxons comparés. Chaque truite a été génétiquement
57 caractérisée et de nouveaux haplotypes de truite tyrrhénienne ont été découverts, appartenant
58 à trois lignées mitochondriales à savoir les lignées adriatique, marbrée et méditerranéenne, les
59 haplotypes adriatiques étant dominants. En combinant les données morphologiques et
60 génétiques, il est montré que la morphologie n'a aucune corrélation évidente avec les lignées
61 mitochondriales. D'autre part, la truite tyrrhénienne n'a aucune affinité particulière avec *S.*
62 *macrostigma* d'Algérie.

63

64 **Mots-clés:** truite commune / mer Tyrrhénienne / morphologie / caractères méristique /
65 ADNmt

66

67 **1 Introduction**

68 The taxonomic status of Eurasian trouts, i.e., all *Salmo* spp. except Atlantic salmon, *Salmo*
69 *salar*, is revised continuously. Among molecular oriented ichthyologists, this part of *Salmo*
70 diversity is most often referred to as brown trout *Salmo trutta* or “brown trout complex”,
71 whereas taxonomic oriented scientists, mainly focusing on morphology, continue to describe
72 new species and recognizes at least 50 distinct taxa (Delling and Doadrio, 2005; Sušnik *et al.*,
73 2006, 2007; Kottelat and Freyhof, 2007; Delling, 2010; Turan *et al.*, 2009, 2011, 2012, 2014a,
74 2014b, 2017, 2020; Doadrio *et al.*, 2015; Froese and Pauly, 2019).

75 The extensive molecular studies of the genus started mainly with allozymes (Ferguson
76 and Mason, 1981; Karakousis and Triantaphyllidis, 1990; García-Marín *et al.*, 1999; Berrebi
77 *et al.*, 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of
78 mitochondrial origin (Giuffra *et al.*, 1994; Apostolidis *et al.*, 1997; Aurelle and Berrebi, 2001;
79 Snoj *et al.*, 2011). These studies led to numerous publications describing genetic variation in
80 the genus. One of the most important findings within the taxonomic context is probably the
81 proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex,
82 Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA)
83 (Bernatchez *et al.*, 1992; Bernatchez, 2001). These lineages are augmented by geographically
84 more limited lineages such as the Duero (DU) lineage (Suarez *et al.*, 2001) and the Dades
85 trout (Snoj *et al.*, 2011), both close to AT, and the Tigris (TI) lineage (Bardakçi *et al.*, 2006)
86 close to DA. A recent analysis of a larger portion of the mtDNA allowed for a division of the
87 AT lineage into two sister clades: a North African lineage (NA, in Morocco, Algeria and
88 Sicily) and a well-known European AT lineage (Tougaard *et al.*, 2018).

89 In several cases, morphological and molecular data are correlated, strengthening
90 hypotheses on taxa delimitation. In several cases, morphological and molecular data are
91 correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018). However, they

92 sometimes disagree: for example, *Salmo marmoratus*, considered as very distinct in
93 morphology (Delling, 2002), is also characterized by the MA mtDNA and the LDH-C1*(120)
94 allozyme allele. However, MA haplotypes are also found in low frequencies outside the taxon
95 (Bernatchez *et al.*, 1992; Snoj *et al.*, 2009; Pustovrh *et al.*, 2011; Tougard *et al.*, 2018) and the
96 120-allele is rare in Trebuscica River (Slovenia) otherwise pure and isolated *S. marmoratus*
97 population (Berrebi *et al.*, 2000b). Another example of marker disagreement is illustrated by
98 *S. obtusirostris*. This species, while fixed for a unique and specific mtDNA haplotype in the
99 Neretva River (Snoj *et al.*, 2002), is fixed for the AD mtDNA lineage in Jadro River
100 population. Other frequent kinds of contradictions have been observed, especially in the
101 Balkans with numerous taxa sharing similar AD haplotypes (Sušnik *et al.*, 2004, 2006; Snoj *et*
102 *al.*, 2010). These kinds of discrepancies may be explained by ancient introgression (Sušnik *et*
103 *al.*, 2007). Another explanation is the Dobzhansky–Muller model which accounts for
104 cytonuclear incompatibilities (Burton and Barreto, 2012).

105 Despite the high number of more or less distinguishable taxa within the genus *Salmo*,
106 large portions of its populations are not easily referred with accuracy to any existing taxon
107 (Splendiani *et al.*, 2019). This is partly due to lack of morphological data, lack of studies
108 including both kinds of data and the fact that several tentatively valid taxa are poorly
109 described lacking clear diagnoses (Kottelat and Freyhof, 2007). Within the native distribution
110 of *Salmo*, a large part of its diversity is found in basins of the Tyrrhenian islands, Corsica,
111 Sardinia and Sicily (Berrebi *et al.*, 2019), and especially in Corsica where numerous
112 differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together
113 with several other Mediterranean trouts, are often referred to as *Salmo macrostigma* (Duméril
114 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr
115 marks retained in adults (Duméril, 1858). This is a common feature in many *Salmo* spp. and
116 may explain the broadened usage of this name, as applied to Corsican trout by Roule (1933)

117 and to Sardinian trout at first by Boulenger (1901) also confirmed by Pomini (1941). Since
118 that, Corsican trout have been characterized both for allozymes (Guyomard and Krieg, 1986;
119 Berrebi, 1995), mtDNA (Bernatchez *et al.*, 1992; Berrebi *et al.*, 2019) and microsatellites
120 (Berrebi *et al.*, 2007, 2019), showing that they mainly belong to the AD lineage and possess
121 the highly diagnostic allozyme allele LDH-4*(040). Morphological data on Corsican trout is
122 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the
123 description of variation in color pattern among populations (Lascaux *et al.*, 2010). In the same
124 way, the non-introgressed Sardinian populations were characterized by only the AD lineage
125 and allele LDH-C1 100/100 (Sabatini *et al.* 2018). Some authors describe, for the Sardinian
126 populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics
127 accompanied by different phenotypes (Sabatini *et al.*, 2011; Zaccara *et al.*, 2015)

128 Regarding distinctiveness of *S. macrostigma sensu stricto*, Tougard *et al.* (2018)
129 analyzed complete mtDNA sequences from one syntype and one topotypic specimen and
130 concluded they belonged to the NA lineage. In the same study, samples from Corsica and
131 Sardinia were associated to AD, ME, MA or AT lineages.

132 The present study is deliberately “cross-disciplinary”, the main focus being to
133 describe and discuss the *Salmo* diversity irrespective of different views on classification and
134 taxonomy. Consequently, the use of different names, e.g. *S. marmoratus* or *S. lourosensis*
135 only serve the purpose of pointing out a certain subset of trouts. Both molecular and
136 morphological data are presented and discussed for indigenous *Salmo* sp. from Corsica and
137 Sardinia, here called Tyrrhenian trout. Regarding comparison to Algerian *S. macrostigma*,
138 morphological data obtained from museum specimens are also included and discussed in the
139 light of recent molecular findings (Tougard *et al.*, 2018).

140

141

142

143 **2 Material and methods**

144

145 **2.1. Sampling**

146 Contemporary specimens (N=38) from one Sardinian and six Corsican localities were
147 captured in the wild by electrofishing, anesthetized with clove essence to death, sampled for
148 tissue (fin clip in 95% ethanol), fixed in formalin 5%, and later transferred to ethanol prior to
149 morphological analyses. The geographic positions of sampling stations are given in Fig. 1.
150 Tyrrhenian *Salmo* is referred to with an abbreviation of the stream, e.g. CAM for Camboni
151 River in text, certain graphs and tables (Table 1). As detailed in Fig. 1, samples for genetic
152 and morphological analyses are not exactly the same.

153 Comparative material includes different sets of *Salmo* spp. depending on analyses
154 and the question of interest: distinction towards i) *Salmo macrostigma* from Algeria, ii)
155 Atlantic basin *Salmo trutta*, iii) *Salmo* sp. from Spain. Comparative material for morphometry
156 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e., within the
157 SL-range of Tyrrhenian trout samples. A description of contemporary and comparative
158 material is given in Table 1, obtained from several museum collections: CMK, Collection of
159 Maurice Kottelat, Cornol, Switzerland; BMNH, British Museum of Natural History, London,
160 UK; MHNG, Museum d'Histoire Naturelle, Geneva, Switzerland; MNCN, Museo Nacional
161 de Ciencias Naturales, Madrid, Spain; MNHN, Museum National d'Histoire Naturelle, Paris,
162 France; NMW, Naturhistorisches Museum, Wien, Austria; NRM, Swedish Museum of
163 Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian Academy of
164 Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg, Germany. The
165 sample from Spain, MNHN 1920 228-229, consists of two specimens only but is included in
166 the study because their morphology resembles Tyrrhenian trout (see below). Comparative
167 material in addition to that in Table 1 (Delling, unpublished) is included for a broader

168 comparison of head length within *Salmo*. A complete list of studied material is provided as
169 supplementary information (Table S1).

170

171 **2.2. Molecular methods**

172 DNA was extracted from fin clips using the Chelex/proteinase K protocol described by
173 Estoup *et al.* (1996). Three to six individuals have been considered by locality in the
174 Tyrrhenian region, being or not the exact individuals included in morphological analyses.
175 Partial mtDNA control region (CR) was amplified by PCR using the PST and FST primers
176 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer
177 (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of MgCl₂ (25 mM), 10 µl of
178 5x PCR buffer, 1 U of Taq polymerase (GoTaq® Promega) and about 50 ng of genomic
179 DNA. The conditions for PCR were an initial denaturation (95°C, 5 min) followed by 30
180 cycles of strand denaturation (94°C, 1 min), primer annealing (52°C, 1 min) and DNA
181 extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR
182 amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA
183 fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR
184 products were purified and sequenced in both directions to confirm polymorphic sites by the
185 MacroGen Company, Seoul, South Korea (<https://dna.macrogen.com/>) and the platform
186 GenSeq of the Institut des Sciences de l'Evolution de Montpellier (Montpellier, France).

187 The sequences of CR were aligned together with reference haplotypes retrieved from
188 GenBank, using MEGA v5.05 (Tamura *et al.*, 2011). Haplotypes for the new sequences were
189 generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and
190 distribution among populations were evaluated with a median-joining network (Bandelt *et al.*,
191 1999) constructed with PopART (Leigh and Bryant, 2015). In order to assign a phylogenetic
192 position to the seven contemporary samples (CAM, SPE, POZ, ESE, CAR, CHA and NIN),

193 the network included published GenBank sequences of the lineages AT, ME, AD, MA, DA
194 and NA, all belonging to the brown trout complex.

195

196 **2.3. Morphology methods**

197 Methodology follows Delling *et al.* (2000) and Delling (2002). The length of the uppermost
198 gill raker on the lower limb of the first gill arch (right side) was measured *in situ* using a pair
199 of dividers. All other measurements were taken on the left side of the specimen with a digital
200 calliper and rounded to the nearest 0.1 mm (Fig. 2). One important morphometric character in
201 the present study is head length (HL) measured from tip of the snout to posterior margin of
202 the operculum. However, the measurement that quantifies the size of the head more accurately
203 is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig.
204 2). Below, the abbreviation HLpp is applied for that measurement.

205 The number of i) pored scales along the lateral line to the end of the caudal peduncle
206 (left side), ii) scales in an oblique row from the base of the adipose fin backwards down to the
207 lateral line including lateral line scales (left side), iii) gill rakers, including rudimentary
208 elements, on lower and upper limbs of the first gill arch separately (right side), and iv)
209 branchiostegal rays on both sides, were counted under a binocular dissection microscope.

210 The number of abdominal vertebrae, caudal vertebrae, pterygiophores supporting
211 anal and dorsal fins, caudal fin upper and lower procurrent rays, and interneurals were taken
212 from radiographs (Fig. 3). Rudimentary vertebrae in the caudal skeleton in addition to the
213 three upturned vertebrae were not included in the counts. In cases of fused centra, the number
214 of neural arches or spines was counted. The last abdominal vertebra is herein defined as the
215 last one having ribs (sometimes rudimentary or missing) and/or having the haemal spine much
216 shorter than in the consecutive first caudal vertebra. The positions of the dorsal and anal fins
217 were estimated in relation to the vertebral column. The most strongly developed anterior

218 pterygiophore was used as a marker of dorsal and anal fin position, respectively. Dorsal and
219 anal fin pterygiophores do not articulate with neural and haemal spines, respectively, and in
220 uncertain cases the lower value was chosen. The dorsal and anal fin positions are treated as
221 meristic characters in statistical analyses. A membranous triangular bone sometimes present,
222 located above the neural spine of the first vertebrae (Fig. 3), was not included in interneural
223 counts.

224 Principal component analyses (PCA) on log transformed measurements and square
225 rooted counts were used as an ordination method (Bookstein *et al.*, 1985). Some informative
226 meristic characters are summarised in frequency tables. The inclusion of ‘soft’ measurements,
227 e.g. body width and body depth in analyses, depends on the state of preservation of
228 specimens. PCAs were performed using SYSTAT 13. Colour pattern descriptions are most
229 often restricted to contrasting markings: size, density and distribution of spots; presence of
230 black and white leading edges of dorsal and anal fins; any other markings such as dark bars
231 were also considered. In preserved material, light spots are interpreted as red spots based on
232 personal observations: after transfer to ethanol, red spots disappear transformed into pale
233 spots. Spots described as ocellated refer to spots enclosed by a light ring.

234

235

236 **3 Results**

237

238 **3.1. Molecular results**

239 Among the 38 contemporary specimens from Corsica and Sardinia, 34 CR sequences were
240 obtained, corresponding to eight new haplotypes (Table 2). The alignment of CR sequences
241 are 998 nucleotides long with 47 phylogenetically informative sites. These haplotypes are
242 genetically very close (distant from each other by up to two mutations, Fig. 4) to 37 published

243 GenBank haplotype sequences used as reference and illustrating the diversity on all the range
244 of the *S. trutta* complex. A comprehensive network of these new and published haplotypes is
245 presented Fig. 4. The sequences of the contemporary analyzed populations were all clustered
246 into ME, AD and MA lineages, according to the reference sequences. AT, DA and NA
247 lineages were represented only by GenBank sequences.

248 New haplotypes from Tyrrhenian trout were called ADcr2 to 6, MAcr1 and 2, and
249 MEcr1 (Table 2). Three populations are characterized by one private haplotype each: NIN,
250 (Corsica) with MEcr1; CAM (Sardinia) with ADcr2 and POZ (Corsica) with ADcr4. CAR,
251 (Corsica) is characterized by a majority of MA private haplotypes (MAcr1 and 2) and one AD
252 haplotype (ADcr5), while CHA (Corsica) is characterized by two AD haplotypes (ADcr3 and
253 ADcr6). SPE and ESE shared ADcr3 with CHA.

254

255 **3.2. Morphology**

256 Morphometric data are given in Table 3, and meristic data are summarized in Tables 4 to 6
257 including extensive comparative material (Tables 1 and S1). Selected results from ordination
258 by means of PCA are given for analyses focusing on variation between Tyrrhenian trout as a
259 whole towards Atlantic basin *S. trutta* (Fig. 5). Corresponding character loadings are given in
260 Supplementary Tables S2 and S3. The distinction of Tyrrhenian trout towards *S. macrostigma*
261 is illustrated with a biplot (Fig. 6) focusing on the major morphological trait of the Tyrrhenian
262 trout, viz. the longer head (HLpp) and slightly shorter caudal peduncle. The two Spanish
263 specimens are included in all analyses and graphs. For discussion related to the comparatively
264 large head in Tyrrhenian trout, HLpp is also presented as box plots in comparison to an
265 extended number of *Salmo* samples (Fig. 7).

266

267 *Variation among Tyrrhenian trout samples*

268 At first glance (Figs. 8A to 8G), the Tyrrhenian trout resembles Atlantic basin *S. trutta*, i.e.
269 rather strong jaws, numerous red and black spots, black and white edges of fins, most
270 prominent in CAM, CAR, CHJ and SPE. Black spots are sometimes irregularly distributed,
271 more or less aggregated along the flanks of the body (SPE, NIN) in contrast to, e.g. CAM
272 having its spots more evenly distributed (Fig. 8A). There are large variations in meristic
273 characters between different populations and the range of variation sometimes exceeds that of
274 the extensive comparative material (Tables 4 to 6). The NIN-sample and the two Spanish
275 specimens were not markedly different in multivariate statistics in comparison to the six
276 remaining samples.

277 Four specimens (1 POZ, 3 ESE) were different in the number of epurals in the caudal
278 skeleton, having three instead of two. All *Salmo* except *S. salar* have two, sometimes fused
279 (anomaly) to one. *Salmo salar* is polymorphic but two is more common (see below).

280

281 *Distinction of Tyrrhenian trout samples towards North Atlantic basin S. trutta*

282 The rather strong jaws and a colour pattern with prominent black and white leading edges on
283 the fins are shared between several populations of Atlantic basin *S. trutta* and the Tyrrhenian
284 trouts. Dark, more or less ocellated, spots on flanks of the body are also common in both.
285 However, some Tyrrhenian trout have their spots aggregated (Figs. 8F, 8G and p. 415 in
286 Kottelat and Freyhof, 2007). The sample from Spain also possesses this uncommon pattern
287 and was therefore especially highlighted in the comparative material. Sparsely or densely
288 distributed, dark spots on caudal fin are also common in Tyrrhenian trout (Figs. 8B and 8G,
289 CAM and SPE, respectively). Spots on caudal fins are rarely found in Atlantic basin *S. trutta*.
290 Multivariate statistics (Fig. 5) indicate distinction but not complete separation of Tyrrhenian
291 trout from Atlantic *S. trutta* due to differences in vertebral counts and head size.

292

293 *Distinction of Tyrrhenian trout samples towards S. macrostigma*
294 The extensive variation in meristic characters in Tyrrhenian trout as a whole (Tables 4 to 6)
295 covers the range of variation in *S. macrostigma* and limits the analyses to morphometric data.
296 PCA (not shown) reveals that HLpp and caudal peduncle length are the two morphometric
297 characters that distinguish them best (Fig. 6). The Tyrrhenian trout has longer head (HLpp)
298 and slightly shorter caudal peduncle compared to *S. macrostigma* (Table 3).

299

300

301 **4 Discussion**

302 **4.1. Genetic diversity: a strong differentiation pattern**

303 According to results from previous (Tougaard *et al.*, 2018; Berrebi *et al.*, 2019) and
304 present studies, the Tyrrhenian trout is mainly characterized by an island specific mtDNA-
305 radiation within the AD lineage, as well as, to a lesser degree, by other lineages (AT, ME and
306 MA). The recently described NA lineage is also naturally present in Sicily. Thus, within a
307 rather limited and nowadays isolated region in the south center of *Salmo* distribution, a
308 comparatively high number of mtDNA lineages (four of the five recognizable major lineages)
309 occur naturally. It is also striking that a majority of the haplotypes recovered in the present
310 study were new (Table 2) despite more than two decades of CR sequencing in *Salmo*. The
311 presence of the ME lineage in the NIN sample, also observed in Corsica by Tougaard *et al.*
312 (2018) and in other Tyrrhenian samples not included in the morphological analyses, is
313 explained by ancient introgressions evidenced elsewhere using nuclear markers (Berrebi *et*
314 *al.*, 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred,
315 according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007).
316 Moreover, the presence of MA lineage in some isolated Corsican rivers including CAR and
317 the range of distribution of the NA lineage (Morocco, Algeria, Sicily: Tougaard *et al.*, 2018;

318 Berrebi *et al.*, 2019) demonstrates the multiple unknown events of migrations, invasions and
319 hybridizations which complicate the trout genetic pattern in the Tyrrhenian region. Finally,
320 the presence of several northern AT haplotypes recorded in the Tyrrhenian trout (Tougaard *et*
321 *al.*, 2018; Berrebi *et al.*, 2019) is due to stocking with commercial AT hatchery strains.

322 Nuclear markers (microsatellites) have also shown that the Tyrrhenian trouts exhibit
323 exceptionally differentiated genotypes, at a continental-like level, within the two small
324 sampled islands, but especially in Corsica where numerous autochthonous isolated small
325 populations still survive (Berrebi *et al.*, 2019). This strong differentiation among neighboring
326 rivers is typical of dry Mediterranean mountainous regions, never frozen by glaciation and
327 providing way for migration (Apostolidis *et al.*, 2008; Berrebi *et al.*, 2019).

328

329 **4.2. Morphological diversity in light of genetic diversity**

330

331 Regarding the strong morphological diversification between studied populations of
332 Tyrrhenian trout, one explanation may involve random effects. Berrebi *et al.* (2019) showed
333 very low levels of genetic variation within populations in Corsican streams based on
334 microsatellites, suggesting small population sizes and repeated bottleneck events.

335 Hypothetically, the frequent (c. 10 %), occurrence of three epurals in Tyrrhenian
336 trout compared to c. 0.1 % in comparative material might be a result of genetic drift
337 accelerated by bottlenecks. Three epurals in the caudal skeleton are typical for, e.g. most
338 Pacific trouts and salmon (*Oncorhynchus*), graylings (*Thymallus*) and whitefishes
339 (*Coregonus*) (Norden, 1961; Stearley and Smith, 1993) and appear to be the ancestral state
340 also retained as a polymorphism in *S. salar* with 12 out of 40 studied specimens having three.

341 Delling and Doadrio (2005) also described a situation with a seemingly
342 plesiomorphic condition in rostrodermethmodid bone in the lake endemic *S. pallaryi* from

343 Lake Sidi Ali, Morocco, not recorded elsewhere in *Salmo*. The genetic characteristics of this
344 extinct trout is unknown but it is likely that these kinds of reversals approaching
345 morphological anomalies may occur under certain conditions involving random processes in
346 temporarily small populations.

347 Comparing Tyrrhenian samples to other *Salmo* spp., it is tempting to search for a
348 pattern connecting certain characters to certain mtDNA lineages. However, ancient
349 introgression in certain populations without strong impacts on morphology seems rather to be
350 the “rule” in many salmonids and other taxa (Martinez *et al.*, 2009; Gratton *et al.*, 2013;
351 Lerceteau-Köhler *et al.*, 2013; Berrebi *et al.*, 2017). The CAR sample also possessing the MA
352 mtDNA lineage shows no typical *S. marmoratus* characters, e.g. marbled color pattern, high
353 vertebral counts or a hypethmoid bone embedded in the rostral cartilage. In contrast, more
354 recent hybrids involving *S. marmoratus* show a variable but, overall, intermediate phenotype
355 (Delling, *et al.*, 2000). Prominent black and white leading edges on fins in several Tyrrhenian
356 populations are similar to Atlantic basin *S. trutta* and could tentatively be regarded as ancient
357 traces of the AT lineage. This pattern is also present in some North African trout, e.g. *S.*
358 *akairos* and *S. macrostigma* belonging to the NA lineage, close to the AT one (Tougard *et al.*,
359 2018). However, neither NA nor AT lineages have so far been reported from Corsica and
360 Sardinia, except AT lineages of hatchery origin.

361 Despite the complex pattern of migrations, hybridizations and genetic radiation in the
362 Tyrrhenian trout history, also giving rise to rather morphologically distinct populations in
363 Corsica and Sardinia (Figs. 8A to 8G), they all share a comparatively large head (Fig. 6 and
364 7). This contradictory pattern of morphological homogeneity for certain characters opposed to
365 strong differentiation in others take probably account of the genetic data in the search for
366 possible explanations. Turning focus to the overall large head in Tyrrhenian trout, it is
367 noticeable that this character varies extensively within and between different kinds of trouts

368 (Fig. 7). It should be noted that sample sizes vary considerably (Fig. 7) but several of them
369 can be characterized as typically small-headed, e.g. *S. salar*, *S. obtusirostris* and *S.*
370 *lourosensis*. Next after the Tyrrhenian trouts, the two North African lake trouts endemic from
371 Morocco, *S. akairos* and *S. pallaryi*, together with *S. marmoratus* and *S. dentex* inhabiting
372 Adriatic basin drainages, possess comparatively large heads. Thus, there is no obvious
373 correlation between size of head and habitat and/or lifestyle, e.g. rapid streams vs. lakes, and
374 it is fully possible that the large head is a result of a founder effect during ancient
375 establishment on the islands. However, body proportions can be affected by, e.g. growth rate.
376 Barlow (1961) refers to studies where it was shown that head length was smaller in faster
377 growing rainbow trout, *Oncorhynchus mykiss*. Thus, the comparatively large head in
378 Tyrrhenian trout can be a consequence of slow growth under harsh condition, or a
379 consequence of earlier sexual maturity. However, Pankhurst and Montgomery (1994) showed,
380 also for *O. mykiss* that retarded growth results in larger eyes. The Tyrrhenian trout possesses
381 on the average slightly smaller eyes compared to Atlantic *S. trutta* (Table 3) and consequently
382 retarded growth seems not to be a likely explanation for the large head.

383 The two Spanish specimens, referred to as *Salmo* sp. MNHN 1920 228-229, possess
384 a color pattern with irregularly distributed spots, also found in some Tyrrhenian trouts, and
385 are comparatively large headed (Fig. 6). They provide an example of what a hypothetical
386 ancestor could have looked like and, if the interpretation of locality information (Ebro basin)
387 is correct, it makes sense as the basin is dominated by AD haplotypes (Cortey *et al.*, 2004).
388 Also, the haplotype ADc13 found in Ebro is very close to the Tyrrhenian AD haplotypes
389 (Fig. 4).

390

391

392 **5 Conclusions**

393 Data presented herein suggest that within the rather unresolved *Salmo* complex in the
394 Mediterranean region, we may start to perceive a kind of large headed trout. However, more
395 populations from the islands and surrounding mainland (France, Spain, and Italy) need to be
396 studied to survey the distribution of this morphology. It would be a large step forward if this
397 kind of trout could get an identity, i.e. a scientific name to balance a perhaps too broad or
398 erroneous usage of names such as *S. trutta* and *S. macrostigma* in the Mediterranean region.
399 This long-term work already began with several recent molecular papers (Sanz, 2018;
400 Tougard *et al.*, 2018; Berrebi *et al.*, 2019) and the present study. The Tyrrhenian trout studied
401 here are left without a taxonomic identity but it is clearly demonstrated, in line with molecular
402 data (Tougaard *et al.*, 2018), that they show no particular affinity to *S. macrostigma* once
403 described from Algeria.

404

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409

410

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610

611

612 **8. Figures captions**

613 **Fig. 1.** Geographic position of the new sampled populations in Corsica and Sardinia.

614

615 **Fig. 2.** Measurements taken on *Salmo* specimens; 1, upper jaw depth, as largest depth of the
616 maxilla and supramaxilla; 2, snout length, from symphysis of premaxilla to osseous orbit
617 margin; 3, orbital horizontal diameter, between osseous orbital margin; 4, head depth, just
618 posterior to orbit; 5, orbital vertical diameter, between osseous orbital margin; 6, length of
619 maxilla, from premaxilla end to posterior end of maxilla; 7, upper jaw length, from symphysis
620 of premaxilla to posterior end of maxilla; 8, lower jaw length, from symphysis of dentary to
621 retroarticular; 9, pectoral fin length, from base of first ray to tip of longest ray; 10, body
622 depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest ray; 12,
623 pelvic fin length, from base of first ray to tip of longest ray; 13, body depth, at level of origin
624 of anal fin; 14, adipose fin length, from origin to tip; 15, anal fin length, from base of first ray
625 to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base of caudal
626 fin; 17, least depth of caudal peduncle; 18, length of upper caudal fin lobe, from base to tip of
627 longest ray; 19, length of middle caudal fin ray, from base to tip of shortest ray; 20, length of
628 lower caudal fin lobe, from base to tip of longest ray; 21 standard length (SL), from upper jaw
629 symphysis to middle base of caudal fin; 22, predorsal length from upper jaw symphysis to
630 origin of dorsal fin; 23, head length, from upper jaw symphysis to posterior tip of operculum;
631 24, premaxilla to preoperculum length, from premaxilla end of maxilla to posterior margin of
632 preoperculum (HLpp); 25, prepelvic length, from upper jaw symphysis to origin of pelvic fin;
633 26, preanal length, from upper jaw symphysis to origin of anal fin; 27, interorbital width,
634 transverse at narrowest part of skull; 28, body width, transverse at widest part of body at level
635 of dorsal fin origin, above abdominal cavity.

636

637 **Fig. 3.** Radiograph of *Salmo* sp. POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu Corsica
638 (same specimen as in Fig. 8E). Upper left; a thin membranous bone indicated with dashed
639 white line. Upper right; the uncommon condition with three (i-iii) epurals in the caudal
640 skeleton.

641

642 **Fig. 4.** Median-joining network of control region haplotypes of some trout samples (new
643 Tyrrhenian haplotypes and AT, AD, DA, MA, ME and NA haplotypes from GenBank).
644 Haplotypes are indicated by numbers as given in Table 2. Black circles are for nodes, and
645 hatch marks are for mutation steps.

646

647 **Fig. 5.** Morphometric PC II plotted against meristic PC I for *Salmo* from Sardinia, Corsica
648 and Spain in comparison to Atlantic basin *S. trutta*.

649

650 **Fig. 6.** Premaxilla to preoperculum length plotted against caudal peduncle length for *Salmo*
651 from Sardinia, Corsica and Spain in comparison to *S. macrostigma*. Linear regression lines
652 with 95% confidence bands are shown for each group separately.

653

654 **Fig. 7.** Box plot of premaxilla to preoperculum length as % of SL, totally 518 specimens 116-
655 208 mm SL. Number of specimens is given for each sample separately. Samples in bold are
656 detailed in Table 1. Additional samples given in capital letters (Delling, unpublished) refer to
657 rivers or streams in the given regions (Table S1). Ezenam is a lake in Daghestan and *labrax*
658 represents trout from Black Sea basin with an anadromous silvery and slender appearance.
659 Turkey and Mediterranean are samples scattered in the regions. Boxes represent median value
660 +/- 25% of the observations, and whiskers the inner fences. Asterisks are outside or far
661 outside values.

662

663 **Fig. 8.** External aspect of seven of the trouts, *Salmo* sp. analyzed in the present study. Picture
664 A-F taken after fixation in formalin prior to transfer to ethanol. **A.** CAM, NRM 61782, 183.7
665 mm SL; Camboni Sardinia. **B.** CAR, NRM 62571, 167.6 mm SL; Carnevale Corsica. **C.** CHJ,
666 NRM62573, 136.6 mm SL; Chjuvone Corsica. **D.** ESE, NRM 61813, 152.0 mm SL; Val
667 d'Ese Corsica. **E.** POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu Corsica. **F.** SPE,
668 NRM61812, 148.1 mm SL Speloncellu Corsica. **G.** NIN, Lake Ninu Corsica © S. Muracciole

1 RESEARCH PAPER

2 **Morphologic and genetic characterisation of Corsican and**
3 **Sardinian trout with comments on *Salmo* taxonomy**

4

5

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16

17 **Short title:** Morphology and genetics of Tyrrhenian trout

18

19 **Abstract** - Both morphological and molecular data are presented and discussed for
20 indigenous *Salmo sp.* from Corsica and Sardinia, here called Tyrrhenian trout. For
21 comparison, morphological data obtained from museum specimens, including the Algerian *S.*
22 *macrostigma*, are discussed in the light of recent and new molecular findings. In total, 29
23 measurements and 20 meristic characters were taken from each specimen. Out of the meristic
24 characters, 12 were obtained by means of X-ray. One important morphometric character in the
25 present study is the size of the head measured from premaxilla to posterior margin of
26 preoperculum. This character was particularly stable in all Tyrrhenian trout, showing
27 relatively large head compared to Atlantic trout and to *S. macrostigma*. On the contrary, other
28 characters like body punctuations, black and white edges of fins, body depth or number of
29 epurals in the caudal skeleton are quite polymorphic. In certain meristic characters, range of
30 variation of Tyrrhenian trout even exceeds that of the extensive comparative material. Each
31 trout has been genetically characterized. New haplotypes from Tyrrhenian trout were
32 discovered, belonging to three mitochondrial lineages viz. Adriatic, marble and
33 Mediterranean, however, Adriatic haplotypes are dominant. Mixing morphological and
34 genetic data, observed morphology lacks any obvious correlation to mitochondrial lineages
35 and it is concluded that Tyrrhenian trout show no particular affinity to *S. macrostigma* from
36 Algeria.

37

38 **Keywords:** brown trout / Tyrrhenian Sea / morphology / meristics / mtDNA

39

40 **Caractérisation morphologique et génétique de la truite corse et sarde avec**
41 **commentaires sur la taxonomie du genre *Salmo*.**

42 **Bo Delling, Andrea Sabatini, Stéphane Muracciole, Christelle Tougard, Patrick Berrebi.**

43 **Résumé** - La présente étude détaille et discute les données morphologiques et moléculaires
44 des truites indigènes, *Salmo* sp. de Corse et de Sardaigne, ici appelée truites tyrrhéniennes. À
45 titre de comparaison, les données morphologiques obtenues à partir de spécimens de musée, y
46 compris *S. macrostigma* d'Algérie, sont discutées à la lumière des découvertes moléculaires
47 récentes et nouvelles. Au total, 29 mesures et 20 caractères méristiques ont été **considérés**
48 **pour** chaque spécimen. Parmi ces caractères méristiques, 12 ont été obtenus au moyen de
49 rayons X. Un caractère morphométrique important dans la présente étude est la taille de la tête
50 mesurée du prémaxillaire à la marge postérieure du préopercule. Ce caractère est
51 particulièrement stable chez toutes les truites tyrrhéniennes, qui ont montré une tête
52 relativement grande par rapport celle de la truite de l'Atlantique et de *S. macrostigma*. Au
53 contraire, d'autres caractères comme les ponctuations du corps, les franges noires et blanches
54 des nageoires, la profondeur du corps ou le nombre d'hypuraux dans le squelette caudal sont
55 assez polymorphes. Pour certains caractères méristiques, la gamme de variation de la truite
56 tyrrhénienne dépasse celle de tous les taxons comparés. Chaque truite a été génétiquement
57 caractérisée et de nouveaux haplotypes de truite tyrrhénienne ont été découverts, appartenant
58 à trois lignées mitochondriales à savoir les **lignées adriatique, marbrée et méditerranéenne**,
59 les haplotypes adriatiques étant dominants. En combinant les données morphologiques et
60 génétiques, il est montré que la morphologie n'a aucune corrélation évidente avec les lignées
61 mitochondriales. D'autre part, la truite tyrrhénienne n'a aucune affinité particulière avec *S.*
62 *macrostigma* d'Algérie.

63

64 **Mots-clés:** truite **commune** / mer Tyrrhénienne / morphologie / **caractères** méristique /
65 ADNmt

66

67 **1 Introduction**

68 The taxonomic status of Eurasian trouts, i.e., all *Salmo* spp. except Atlantic salmon, *Salmo*
69 *salar*, is revised continuously. Among molecular oriented ichthyologists, this part of *Salmo*
70 diversity is most often referred to as brown trout *Salmo trutta* or “brown trout complex”,
71 whereas taxonomic oriented scientists, mainly focusing on morphology, continue to describe
72 new species and recognizes **at least** 50 distinct taxa (Delling and Doadrio, 2005; Sušnik *et al.*,
73 2006, 2007; Kottelat and Freyhof, 2007; Delling, 2010; Turan *et al.*, 2009, 2011, 2012, 2014a,
74 2014b, 2017, **2020**; Doadrio *et al.*, 2015; **Froese and Pauly, 2019**).

75 The extensive molecular studies of the genus started mainly with allozymes (Ferguson
76 and Mason, 1981; Karakousis and Triantaphyllidis, 1990; García-Marín *et al.*, 1999; Berrebi
77 *et al.*, 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of
78 mitochondrial origin (Giuffra *et al.*, 1994; Apostolidis *et al.*, 1997; Aurelle and Berrebi, 2001;
79 Snoj *et al.*, 2011). These studies led to numerous publications describing genetic variation in
80 the genus. One of the most important findings within the taxonomic context is probably the
81 proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex,
82 Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA)
83 (Bernatchez *et al.*, 1992; Bernatchez, 2001). These lineages are augmented by geographically
84 more limited lineages such as the Duero (DU) lineage (Suarez *et al.*, 2001) and the Dades
85 trout (Snoj *et al.*, 2011), both close to AT, and the Tigris (TI) lineage (Bardakçi *et al.*, 2006)
86 close to DA. A recent analysis of a larger portion of the mtDNA allowed for a division of the
87 AT lineage into two sister clades: a North African lineage (NA, in Morocco, Algeria and
88 Sicily) and a well-known European AT lineage (Tougaard *et al.*, 2018).

89 In several cases, morphological and molecular data are correlated, strengthening
90 hypotheses on taxa delimitation. **In several cases, morphological and molecular data are**
91 **correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018)**. However, they

92 sometimes disagree: for example, *Salmo marmoratus*, considered as very distinct in
93 morphology (Delling, 2002), is also characterized by the MA mtDNA and the LDH-C1*(120)
94 allozyme allele. However, MA haplotypes are also found in low frequencies outside the taxon
95 (Bernatchez *et al.*, 1992; Snoj *et al.*, 2009; Pustovrh *et al.*, 2011; Tougard *et al.*, 2018) and the
96 120-allele is rare in Trebuscica River (Slovenia) otherwise pure and isolated *S. marmoratus*
97 population (Berrebi *et al.*, 2000b). Another example of marker disagreement is illustrated by
98 *S. obtusirostris*. This species, while fixed for a unique and specific mtDNA haplotype in the
99 Neretva River (Snoj *et al.*, 2002), is fixed for the AD mtDNA lineage in Jadro River
100 population. Other frequent kinds of contradictions have been observed, especially in the
101 Balkans with numerous taxa sharing similar AD haplotypes (Sušnik *et al.*, 2004, 2006; Snoj *et*
102 *al.*, 2010). These kinds of discrepancies may be explained by ancient introgression (Sušnik *et*
103 *al.*, 2007). Another explanation is the Dobzhansky–Muller model which accounts for
104 cytonuclear incompatibilities (Burton and Barreto, 2012).

105 Despite the high number of more or less distinguishable taxa within the genus *Salmo*,
106 large portions of its populations are not easily referred with accuracy to any existing taxon
107 (Splendiani *et al.*, 2019). This is partly due to lack of morphological data, lack of studies
108 including both kinds of data and the fact that several tentatively valid taxa are poorly
109 described lacking clear diagnoses (Kottelat and Freyhof, 2007). Within the native distribution
110 of *Salmo*, a large part of its diversity is found in basins of the Tyrrhenian islands, Corsica,
111 Sardinia and Sicily (Berrebi *et al.*, 2019), and especially in Corsica where numerous
112 differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together
113 with several other Mediterranean trouts, are often referred to as *Salmo macrostigma* (Duméril
114 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr
115 marks retained in adults (Duméril, 1858). This is a common feature in many *Salmo* spp. and
116 may explain the broadened usage of this name, as applied to Corsican trout by Roule (1933)

117 and to Sardinian trout at first by Boulenger (1901) also confirmed by Pomini (1941). Since
118 that, Corsican trout have been characterized both for allozymes (Guyomard and Krieg, 1986;
119 Berrebi, 1995), mtDNA (Bernatchez *et al.*, 1992; Berrebi *et al.*, 2019) and microsatellites
120 (Berrebi *et al.*, 2007, 2019), showing that they mainly belong to the AD lineage and possess
121 the highly diagnostic allozyme allele LDH-4*(040). Morphological data on Corsican trout is
122 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the
123 description of variation in color pattern among populations (Lascaux *et al.*, 2010). In the same
124 way, the **non**-introgressed Sardinian populations were characterized by only the AD lineage
125 and allele LDH-C1 100/100 (Sabatini *et al.* 2018). Some authors describe, for the Sardinian
126 populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics
127 accompanied by different phenotypes (Sabatini *et al.*, 2011; Zaccara *et al.*, 2015)

128 Regarding distinctiveness of *S. macrostigma sensu stricto*, Tougard *et al.* (2018)
129 analyzed complete mtDNA sequences from one syntype and one topotypic specimen and
130 concluded they belonged to the NA lineage. In the same study, samples from Corsica and
131 Sardinia were associated to AD, ME, MA or AT lineages.

132 The present study is deliberately “cross-disciplinary”, the main focus being to
133 describe and discuss the *Salmo* diversity irrespective of different views on classification and
134 taxonomy. Consequently, the use of different names, e.g. *S. marmoratus* or *S. lourosensis*
135 only serve the purpose of pointing out a certain subset of trouts. Both molecular and
136 morphological data are presented and discussed for indigenous *Salmo* sp. from Corsica and
137 Sardinia, here called Tyrrhenian trout. Regarding comparison to Algerian *S. macrostigma*,
138 morphological data obtained from museum specimens are also included and discussed in the
139 light of recent molecular findings (Tougard *et al.*, 2018).

140

141

142

143 **2 Material and methods**

144

145 **2.1. Sampling**

146 Contemporary specimens (N=38) from one Sardinian and six Corsican localities were
147 captured in the wild by electrofishing, anesthetized with clove essence to death, sampled for
148 tissue (fin clip in 95% ethanol), fixed in formalin 5%, and later transferred to ethanol prior to
149 morphological analyses. The geographic positions of sampling stations are given in Fig. 1.
150 Tyrrhenian *Salmo* is referred to with an abbreviation of the stream, e.g. CAM for Camboni
151 River in text, certain graphs and tables (Table 1). As detailed in Fig. 1, samples for genetic
152 and morphological analyses are not exactly the same.

153 Comparative material includes different sets of *Salmo* spp. depending on analyses
154 and the question of interest: distinction towards i) *Salmo macrostigma* from Algeria, ii)
155 Atlantic basin *Salmo trutta*, iii) *Salmo* sp. from Spain. Comparative material for morphometry
156 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e., within the
157 SL-range of Tyrrhenian trout samples. A description of contemporary and comparative
158 material is given in Table 1, obtained from several museum collections: **CMK, Collection of**
159 **Maurice Kottelat, Cornol, Switzerland;** BMNH, British Museum of Natural History,
160 London, UK; **MHNG, Museum d'Histoire Naturelle, Geneva, Switzerland;** MNCN,
161 Museo Nacional de Ciencias Naturales, Madrid, Spain; MNHN, Museum National d'Histoire
162 Naturelle, Paris, France; NMW, Naturhistorisches Museum, Wien, Austria; NRM, Swedish
163 Museum of Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian
164 Academy of Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg,
165 Germany. The sample from Spain, MNHN 1920 228-229, consists of two specimens only but
166 is included in the study because their morphology resembles Tyrrhenian trout (see below).

167 Comparative material in addition to that in Table 1 (Delling, unpublished) is included for a
168 broader comparison of head length within *Salmo*. **A complete list of studied material is**
169 **provided as supplementary information (Table S1).**

170

171 **2.2. Molecular methods**

172 DNA was extracted from fin **clips** using the Chelex/proteinase K protocol described by
173 Estoup *et al.* (1996). Three to six individuals have been considered by locality in the
174 Tyrrhenian region, being or not the exact individuals included in morphological analyses.
175 Partial mtDNA control region (CR) was amplified by PCR using the PST and FST primers
176 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer
177 (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of MgCl₂ (25 mM), 10 µl of
178 5x PCR buffer, 1 U of Taq polymerase (GoTaq® Promega) and about 50 ng of genomic
179 DNA. The conditions for PCR were an initial denaturation (95°C, 5 min) followed by 30
180 cycles of strand denaturation (94°C, 1 min), primer annealing (52°C, 1 min) and DNA
181 extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR
182 amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA
183 fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR
184 products were purified and sequenced in both directions to confirm polymorphic sites by the
185 Macrogen Company, Seoul, South Korea (<https://dna.macrogen.com/>) and the platform
186 GenSeq of the Institut des Sciences de l'Evolution de Montpellier (Montpellier, France).

187 The sequences of CR were aligned together with reference haplotypes retrieved from
188 GenBank, using MEGA v5.05 (Tamura *et al.*, 2011). Haplotypes for the new sequences were
189 generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and
190 distribution among populations were evaluated with a median-joining network (Bandelt *et al.*,
191 1999) constructed with PopART (Leigh and Bryant, 2015). In order to assign a phylogenetic

192 position to the seven contemporary samples (CAM, SPE, POZ, ESE, CAR, CHA and NIN),
193 the network included published GenBank sequences of the lineages AT, ME, AD, MA, DA
194 and NA, all belonging to the brown trout complex.

195

196 **2.3. Morphology methods**

197 Methodology follows Delling *et al.* (2000) and **Delling** (2002). The length of the uppermost
198 gill raker on the lower limb of the first gill arch (right side) was measured *in situ* using a pair
199 of dividers. All other measurements were taken on the left side of the specimen with a digital
200 calliper and rounded to the nearest 0.1 mm (Fig. 2). One important morphometric character in
201 the present study is head length (HL) measured from tip of the snout to posterior margin of
202 the operculum. However, the measurement that quantifies the size of the head more accurately
203 is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig.
204 2). Below, the abbreviation HLpp is applied for that measurement.

205 The number of i) pored scales along the lateral line to the end of the caudal peduncle
206 (left side), ii) scales in an oblique row from the base of the adipose fin backwards down to the
207 lateral line including lateral line scales (left side), iii) gill rakers, including rudimentary
208 elements, on lower and upper limbs of the first gill arch separately (right side), and iv)
209 branchiostegal rays on both sides, were counted under a binocular dissection microscope.

210 The number of abdominal vertebrae, caudal vertebrae, pterygiophores supporting
211 anal and dorsal fins, caudal fin upper and lower procurrent rays, and interneurals were taken
212 from radiographs (**Fig. 3**). Rudimentary vertebrae in the caudal skeleton in addition to the
213 three upturned vertebrae were not included in the counts. In cases of fused centra, the number
214 of neural arches or spines was counted. The last abdominal vertebra is herein defined as the
215 last one having ribs (sometimes rudimentary or missing) and/or having the haemal spine much
216 shorter than in the consecutive first caudal vertebra. The positions of the dorsal and anal fins

217 were estimated in relation to the vertebral column. The most strongly developed anterior
218 pterygiophore was used as a marker of dorsal and anal fin position, respectively. Dorsal and
219 anal fin pterygiophores do not articulate with neural and haemal spines, respectively, and in
220 uncertain cases the lower value was chosen. The dorsal and anal fin positions are treated as
221 meristic characters in statistical analyses. A membranous triangular bone sometimes present,
222 located above the neural spine of the first vertebrae (**Fig. 3**), was not included in interneural
223 counts.

224 Principal component analyses (PCA) on log transformed measurements and square
225 rooted counts were used as an ordination method (Bookstein *et al.*, 1985). Some informative
226 meristic characters are summarised in frequency tables. The inclusion of ‘soft’ measurements,
227 e.g. body width and body depth in analyses, depends on the state of preservation of
228 specimens. PCAs were **performed** using SYSTAT 13. Colour pattern descriptions are most
229 often restricted to contrasting markings: size, density and distribution of spots; presence of
230 black and white leading edges of dorsal and anal fins; any other markings such as dark bars
231 were also considered. In preserved material, light spots are interpreted as red spots based on
232 personal observations: after transfer to ethanol, red spots disappear transformed into pale
233 spots. Spots described as ocellated refer to spots enclosed by a light ring.

234

235

236 **3 Results**

237

238 **3.1. Molecular results**

239 Among the 38 contemporary specimens from Corsica and Sardinia, 34 CR sequences were
240 obtained, corresponding to eight new haplotypes (Table 2). **The alignment of CR sequences**
241 **are 998 nucleotides long with 47 phylogenetically informative sites. These haplotypes are**

242 genetically very close (distant from each other by up to two mutations, **Fig. 4**) to 37 published
243 GenBank haplotype sequences used as reference and illustrating the diversity on all the range
244 of the *S. trutta* complex. A comprehensive network of these new and published haplotypes is
245 presented **Fig. 4**. The sequences of the contemporary analyzed populations were all clustered
246 into ME, AD and MA lineages, according to the reference sequences. AT, DA and NA
247 lineages were represented only by GenBank sequences.

248 New haplotypes from Tyrrhenian trout were called ADcr2 to 6, MAcr1 and 2, and
249 MEcr1 (Table 2). Three populations are characterized by one private haplotype each: NIN,
250 (Corsica) with MEcr1; CAM (Sardinia) with ADcr2 and POZ (Corsica) with ADcr4. CAR,
251 (Corsica) is characterized by a majority of MA private haplotypes (MAcr1 and 2) and one AD
252 haplotype (ADcr5), while CHA (Corsica) is characterized by two AD haplotypes (ADcr3 and
253 ADcr6). SPE and ESE shared ADcr3 with CHA.

254

255 **3.2. Morphology**

256 Morphometric data are given in Table 3, and meristic data are summarized in Tables 4 to 6
257 including extensive comparative material (**Tables 1 and S1**). Selected results from ordination
258 by means of PCA are given for analyses focusing on variation between Tyrrhenian trout as a
259 whole towards Atlantic basin *S. trutta* (**Fig. 5**). Corresponding character loadings are given in
260 Supplementary **Tables S2 and S3**. The distinction of Tyrrhenian trout towards *S.*
261 *macrostigma* is illustrated with a biplot (**Fig. 6**) focusing on the major morphological trait of
262 the Tyrrhenian trout, viz. the longer head (HLpp) and slightly shorter caudal peduncle. The
263 two Spanish specimens are included in all analyses and graphs. For discussion related to the
264 comparatively large head in Tyrrhenian trout, HLpp is also presented as box plots in
265 comparison to an extended number of *Salmo* samples (**Fig. 7**).

266

267 *Variation among Tyrrhenian trout samples*

268 At first glance (**Figs. 8A to 8G**), the Tyrrhenian trout resembles Atlantic basin *S. trutta*, i.e.
269 rather strong jaws, numerous red and black spots, black and white edges of fins, most
270 prominent in CAM, CAR, CHJ and SPE. Black spots are sometimes irregularly distributed,
271 more or less aggregated along the flanks of the body (SPE, NIN) in contrast to, e.g. CAM
272 having its spots more evenly distributed (**Fig. 8A**). There are large variations in meristic
273 characters between different populations and the range of variation sometimes exceeds that of
274 the extensive comparative material (Tables 4 to 6). The NIN-sample and the two Spanish
275 specimens were not markedly different in multivariate statistics in comparison to the six
276 remaining samples.

277 Four specimens (1 POZ, 3 ESE) were different in the number of epurals in the caudal
278 skeleton, having three instead of two. All *Salmo* except *S. salar* have two, sometimes fused
279 (anomaly) to one. *Salmo salar* is polymorphic but two is more common (**see below**).

280

281 *Distinction of Tyrrhenian trout samples towards North Atlantic basin S. trutta*

282 The rather strong jaws and a colour pattern with prominent black and white leading edges on
283 the fins are shared between several populations of Atlantic basin *S. trutta* and the Tyrrhenian
284 trouts. Dark, more or less ocellated, spots on flanks of the body are also common in both.
285 However, some Tyrrhenian trout have their spots aggregated (**Figs. 8F, 8G** and p. 415 in
286 Kottelat and Freyhof, 2007). The sample from Spain also possesses this uncommon pattern
287 and was therefore especially highlighted in the comparative material. Sparsely or densely
288 distributed, dark spots on caudal fin are also common in Tyrrhenian trout (**Figs. 8B and 8G**,
289 CAM and SPE, respectively). Spots on caudal fins are rarely found in Atlantic basin *S. trutta*.
290 Multivariate statistics (**Fig. 5**) indicate distinction but not complete separation of **Tyrrhenian**
291 trout from Atlantic *S. trutta* due to differences in vertebral counts and head size.

292

293 *Distinction of Tyrrhenian trout samples towards S. macrostigma*

294 The extensive variation in meristic characters in Tyrrhenian trout as a whole (Tables 4 to 6)
295 covers the range of variation in *S. macrostigma* and limits the analyses to morphometric data.
296 PCA (not shown) reveals that HLpp and caudal peduncle length are the two morphometric
297 characters that distinguish them best (**Fig. 6**). The Tyrrhenian trout has longer head (HLpp)
298 and slightly shorter caudal peduncle compared to *S. macrostigma* (Table 3).

299

300

301 **4 Discussion**

302 **4.1. Genetic diversity: a strong differentiation pattern**

303 According to results from previous (Tougard *et al.*, 2018; Berrebi *et al.*, 2019) and
304 present studies, the Tyrrhenian trout is mainly characterized by an island specific mtDNA-
305 radiation within the AD lineage, as well as, to a lesser degree, by other lineages (AT, ME and
306 MA). The recently described NA lineage is also naturally present in Sicily. Thus, within a
307 rather limited and nowadays isolated region in the south center of *Salmo* distribution, a
308 comparatively high number of mtDNA lineages (four of the five recognizable major lineages)
309 occur naturally. It is also striking that a majority of the haplotypes recovered in the present
310 study were new (Table 2) despite more than two decades of CR sequencing in *Salmo*. The
311 presence of the ME lineage in the NIN sample, also observed in Corsica by Tougard *et al.*
312 (2018) and in other Tyrrhenian samples not included in the morphological analyses, is
313 explained by ancient introgressions evidenced elsewhere using nuclear markers (Berrebi *et*
314 *al.*, 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred,
315 according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007).
316 Moreover, the presence of MA lineage in some isolated Corsican rivers including CAR and

317 the range of distribution of the NA lineage (Morocco, Algeria, Sicily: Tougard *et al.*, 2018;
318 Berrebi *et al.*, 2019) demonstrates the multiple unknown events of migrations, invasions and
319 hybridizations which complicate the trout genetic pattern in the Tyrrhenian region. Finally,
320 the presence of several northern AT haplotypes recorded in the Tyrrhenian trout (Tougaard *et*
321 *al.*, 2018; Berrebi *et al.*, 2019) is due to stocking with commercial AT **hatchery** strains.

322 Nuclear markers (microsatellites) have also shown that the Tyrrhenian trouts exhibit
323 exceptionally differentiated genotypes, at a continental-like level, within the two small
324 sampled islands, but especially in Corsica where numerous autochthonous isolated small
325 populations still survive (Berrebi *et al.*, 2019). This strong differentiation among neighboring
326 rivers is typical of dry Mediterranean mountainous regions, **never frozen by glaciation and**
327 **providing way for migration** (Apostolidis *et al.*, 2008; Berrebi *et al.*, 2019).

328

329 **4.2. Morphological diversity in light of genetic diversity**

330

331 Regarding the strong morphological diversification between studied populations of
332 Tyrrhenian trout, one explanation may involve random effects. Berrebi *et al.* (2019) **showed**
333 very low levels of genetic variation within populations in Corsican streams based on
334 microsatellites, suggesting small population sizes and repeated bottleneck events.

335 Hypothetically, the frequent (c. 10 %), occurrence of three epurals in Tyrrhenian
336 trout compared to c. 0.1 % in comparative material might be a result of genetic drift
337 accelerated by bottlenecks. Three epurals in the caudal skeleton are typical for, e.g. most
338 Pacific trouts and salmon (*Oncorhynchus*), graylings (*Thymallus*) and whitefishes
339 (*Coregonus*) (Norden, 1961; Stearley and Smith, 1993) and appear to be the ancestral state
340 also retained as a polymorphism in *S. salar* with **12 out of 40 studied specimens having three.**

341 Delling and Doadrio (2005) also described a situation with a seemingly
342 plesiomorphic condition in rostrodermethmodid bone in the lake endemic *S. pallaryi* from
343 Lake Sidi Ali, Morocco, not recorded elsewhere in *Salmo*. The genetic characteristics of this
344 extinct trout is unknown but it is likely that these kinds of reversals approaching
345 morphological anomalies may occur under certain conditions involving random processes in
346 temporarily small populations.

347 Comparing Tyrrhenian samples to other *Salmo* spp., it is tempting to search for a
348 pattern connecting certain characters to certain mtDNA lineages. However, ancient
349 introgression in certain populations without strong impacts on morphology seems rather to be
350 the “rule” in many salmonids and other taxa (Martinez *et al.*, 2009; Gratton *et al.*, 2013;
351 Lerceteau-Köhler *et al.*, 2013; Berrebi *et al.*, 2017). The CAR sample also possessing the MA
352 mtDNA lineage shows no typical *S. marmoratus* characters, e.g. marbled color pattern, high
353 vertebral counts or a hypethmoid bone embedded in the rostral cartilage. In contrast, more
354 recent hybrids involving *S. marmoratus* show a variable but, overall, intermediate phenotype
355 (Delling, *et al.*, 2000). Prominent black and white leading edges on fins in several Tyrrhenian
356 populations are similar to Atlantic basin *S. trutta* and could tentatively be regarded as ancient
357 traces of the AT lineage. This pattern is also present in some North African trout, e.g. *S.*
358 *akairos* and *S. macrostigma* belonging to the NA lineage, close to the AT one (Tougard *et al.*,
359 2018). However, neither NA nor AT lineages have so far been reported from Corsica and
360 Sardinia, except AT lineages of hatchery origin.

361 Despite the complex pattern of migrations, hybridizations and genetic radiation in the
362 Tyrrhenian trout history, also giving rise to rather morphologically distinct populations in
363 Corsica and Sardinia (**Figs. 8A to 8G**), they all share a comparatively large head (**Fig. 6 and**
364 **7**). This contradictory pattern of morphological homogeneity for certain characters opposed to
365 strong differentiation in others take probably account of the genetic data in the search for

366 possible explanations. Turning focus to the overall large head in Tyrrhenian trout, it is
367 noticeable that this character varies extensively within and between different kinds of trouts
368 (**Fig. 7**). It should be noted that sample sizes vary considerably (**Fig. 7**) but several of them
369 can be characterized as typically small-headed, e.g. *S. salar*, *S. obtusirostris* and *S.*
370 *lourosensis*. Next after the Tyrrhenian trouts, the two North African lake trouts endemic from
371 Morocco, *S. akairos* and *S. pallaryi*, together with *S. marmoratus* and *S. dentex* inhabiting
372 Adriatic basin drainages, possess comparatively large heads. Thus, there is no obvious
373 correlation between size of head and habitat and/or lifestyle, e.g. rapid streams vs. lakes, and
374 it is fully possible that the large head is a result of a founder effect during ancient
375 establishment on the islands. However, body proportions can be affected by, e.g. growth rate.
376 Barlow (1961) refers to studies where it was shown that head length was smaller in faster
377 growing rainbow trout, *Oncorhynchus mykiss*. Thus, the comparatively large head in
378 Tyrrhenian trout can be a consequence of slow growth under harsh condition, or a
379 consequence of earlier sexual maturity. However, Pankhurst and Montgomery (1994) showed,
380 also for *O. mykiss* that retarded growth results in larger eyes. The Tyrrhenian trout possesses
381 on the average slightly smaller eyes compared to Atlantic *S. trutta* (Table 3) and consequently
382 retarded growth seems not to be a likely explanation for the large head.

383 The two Spanish specimens, referred to as *Salmo* sp. MNHN 1920 228-229, possess
384 a color pattern with irregularly distributed spots, also found in some Tyrrhenian trouts, and
385 are comparatively large headed (**Fig. 6**). They provide an example of what a hypothetical
386 ancestor could have looked like and, if the interpretation of locality information (Ebro basin)
387 is correct, it makes sense as the basin is dominated by AD haplotypes (Cortey *et al.*, 2004).
388 Also, the haplotype ADc13 found in Ebro is very close to the Tyrrhenian AD haplotypes
389 (**Fig. 4**).

390

391

392 **5 Conclusions**

393 Data presented herein suggest that within the rather unresolved *Salmo* complex in the
394 Mediterranean region, we may start to perceive a kind of large headed trout. However, more
395 populations from the islands and surrounding mainland (France, Spain, and Italy) need to be
396 studied to survey the distribution of this morphology. It would be a large step forward if this
397 kind of trout could get an identity, i.e. a scientific name to balance a perhaps too broad or
398 erroneous usage of names such as *S. trutta* and *S. macrostigma* in the Mediterranean region.
399 This long-term work already began with several recent molecular papers (Sanz, 2018;
400 Tougard *et al.*, 2018; Berrebi *et al.*, 2019) and the present study. The Tyrrhenian trout studied
401 here are left without a taxonomic identity but it is clearly demonstrated, in line with molecular
402 data (Tougard *et al.*, 2018), that they show no particular affinity to *S. macrostigma* once
403 described from Algeria.

404

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409

410

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412

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611

612 **8. Figures captions**

613 **Fig. 1.** Geographic position of the new sampled populations in Corsica and Sardinia.

614

615 **Fig. 2.** Measurements taken on *Salmo* specimens; 1, upper jaw depth, as largest depth of the
616 maxilla and supramaxilla; 2, snout length, from symphysis of premaxilla to osseous orbit
617 margin; 3, orbital horizontal diameter, between osseous orbital margin; 4, head depth, just
618 posterior to orbit; 5, orbital vertical diameter, between osseous orbital margin; 6, length of
619 maxilla, from **premaxilla** end to posterior end of maxilla; 7, upper jaw length, from
620 symphysis of premaxilla to posterior end of maxilla; 8, lower jaw length, from symphysis of
621 dentary to retroarticular; 9, pectoral fin length, from base of first ray to tip of longest ray; 10,
622 body depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest
623 ray; 12, pelvic fin length, from base of first ray to tip of longest ray; 13, body depth, at level
624 of origin of anal fin; 14, adipose fin length, from origin to tip; 15, anal fin length, from base of
625 first ray to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base
626 of caudal fin; 17, least depth of caudal peduncle; 18, length of upper caudal fin lobe, from
627 base to tip of longest ray; 19, length of middle caudal fin ray, from base to tip of shortest ray;
628 20, length of lower caudal fin lobe, from base to tip of longest ray; 21 standard length (SL),
629 from upper jaw symphysis to middle base of caudal fin; 22, predorsal length from upper jaw
630 symphysis to origin of dorsal fin; 23, head length, from upper jaw symphysis to posterior tip
631 of operculum; 24, premaxilla to preoperculum length, from **premaxilla** end of maxilla to
632 posterior margin of preoperculum (HLpp); 25, prepelvic length, from upper jaw symphysis to
633 origin of pelvic fin; 26, preanal length, from upper jaw symphysis to origin of anal fin; 27,
634 interorbital width, transverse at narrowest part of skull; 28, body width, transverse at widest
635 part of body at level of dorsal fin origin, above abdominal cavity.

636

637 **Fig. 3.** Radiograph of *Salmo* sp. POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu Corsica
638 (same specimen as in Fig. 8E). Upper left; a thin membranous bone indicated with dashed
639 white line. Upper right; the uncommon condition with three (i-iii) epurals in the caudal
640 skeleton.

641

642 **Fig. 4.** Median-joining network of control region haplotypes of some trout samples (new
643 Tyrrhenian haplotypes and AT, AD, DA, MA, ME and NA haplotypes from GenBank).
644 Haplotypes are indicated by numbers as given in Table 2. Black circles are for nodes, and
645 hatch marks are for mutation steps.

646

647 **Fig. 5.** Morphometric PC II plotted against meristic PC I for *Salmo* from Sardinia, Corsica
648 and Spain in comparison to Atlantic basin *S. trutta*.

649

650 **Fig. 6.** Premaxilla to preoperculum length plotted against caudal peduncle length for *Salmo*
651 from Sardinia, Corsica and Spain in comparison to *S. macrostigma*. Linear regression lines
652 with 95% confidence bands are shown for each group separately.

653

654 **Fig. 7.** Box plot of premaxilla to preoperculum length as % of SL, totally 518 specimens 116-
655 208 mm SL. Number of specimens is given for each sample separately. Samples in bold are
656 detailed in Table 1. Additional samples given in capital letters (Delling, **unpublished**) refer to
657 rivers or streams in the given regions (**Table S1**). Ezenam is a lake in Daghestan and *labrax*
658 represents trout from Black Sea basin with an anadromous silvery and slender **appearance**.
659 Turkey and Mediterranean are samples scattered in the regions. Boxes represent median value
660 +/- 25% of the observations, and whiskers the inner fences. Asterisks are outside or far
661 outside values.

662

663 **Fig. 8. External aspect of seven of the trouts, *Salmo* sp. analyzed in the present study.**

664 **Picture A-F taken after fixation in formalin prior to transfer to ethanol. A. CAM, NRM**
665 **61782, 183.7 mm SL; Camboni Sardinia. B. CAR, NRM 62571, 167.6 mm SL; Carnevale**
666 **Corsica. C. CHJ, NRM62573, 136.6 mm SL; Chjuvone Corsica. D. ESE, NRM 61813,**
667 **152.0 mm SL; Val d'Ese Corsica. E. POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu**
668 **Corsica. F. SPE, NRM61812, 148.1 mm SL Speloncellu Corsica. G. NIN, Lake Ninu**
669 **Corsica © S. Muracciole**

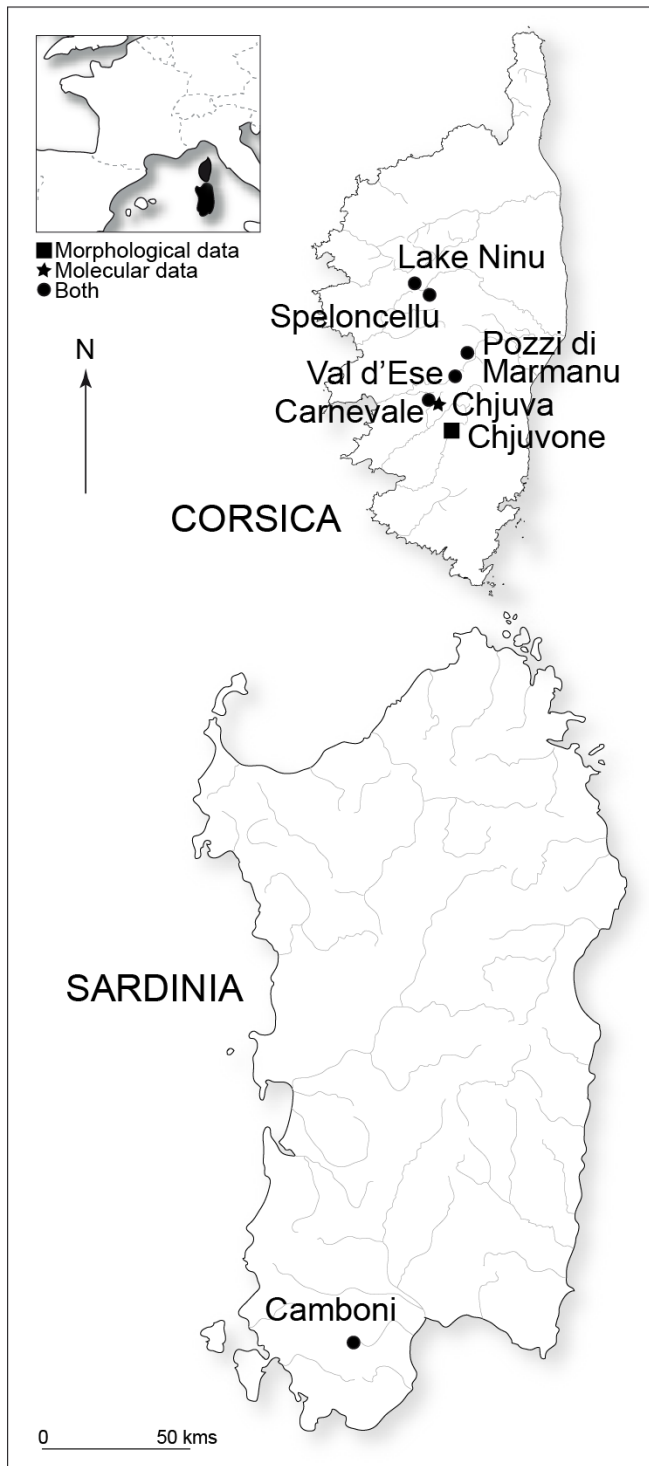


Fig. 1. Geographic position of the new sampled populations in Corsica and Sardinia.

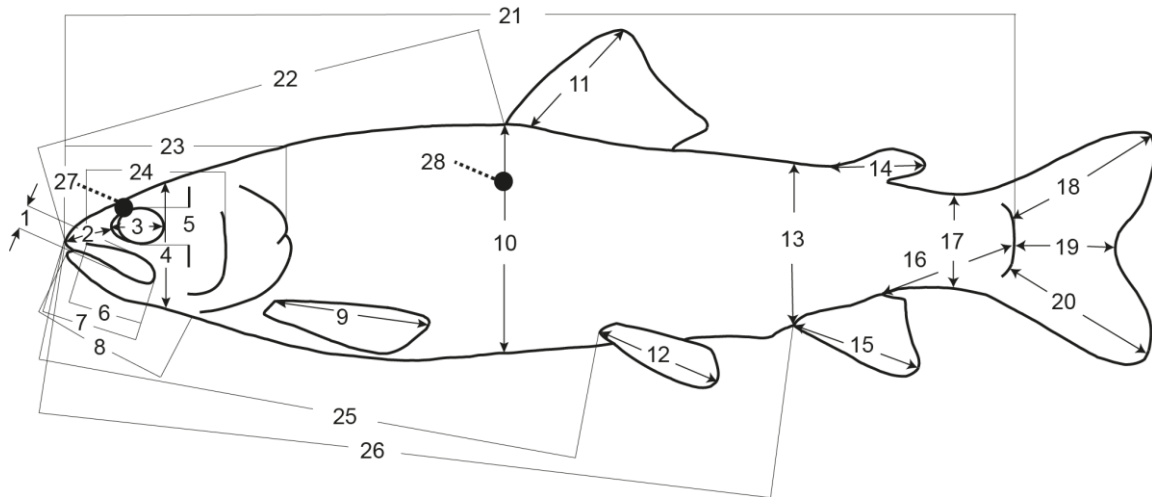


Fig. 2. Measurements taken on *Salmo* specimens; 1, upper jaw depth, as largest depth of the maxilla and supramaxilla; 2, snout length, from symphysis of premaxilla to osseous orbit margin; 3, orbital horizontal diameter, between osseous orbital margin; 4, head depth, just posterior to orbit; 5, orbital vertical diameter, between osseous orbital margin; 6, length of maxilla, from premaxilla end to posterior end of maxilla; 7, upper jaw length, from symphysis of premaxilla to posterior end of maxilla; 8, lower jaw length, from symphysis of dentary to retroarticular; 9, pectoral fin length, from base of first ray to tip of longest ray; 10, body depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest ray; 12, pelvic fin length, from base of first ray to tip of longest ray; 13, body depth, at level of origin of anal fin; 14, adipose fin length, from origin to tip; 15, anal fin length, from base of first ray to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base of caudal fin; 17, least depth of caudal peduncle; 18, length of upper caudal fin lobe, from base to tip of longest ray; 19, length of middle caudal fin ray, from base to tip of shortest ray; 20, length of lower caudal fin lobe, from base to tip of longest ray; 21 standard length (SL), from upper jaw symphysis to middle base of caudal fin; 22, predorsal length from upper jaw symphysis to origin of dorsal fin; 23, head length, from upper jaw symphysis to posterior tip of operculum; 24, premaxilla to preoperculum length, from premaxilla end of maxilla to posterior margin of preoperculum (HLpp); 25, prepelvic length, from upper jaw symphysis to origin of pelvic fin; 26, preanal length, from upper jaw symphysis to origin of anal fin; 27, interorbital width, transverse at narrowest part of skull; 28, body width, transverse at widest part of body at level of dorsal fin origin, above abdominal cavity.

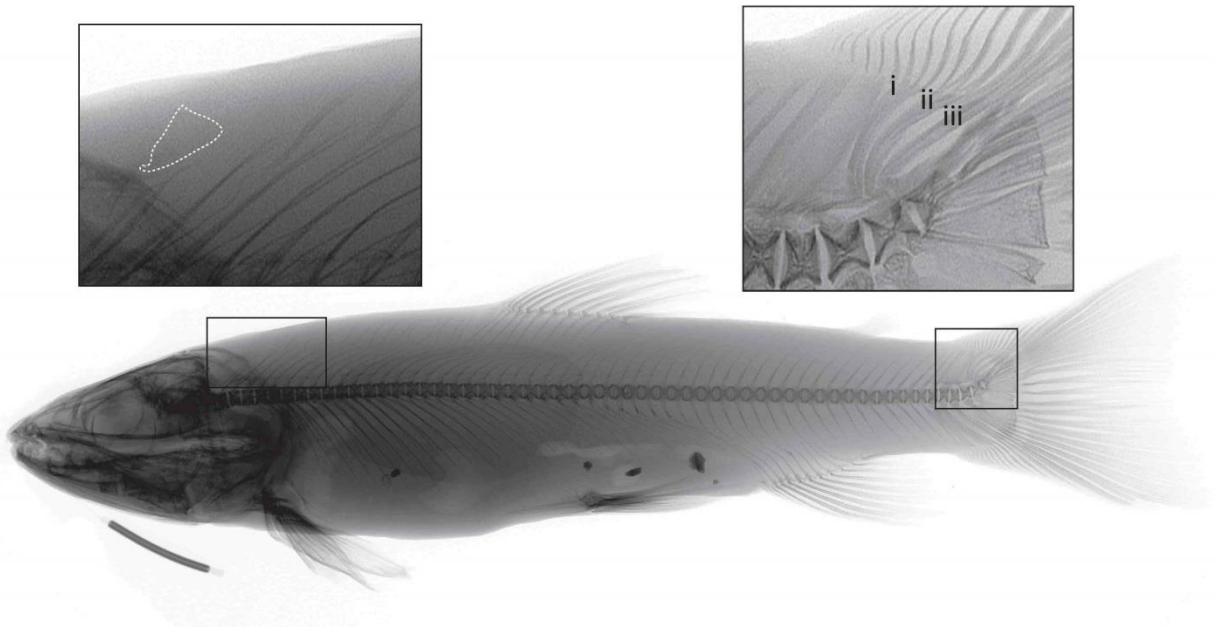


Fig. 3. Radiograph of *Salmo* sp. POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu Corsica (same specimen as in Fig. 8E). Upper left; a thin membranous bone indicated with dashed white line. Upper right; the uncommon condition with three (i-iii) epurals in the caudal skeleton.

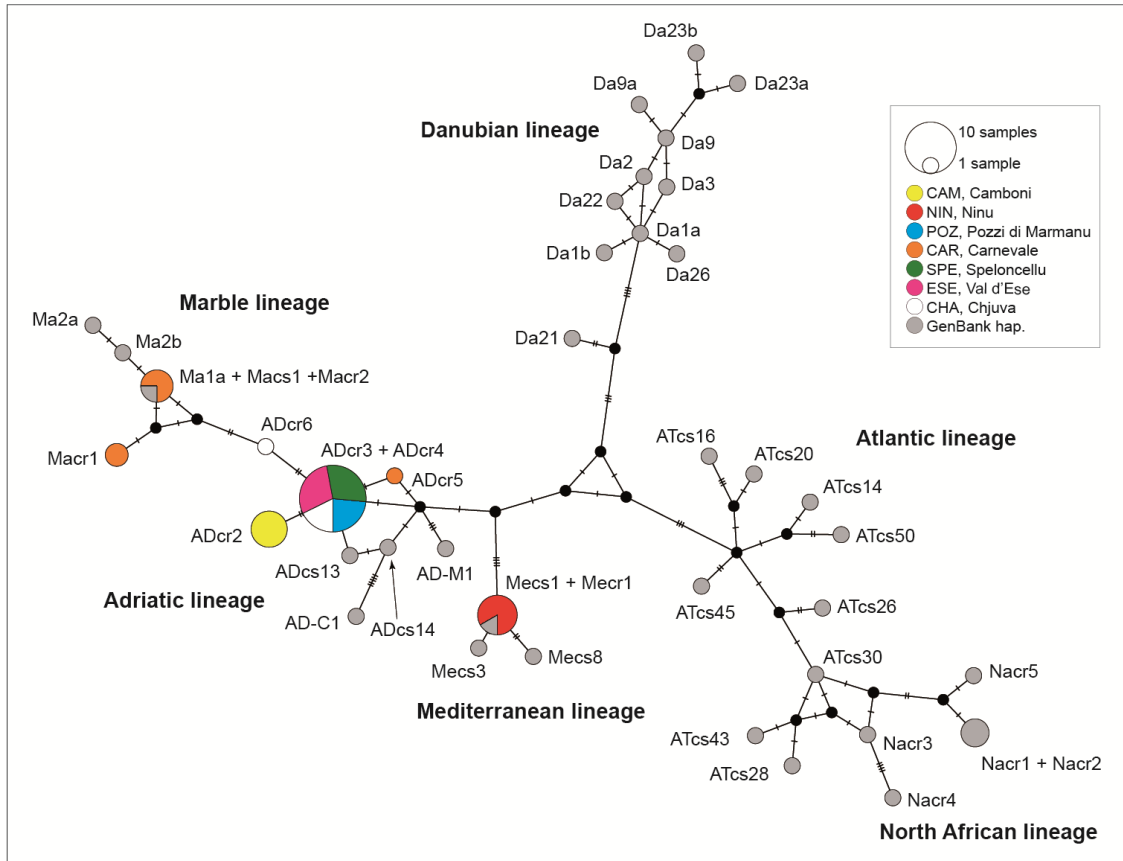


Fig. 4. Median-joining network of control region haplotypes of some trout samples (new Tyrrhenian haplotypes and AT, AD, DA, MA, ME and NA haplotypes from GenBank). Haplotypes are indicated by numbers as given in Table 2. Black circles are for nodes, and hatch marks are for mutation steps.

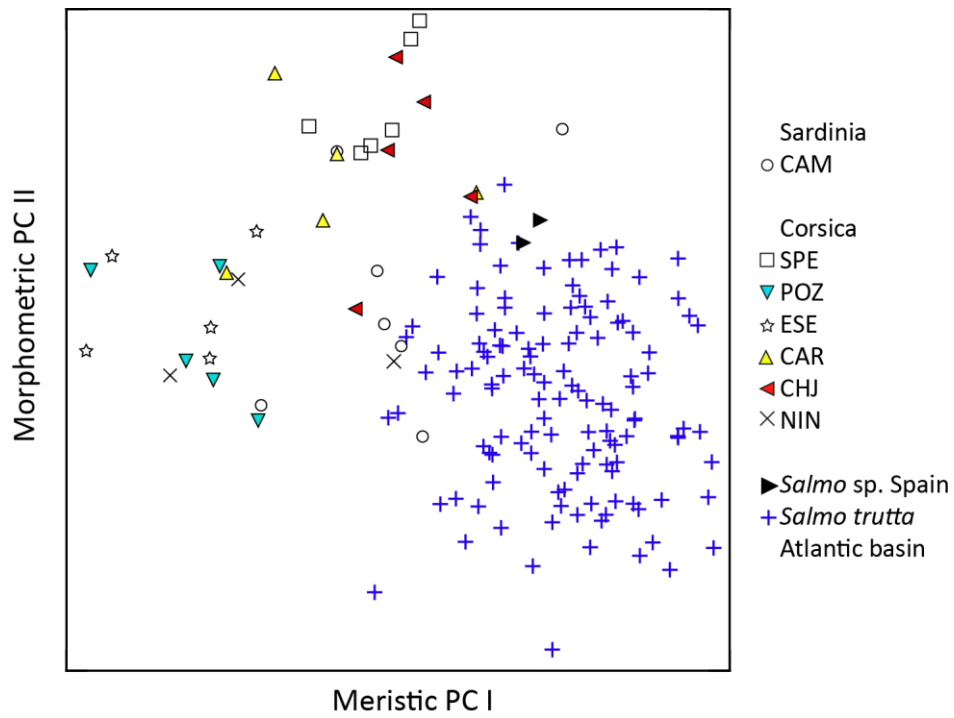


Fig. 5. Morphometric PC II plotted against meristic PC I for *Salmo* from Sardinia, Corsica and Spain in comparison to Atlantic basin *S. trutta*.

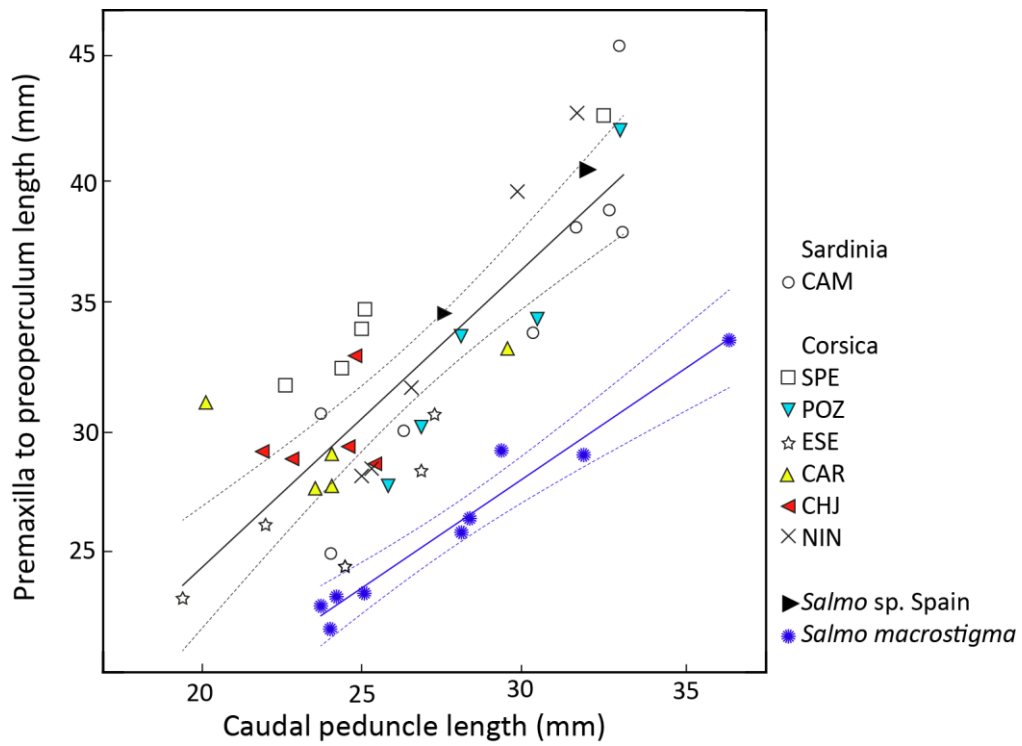


Fig. 6. Premaxilla to preoperculum length plotted against caudal peduncle length for *Salmo* from Sardinia, Corsica and Spain in comparison to *S. macrostigma*. Linear regression lines with 95% confidence bands are shown for each group separately.

Premaxilla to preoperculum length, HLpp (% of SL)

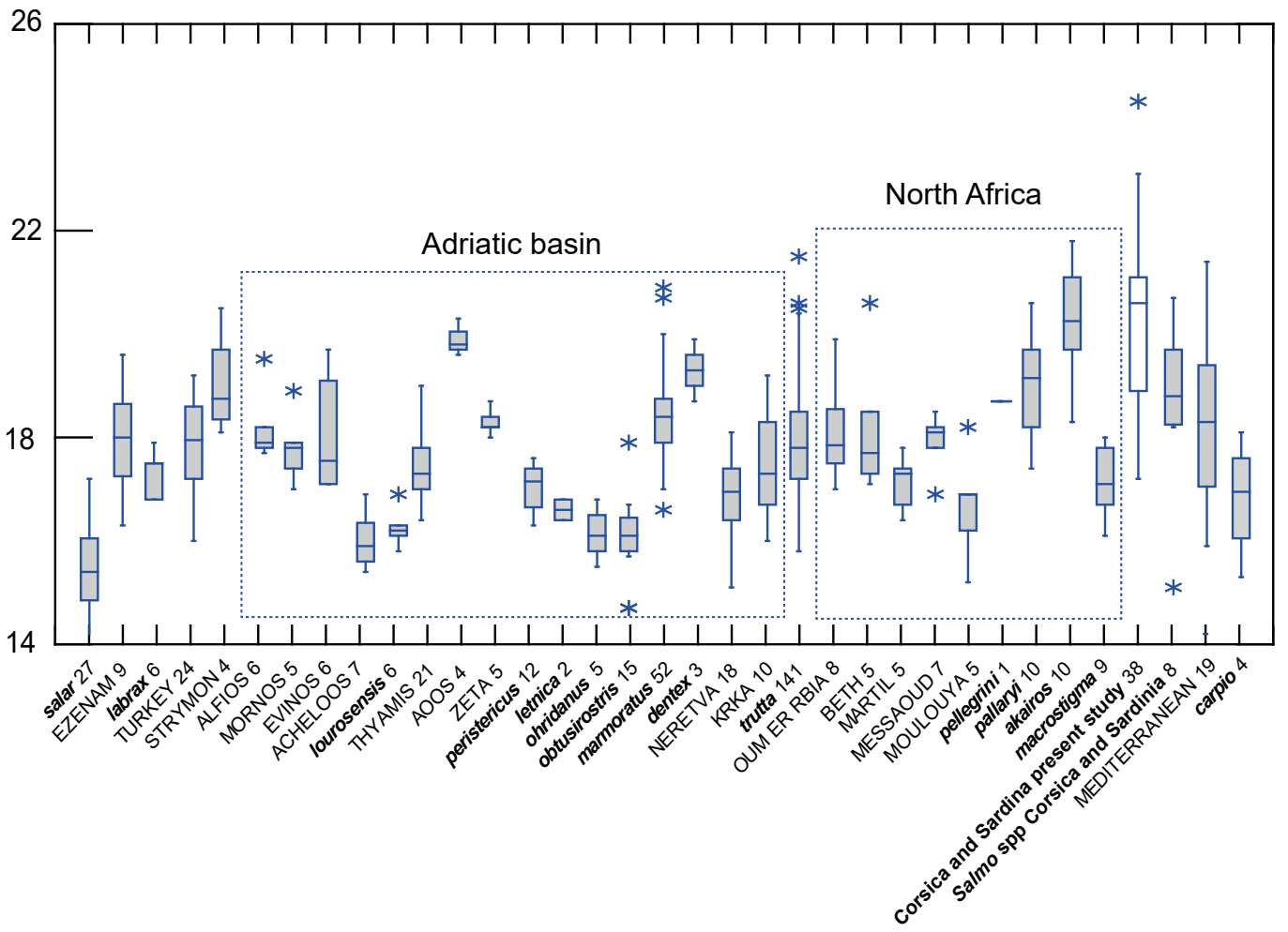


Fig. 8. External aspect of seven of the trouts analyzed in the present study. For A to F, the pictures were taken after fixation in formalin prior to transfer to ethanol.



A. *Salmo* sp. CAM, NRM 61782, 183.7 mm SL; Camboni Sardinia.



B. *Salmo* sp. CAR, NRM 62571, 167.6 mm SL; Carnevale Corsica.



C. *Salmo* sp. CHJ, NRM62573, 136.6 mm SL; Chjuvone Corsica.



D. *Salmo* sp. ESE, NRM 61813, 152.0 mm SL; Val d'Ese Corsica.



E. *Salmo* sp. POZ, NRM62572, 197.3 mm SL; Pozzi di Marmanu Corsica.



F. *Salmo* sp. SPE, NRM61812, 148.1 mm SL Speloncellu Corsica.



G. *Salmo* sp. NIN, Lake Ninu Corsica.
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Table 1. Studied material of *Salmo* from Sardinia and Corsica and selected comparative material (Tables 3-6). Catalog numbers are given for the Tyrrhenian samples only.

Taxon	River/Lake	Region	Date of capture	N	Museum Collection	Reference	Remarks
<i>Salmo</i> spp. Sardinia		Sardinia	1909-1960	9	ZMH, MHNG	Delling and Doadrio (2005)	Incomplete locality data
CAM	Camboni	Sardinia	2010-2012	8	NRM 61782, 61783, 65092	this study	
<i>Salmo</i> spp. Corsica		Corsica	1895-1909	10	MNHN, NMW, BMNH	Delling and Doadrio (2005)	Incomplete locality data
SPE	Speloncellu	Corsica	2011	5	NRM 61812	this study	
POZ	Pozzi di Marmanu	Corsica	2012	5	NRM 62572	this study	
ESE	Val d'Ese	Corsica	2011	5	NRM 61813	this study	
CAR	Carnevale	Corsica	2012	5	NRM 62571	this study	
CHJ	Chjuvone	Corsica	2012	5	NRM 62573	this study	Morphology only
CHA	Chjuva	Corsica	2004	5		this study	Genetics only
NIN	Lake Ninu	Corsica	2013	5	NRM 65092	this study	
<i>Salmo</i> sp. Spain **		Spain	1920	2	MNHN	this study	
<i>Salmo macrostigma</i> *	El Abaich River	Algeria	1866-1907	9	MNHN, BMNH, NMW	Delling and Doadrio (2005)	Syntype included
<i>Salmo pallaryi</i> *	Lake Sidi Ali	Morocco	1927-1936	17	MNHN, BMNH, NRM	Delling and Doadrio (2005)	Syntypes included
<i>Salmo pellegrini</i> *	Tensift River	Morocco	1931	1	NMW	Delling and Doadrio (2005)	Syntype
<i>Salmo akairos</i> *	Lake Ifni	Morocco	1995	10	NRM, MNCN	Delling and Doadrio (2005)	Holotype and paratypes
<i>Salmo trutta</i>	Details in reference	European Atlantic basin	1827-1999	243		Delling (2002)	Numerous samples from several countries and collections
<i>Salmo salar</i>	Details in reference	European Atlantic basin	1882-1998	40		Delling (2002)	Numerous samples from several countries and collections
<i>Salmo marmoratus</i>	Po, Adige, Soca and Neretva Rivers	Italy, Slovenia and Bosnia and Herzegovina	1823-1998	77	NRM, ZISP, MNHN, BMNH,	Delling (2002)	

					NMW		
<i>Salmo carpio</i> *	Lake Garda	Italy	1971-1998	13	NRM, ZISP, BMNH	Delling (2002)	Neotype included
<i>Salmo ischchan</i> *	Lake Sevan	Armenia		24	NRM, ZISP		
<i>Salmo letnica</i> *	Lakes Ohrid and Prespa	Albania, Macedonia and Greece	1890-1995	11	NRM, ZISP, MHCH, ZMH	Delling (2003)	Endemic to Ohrid but stocked into Prespa lakes
<i>Salmo platycephalus</i> *	Seyhan	Turkey	1966-2003	3	NRM, ZMH	Turan et al.(2012)	Holotype included
<i>Salmo obtusirostris</i>	Jadro and Neretva Rivers	Croatia, Bosnia and Herzegovina	1883-2000	19	NRM, ZMH, ZISP, MNHN, MHCH	Delling (2003)	
<i>Salmo ohridanus</i> *	Lakes Ohrid and Prespa	Albania, Macedonia and Greece	1924-2002	13	ZMH, MNHN	Delling & Doadrio (2005)	Endemic to Ohrid but stocked into Prespa lakes
<i>Salmo lourosensis</i> *	Louros Stream	Greece	1977	7	NRM	Delling (2010)	
<i>Salmo peristericus</i> *	Agios Germanos Stream	Greece	1977, 1998	9	NRM	Delling (2010)	
<i>Salmo dentex</i>	Neretva and Cetina Rivers	Bosnia and Herzegovina	1843-	5	NMW, NRM	Delling (2010)	Lectotype included

*) Endemic taxa and/or samples restricted to type locality

**) Locality data for MNHN 1920 0228-0229: Spain, Lerida, Sarrade, 2052 m.a.s.l. is interpreted as close to the mountain Pic de la Pala Alta de Sarradé (2893 m, 42° 34' 27.1" N, 0° 53' 16.82" E) in the Lerida/Lleida region in Catalonia, Spain, most probably part of Ebro basin.

Table 2. Distribution of the haplotypes involved in this study

Haplotypes	Accession Number	References / Samples	Locality of first observation
ATcs14	EF530476	Cortey <i>et al.</i> (2009)	Iceland (Skorradalsvatn R.)
ATcs16	EF530478	Cortey <i>et al.</i> (2009)	Spain (Several Cantabric rivers)
ATcs20	EF530482	Cortey <i>et al.</i> (2009)	Russia (Vorobiex R.)
ATcs26	EF530488	Cortey <i>et al.</i> (2009)	Spain (Duero R.)
ATcs28	EF530490	Cortey <i>et al.</i> (2009)	Spain (Tajo R.)
ATcs30	EF530492	Cortey <i>et al.</i> (2009)	Spain (Tajo R.)
ATcs43	EF530504	Cortey <i>et al.</i> (2009)	Spain (Duero R.)
ATcs45	EF530505	Cortey <i>et al.</i> (2009)	Iceland (Skorradalsvatn R.)
ATcs50	EF530510	Cortey <i>et al.</i> (2009)	UK (Stour R.)
ADC1	DQ381567	Sušnik <i>et al.</i> (2007)	Montenegro + Serbia + Albania (3 rivers)
ADM1	DQ381566	Sušnik <i>et al.</i> (2007)	Montenegro + Serbia + Albania (3 rivers)
ADcs14	AY836343	Cortey <i>et al.</i> (2004)	France (Corsica)
ADcs15	AY836344	Cortey <i>et al.</i> (2004)	France (Corsica)
Ma1a	DQ841191	Meraner <i>et al.</i> (2007)	Italy (Po R.)
Ma2a	DQ841189	Meraner <i>et al.</i> (2007)	Italy (Po R.)
Ma2b	DQ841190	Meraner <i>et al.</i> (2007)	Italy (Po R.)
MAcs1	AY836365	Cortey <i>et al.</i> (2004)	Slovenia (2 Adriatic rivers)
MEcs1	AY836350	Cortey <i>et al.</i> (2004)	Spain (8 watersheds)
MEcs3	AY836352	Cortey <i>et al.</i> (2004)	Spain (8 watersheds)
MEcs8	AY836357	Cortey <i>et al.</i> (2004)	Spain (8 watersheds)
Da1a	AY185568	Duftner <i>et al.</i> (2003)	Austria (5 Danubian rivers)
Da1b	AY185569	Duftner <i>et al.</i> (2003)	Austria (Lake Gossenköllesee)
Da2	AY185570	Duftner <i>et al.</i> (2003)	Austria (Fressnitzbach R.)
Da3	AY185571	Duftner <i>et al.</i> (2003)	Austria (Kleiner Kamp R.)
Da9	AY185572	Duftner <i>et al.</i> (2003)	Austria (Kleiner Kamp R.)
Da9a	GQ222380	Jadan <i>et al.</i> , unpubl.	Croatia (Plitvica R.)
Da22	AY185573	Duftner <i>et al.</i> (2003)	Austria (2 Danubian rivers)
Da23a	AY185574	Duftner <i>et al.</i> (2003)	Austria (Kleiner Kamp R.)
Da23b	AY185575	Duftner <i>et al.</i> (2003)	Austria (Lohnbach R.)
Da24	AY185576	Duftner <i>et al.</i> (2003)	Austria (Waldaist R.)
Da26	DQ841194	Meraner <i>et al.</i> (2007)	Italy (Po R.)
NAcr1	LT617612	Tougard <i>et al.</i> (2018)	Italy (Anapo R., Sicily)
NAcr2	LT617613, LT617614	Tougard <i>et al.</i> (2018)	Italy (Anapo R., Sicily)
NAcr3	LT617630	Tougard <i>et al.</i> (2018)	Algeria (El-Abaïch oued)
NAcr4	LT617631	Tougard <i>et al.</i> (2018)	Algeria (El-Abaïch oued)
NAcr5	LT617632	Tougard <i>et al.</i> (2018)	Morocco
ADcr2	MK184916-20	CAM (this survey)	Italy (Sardinia)
ADcr3	MK184921-25, 30-34, 41-42, 44	SPE, ESE, CHA (this survey)	France (Corsica)
ADcr4	MK184926-29	POZ (this survey)	France (Corsica)

ADcr5	MK184935	CAR (this survey)	France (Corsica)
ADcr6	MK184943	CHA (this survey)	France (Corsica)
MAcr1	MK184938-40	CAR (this survey)	France (Corsica)
MAcr2	MK184936-37	CAR (this survey)	France (Corsica)
MEcr1	MK184945-49	NIN (this survey)	France (Corsica)

Table 3. Morphometry of *Salmo* spp. Number of studied specimens (N) for certain measurements varies due to condition of preserved specimens.

	Tyrrhenian <i>Salmo</i>					<i>Salmo macrostigma</i>					<i>Salmo trutta</i>				
	N	min	max	mean	SD	N	min	max	mean	SD	N	min	max	mean	SD
Standard length (mm)	38	116.3	208.5	158.6	23.5	9	129.9	208.0	151.5	25.92	138	118.5	207.3	160.6	26.6
In percent of standard length															
Preanal length	38	74.2	80.2	76.7	1.29	9	73.4	78.1	75.9	1.45	138	72.8	81.9	76.4	1.56
Prepelvic length	38	54.6	63.0	56.8	1.49	9	51.6	56.3	53.6	1.83	138	50.4	60.8	55.2	1.79
Predorsal length	38	46.2	51.6	48.8	1.38	9	44.8	49.5	47.1	1.58	138	44.0	50.9	47.6	1.42
Head length	38	25.3	32.7	28.1	1.80	9	23.3	26.2	24.9	0.89	138	22.6	29.5	25.5	1.27
Premaxilla to preoperculum length	38	17.2	24.5	20.3	1.64	9	16.1	18.0	17.2	0.65	138	15.8	21.5	17.9	1.02
Caudal peduncle length	38	15.0	18.3	16.6	0.74	9	17.3	19.5	18.3	0.77	138	14.6	19.8	17.3	0.97
Caudal peduncle depth	38	9.7	12.4	11.2	0.51	9	9.9	12.0	11.1	0.69	138	8.7	11.8	10.1	0.68
Length of upper caudal fin lobe	32	17.5	22.5	19.9	1.02	9	18.4	22.9	20.7	1.71	134	16.8	23.4	20.4	1.28
Length of lower caudal fin lobe	33	17.6	21.5	19.9	0.96	9	19.3	22.3	20.9	1.22	134	15.1	23.3	20.4	1.39
Length of middle caudal fin ray	38	11.0	16.9	14.4	1.10	9	11.9	14.4	13.0	0.81	137	10.9	15.5	13.4	0.90
Dorsal fin height	38	15.0	19.9	17.1	1.31	9	15.0	19.5	17.3	1.33	138	12.1	19.1	16.1	1.22
Pectoral fin length	38	16.3	23.5	19.3	1.74	9	16.6	20.6	18.7	1.27	138	15.0	21.6	18.4	1.22
Pelvic fin length	38	12.7	18.6	15.4	1.29	9	13.0	16.7	14.9	1.03	137	11.8	17.2	14.3	0.94
Adipose fin length	38	5.7	11.4	8.6	1.57	9	5.5	8.3	7.2	0.91	138	4.3	10.5	8.4	1.12
Anal fin length	37	12.8	23.1	17.4	2.20	9	17.0	19.8	18.3	0.79	138	13.5	18.8	16.1	1.16
Body width	38	12.4	17.3	14.7	1.18	9	10.3	12.1	11.4	0.64	138	7.6	16.0	12.3	1.60
Body depth at origin of dorsal fin	38	19.7	27.3	23.5	1.65	9	24.7	28.2	26.6	1.02	138	19.7	28.2	23.9	1.55
Body depth at origin of anal fin	38	16.2	20.5	18.2	0.91	9	19.2	21.9	20.1	0.90	138	13.1	24.7	17.8	1.26
Head depth	36	13.2	19.6	15.9	1.59	9	13.1	16.1	14.3	0.90	138	12.0	17.3	13.8	0.82
In percent of head length															
Horizontal orbit diameter	38	22.1	30.6	26.6	1.99	9	26.5	34.1	30.0	2.33	138	22.8	33.4	28.4	2.16
Vertical orbit diameter	38	18.9	27.0	23.4	2.00	9	20.8	27.7	24.6	2.31	138	18.9	28.2	23.7	1.97
Interorbital width	38	23.2	29.8	26.3	1.79	9	26.4	28.6	27.8	0.77	138	24.2	33.5	28.5	1.83
Snout length	38	23.4	31.1	27.0	1.66	9	21.8	28.8	25.6	2.22	138	21.0	29.0	25.2	1.49
Upper jaw length	38	50.2	65.9	57.2	3.57	9	49.3	56.0	52.5	1.91	138	43.4	61.8	52.1	2.51
Length of maxilla	38	40.8	54.5	46.0	2.89	9	38.3	45.3	42.4	2.04	138	34.3	50.1	41.6	2.15
Height of maxilla	38	9.3	15.2	11.6	1.29	9	9.4	11.9	10.7	0.77	138	9.0	13.1	11.0	0.83
Lower jaw length	38	59.3	71.9	64.6	3.33	9	58.8	66.9	61.1	2.44	138	53.4	69.0	61.5	2.66
Gill raker length	38	6.0	11.0	7.6	1.09	9	7.4	10.8	8.8	1.08	135	5.0	10.2	7.6	0.89

Table 4. Frequency distribution of scale counts from base of adipose fin to lateral line and left side branchiostegal counts in *Salmo* spp.

	scales from base of adipose fin to lateral line											left side branchiostegals							
	11	12	13	14	15	16	17	18	19	20	21	22	23	8	9	10	11	12	13
<i>Salmo</i> spp. Sardinia						3	2	4								5	3	2	
CAM					2	1	3		2							4	4		
<i>Salmo</i> spp. Corsica					1	2	1	1	4							2	8		
SPE					1	1	3									2	3		
POZ								4		1						2	3		
ESE						1	2	1	1					1	2	2			
CAR										3		1	1				3	2	
CHJ								1	1	3						1	4		
NIN						3	1	1							2	3			
<i>Salmo</i> sp. Spain						1	1										2		
<i>Salmo macrostigma</i>						5	3									2	6	1	
<i>Salmo pallaryi</i>							1	4	4	5	3						3	3	11
<i>Salmo pellegrini</i>					1												1		
<i>Salmo akairos</i>		1	2	5	2												1	4	5
<i>Salmo trutta</i>				8	55	90	61	23	4	1					4	40	111	80	8
<i>Salmo salar</i>	2	5	8	15	9		1									9	16	15	
<i>Salmo marmoratus</i>			4	8	33	24	6	2							1	7	29	35	5
<i>Salmo carpio</i>			1	2	5	4	1								2	5	2	4	1
<i>Salmo ischchan</i>					1	6	5	4	4	4					1	8	12	3	
<i>Salmo letnica</i>				2	5	4											7	3	1
<i>Salmo platycephalus</i>						1											1		
<i>Salmo obtusirostris</i>		3	9	7												3	8	8	
<i>Salmo ohridanus</i>	6	6	1												4	9			
<i>Salmo lourosensis</i>						3	4									5	2		
<i>Salmo peristericus</i>					4	6	2								2	9	1		
<i>Salmo dentex</i>					2	2	1										1	2	2

Table 5. Frequency distribution of vertebral counts and caudal fin upper procurrent rays in *Salmo* spp.

	vertebrae													caudal fin upper procurrent rays						
	52	53	54	55	56	57	58	59	60	61	62	63	10	11	12	13	14	15	16	17
<i>Salmo</i> spp. Sardinia			1			2	1	1							1	3	1	1		
CAM						1	6		1							4	4			
<i>Salmo</i> spp. Corsica					1	3	5									3	2	2	2	
SPE					1		2	2											1	4
POZ				2	3												5			
ESE				1	4											3	1	1		
CAR					1	3		1								1	1			3
CHJ						1	3	1								1	1	3		
NIN				1	2	2										4		1		
<i>Salmo</i> sp. Spain							1	1									1			1
<i>Salmo macrostigma</i>						4	3									5	2			
<i>Salmo pallaryi</i>					3	13	2	1									4	11	4	
<i>Salmo pellegrini</i>			1														1			
<i>Salmo akairos</i>					6	3	1								1	3	5	1		
<i>Salmo platycephalus</i>								3								1	2			
<i>Salmo obtusirostris</i>					1	4	9								1	8	5			
<i>Salmo ohridanus</i>	1	3	3		1									1	7					
<i>Salmo trutta</i>					1	15	48	69	38	15	1					4	31	60	72	28
<i>Salmo salar</i>							1	16	15	7		1	1	8	31					
<i>Salmo marmoratus</i>								5	14	42	15	1		1	30	32	10	5		
<i>Salmo carpio</i>								5	7	2						2	7	5		
<i>Salmo ischchan</i>			2	6	12	2									2	7	9	3	1	
<i>Salmo letnica</i>					1	3	1	3								1	6	1		
<i>Salmo lourosensis</i>						2	4	1								5	2			
<i>Salmo peristericus</i>							3	9								4	6	1	1	
<i>Salmo dentex</i>								2	3								2	2	1	

Table 6. Frequency distribution of gill raker counts in *Salmo* spp.

	total number of gill rakers on first arch												
	13	14	15	16	17	18	19	20	21	22	23	24	≥25
<i>Salmo</i> spp. Sardinia			2	1	1	4			1				
CAM					3	5							
<i>Salmo</i> spp. Corsica		1	2	1	3	2	1						
SPE					2	3							
POZ						3	1	1					
ESE					3	1	1						
CAR						2	3						
CHJ					1	1	3						
NIN				2	2	1							
<i>Salmo</i> sp. Spain					1	1							
<i>Salmo macrostigma</i>				1	2	3	2	1					
<i>Salmo pallaryi</i>							2	6	6	1	2		
<i>Salmo pellegrini</i>									1				
<i>Salmo akairos</i>								4	3	1	2		
<i>Salmo platycephalus</i>									1	1			1
<i>Salmo obtusirostris</i>											2		17
<i>Salmo ohridanus</i>					2	4	5	2					
<i>Salmo trutta</i>		1	14	46	99	54	25	4					
<i>Salmo salar</i>						4	12	18	5	1			
<i>Salmo marmoratus</i>	1	4	12	18	28	14							
<i>Salmo carpio</i>				2	6	5							
<i>Salmo ischchan</i>						6	8	7	3				
<i>Salmo letnica</i>							2	6	1	1			1
<i>Salmo lourosensis</i>						2	2	2	1				
<i>Salmo peristericus</i>				3	5	3		1					
<i>Salmo dentex</i>							3	1	1				

SUPPLEMENTARY INFORMATION: Studied material of *Salmo* spp.

Primary material of Tyrrhenian trout *Salmo* sp. from Corsica and Sardinia

NRM 61783, 61950, 61812, 61813, 62571, 62572, 62573, 65092

Comparative material (Figures 5-6, Tables 3, S2 and S3)

Primary and comparative material is also included in Fig. 7 and Tables 4-6 together with additional comparative material listed below.

Salmo macrostigma from Algeria

BMNH 1866.1.18:1-3

MNHN A7585 (SYNTYPE), 0000-1909, 1899-0242

NMW 67970

Salmo sp. Spain

MNHN 1920 0228-0229

Salmo trutta, Atlantic basin

BMNH 1865.7.10:2-12, 1866.1.8:1-4, 1947.9.12:1-3, 1982.11.15:6-9, 1986.5.20:208-213,
1986.5.20:94-95, 1991.7.12:18-20

MHNG 816.8

MNHN 0000-3639, 0000-3640, 0000-3643, 1923-0216, 1939-0017, A5532, A5533, B0722

NMW66013

NRM 20125, 20126, 23404, 23406, 23661, 24343, 24344, 24345, 24357, 24361, 24841, 36995,
41373, 41781, 41785, 41785, 41790, 41791, 41793, 41794, 42540, 42541, 42542, 42548, 42549,
42551, 42558, 43978, 44037, 44475, 44780

ZMH 10712, 10733, 10734, 10738, 1206, 5672, 5735, 6346

Additional comparative material (Figure 7, Tables 4-6)

Sequence of listed taxon or geographic region follows Fig. 7 (left to right) and inclusion of a particular specimen in Fig. 7 and/or Tables 4-6 depends on SL, state of preservation and, availability of X-ray data for Table 5.

Salmo salar

MNHN 0000-1441, 1898-1143, 1894-0004, 1894-0005, 1939-0016

NRM 21142, 24914, 41372, 42545, 42546, 42547, 46136

ZMH 10727, 10714

EZENAM (*Salmo ezenami*)

ZISP 28356, 48317

Salmo cf. *labrax*

BMNH 1913.5.25:1-3, 1962.9.25:1, 1991.7.12:21-22

NMW 65628, two uncatalogued specimens

TURKEY

NMW 80837, 90952, 50581, 50582, 50583

ZMH 2450, 3578, 4222, 4223, 4224

STRYMON

NRM 60790, 60791

ALFIOS

NRM 46352, 60785

MORNOS

CMK 16980

NRM 60789

EVINOS

CMK 16975

NRM 46353, 60786

ACHELOOS

NRM 46357

Salmo lourosensis

NRM 60787 (HOLOTYPE), 60788

THYAMIS

NRM 46355, 46356

AOOS

NRM 46354

ZETA

NMW 22904, 22905, 22906, 22907, 22908

Salmo peristericus

NRM 42538, 60784

Salmo letnica

MHCH 2573.92

MNHN 1977-0262

NMW 65650

ZMH 791, 9182

ZISP39456

Salmo ohridanus

CMK17387

MNHN1924-0227, one uncatalogued specimen

ZISP 39455

ZMH 790, 1461

Salmo obtusirostris

MHCH 608.66

MNHN A6037, A7589, 1904-0032

NRM46364

ZISP39451

ZMH10743

Salmo marmoratus

BMHN 1924.3.14:2-3, 1924.3.14:4-6, 1924.3.14:7-10, 1924.3.14:11-13
MNHN 0000-3635, 0000-3636, 0000-4920, B1139
NMW 65890, 65895
NRM 41516, 41519, 41522, 41523, 41528, 41529, 44701, 44702, 44703
ZISP 48210

Salmo dentex

NMW 65864, 65887, 65895 (LECTOTYPE), 95248
NRM 25000

NERETVA

NRM 46358, 46359, 46361, 46362, 46370

KRKA

NMW 65860, 65915, 65930

Salmo trutta (Atlantic basin, not listed above)

BMNH 1885.9.18:11-21, 1908.4.28:4, 1908.4.28:5, 1937.9.15:9-14, 1947.8.15:1-2, 1991.7.12:18-20
MHNG 642.70, 816.8
MNHN 0000-2898, 0000-2909, 0000-3634, 0000-3638, 0000-3639, 0000-3641, 0000-3642, 0000-3649, 0000-3650, 0000-3651, 0000-6321, 1923-0215, 1982-0480, A5532, A5533
NRM 24849, 24866, 24885, 24894, 24895, 24903, 45239, 42543, 42544, 42559
ZMH 1206, 6344, 10718, 10721, 10731, 10736

OUM ER RBIA

BMNH 1934.10.25:1-2
MNCN 208127-139
MNHN 1925-0350, 1925-0351, 1977-0282

BETH

MNHN 1920-0200, 1926-0013, 1977-0272, 1977-0285

MARTIL

BMNH 1887.12.23:4-10

MESSAOUD

MNHN 1926-0018, 1926 0019, 1926-0020, 1926-0021, 1926-0022, 1926-0023, 1926-0024, 1926-0025, 1926-0026, 1926-0027

MOULOYA

MNHN 1926-0014, 1926-0015, 1926-0016, 1926-0017, 1947-0018, 1977-0269

Salmo pellegrini

NMW 19546 (SYNTYPE)

Salmo pallaryi

BMHN 1926.5.5:1
BMHN 1926.6.24:1
BMHN 1934.10:3-5

MNHN 1923-0066 (HOLOTYPE), 1925-0341, 1925-0342, 1925-0343, 1925-0344, 1925-0346, 1925-0347, 1925-0348, 1925-0349, 1977-0261, 1977-0273, 1977-0280
NRM41452

Salmo akairos

MNCN 115018-029, 115022 (HOLOTYPE)

Salmo sp. (Corsica and Sardinia)

BMNH 1901.6.4:1-6, 1909.2.25:14

MHCH 730.22

MNHN 1896-0005, 1896-0006, 1896-0007, 1896-0008, 1896-0009, 1896-0010, 1896-0011

NMW66115

ZMH4302

MEDITERRANEAN

BMNH 1877.1.6:3-4, 1887.1.6:1-2, 1901.8.6:6-7, 1940.2.10:1-3

MHCH 52.82

MNHN 0000-0002, 0000-2575, 0000-3646, 0000-3652, 0000-3653, 000-3354, 1960-0347

nrm7190

ZMH4221

Salmo carpio

NMW 59704, 65957

NRM 28000 (NEOTYPE), 41539

ZISP 40513, 40514, 48207

Table S2. Character loadings on principal component I-V for 24 measurements taken on *Salmo* from Corsica, Sardinia, Spain and Atlantic basin.

	PC I	PC II	PC III	PC IV	PC V
Standard length (mm)	0.165	-0.039	-0.003	0.013	0.011
Preanal length	0.170	-0.036	-0.002	0.014	0.014
Prepelvic length	0.178	-0.029	0.003	0.010	0.013
Predorsal length	0.169	-0.027	-0.001	0.006	0.013
Head length	0.175	0.016	0.005	0.002	0.008
Premaxilla to preoperculum length	0.188	0.028	0.006	0.003	0.007
Caudal peduncle length	0.144	-0.065	0.001	0.015	0.023
Caudal peduncle depth	0.153	-0.016	0.014	-0.026	-0.027
Length of middle caudal fin ray	0.167	0.005	-0.008	0.013	-0.016
Pectoral fin length	0.150	0.004	-0.010	0.018	-0.016
Pelvic fin length	0.165	0.004	-0.006	0.007	-0.015
Adipose fin length	0.157	-0.056	0.009	0.004	-0.020
Body depth at origin of dorsal fin	0.161	-0.048	0.011	-0.016	-0.030
Body depth at origin of anal fin	0.168	0.018	0.016	-0.002	0.003
Head depth	0.137	0.035	-0.010	0.051	-0.005
Horizontal orbit diameter	0.144	0.055	-0.012	0.040	-0.002
Vertical orbit diameter	0.180	-0.029	0.013	-0.006	0.000
Interorbital width	0.193	0.024	0.028	-0.038	0.027
Snout length	0.204	0.055	0.022	-0.018	0.019
Upper jaw length	0.163	0.040	0.000	-0.014	-0.047
Length of maxilla	0.199	0.035	0.019	-0.012	0.011
Height of maxilla	0.177	0.003	-0.106	-0.034	0.010
Lower jaw length	0.165	-0.039	-0.003	0.013	0.011
Gill raker length	0.170	-0.036	-0.002	0.014	0.014
Variance explained (%)	87.9	3.78	1.99	1.37	1.06

Table S3. Character loadings on principal component I-V for 16 meristic characters taken on *Salmo* from Corsica, Sardinia, Spain and Atlantic basin

	PC I	PC II	PC III	PC IV	PC V
Scales along lateral line					
Scales from base of adipose fin to lateral line	0.706	0.151	0.136	0.106	0.078
Left side branchiostegals	-0.369	0.486	0.022	-0.035	-0.184
Right side branchiostegals	0.578	0.324	-0.265	0.105	-0.454
Gill rakers on lower limb	0.552	0.307	-0.201	0.058	-0.579
Gill rakers on upper limb	0.019	0.751	0.368	-0.017	-0.069
Total number of gill rakers	-0.377	0.515	0.457	0.092	0.181
Vertebral counts	-0.218	0.804	0.518	0.044	0.066
Abdominal vertebrae	0.875	0.017	0.220	0.107	0.135
Caudal vertebrae	0.764	-0.111	0.347	-0.358	-0.019
Dorsal fin position	0.360	0.182	-0.124	0.704	0.220
Anal fin position	0.623	-0.291	0.338	0.075	0.199
Dorsal fin pterygiophores	0.794	-0.104	0.346	-0.292	0.039
Interneurales	0.448	0.229	-0.412	0.057	-0.208
Anal fin pterygiophores	0.608	0.092	-0.035	0.015	0.213
Caudal fin upper procurrent rays	0.160	0.136	-0.353	0.551	0.312
Caudal fin lower procurrent rays	0.158	0.371	-0.581	-0.412	0.409
Variance explained (%)	27.149	15.194	11.905	8.422	6.897