

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

Bo Delling, Andrea Sabatini, Stéphane Muracciole, Christelle Tougard,

Patrick Berrebi

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2	Morphologic and genetic characterisation of Corsican and
3	Sardinian trout with comments on Salmo taxonomy
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6	Bo Delling ¹ , Andrea Sabatini ² , Stephane Muracciole ³ ,
7	Christelle Tougard ⁴ and Patrick Berrebi ^{4,5,*}
8	
9	¹ Department of Zoology, Swedish Museum of Natural History, 10405 Stockholm, Sweden.
10	² Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, Via Fiorelli 1,
11	Cagliari, Italy.
12	³ Office National des Forêts, Pont de l'Orta, 20250 Corte, France.
13	⁴ ISEM, Université de Montpellier, CNRS, IRD, EPHE, 34095 Montpellier cedex, France.
14	⁵ Present address: Genome-R&D, 697 avenue de Lunel, 34400 Saint-Just, France.
15	* Corresponding author: Patrick.berrebi@laposte.net
16	
17	Short title: Morphology and genetics of Tyrrhenian trout
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Abstract - Both morphological and molecular data are presented and discussed for 19 20 indigenous Salmo sp. from Corsica and Sardinia, here called Tyrrhenian trout. For comparison, morphological data obtained from museum specimens, including the Algerian S. 21 22 *macrostigma*, are discussed in the light of recent and new molecular findings. In total, 29 measurements and 20 meristic characters were taken from each specimen. Out of the meristic 23 characters, 12 were obtained by means of X-ray. One important morphometric character in the 24 25 present study is the size of the head measured from premaxilla to posterior margin of preoperculum. This character was particularly stable in all Tyrrhenian trout, showing 26 relatively large head compared to Atlantic trout and to S. macrostigma. On the contrary, other 27 28 characters like body punctuations, black and white edges of fins, body depth or number of epurals in the caudal skeleton are quite polymorphic. In certain meristic characters, range of 29 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 discovered, belonging to three mitochondrial lineages viz. Adriatic, marble and 32 Mediterranean, however, Adriatic haplotypes are dominant. Mixing morphological and 33 genetic data, observed morphology lacks any obvious correlation to mitochondrial lineages 34 and it is concluded that Tyrrhenian trout show no particular affinity to S. macrostigma from 35 36 Algeria.

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

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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.

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

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

67 **1 Introduction**

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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", whereas taxonomic oriented scientists, mainly focusing on morphology, continue to describe 71 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, 2014b, 2017, 2020; Doadrio et al., 2015; Froese and Pauly, 2019). 74 75 The extensive molecular studies of the genus started mainly with allozymes (Ferguson and Mason, 1981; Karakousis and Triantaphyllidis, 1990; García-Marín et al., 1999; Berrebi 76 et al., 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of 77 78 mitochondrial origin (Giuffra et al., 1994; Apostolidis et al., 1997; Aurelle and Berrebi, 2001; Snoj *et al.*, 2011). These studies led to numerous publications describing genetic variation in 79 the genus. One of the most important findings within the taxonomic context is probably the 80 proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex, 81 Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA) 82 (Bernatchez et al., 1992; Bernatchez, 2001). These lineages are augmented by geographically 83 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 AT lineage into two sister clades: a North African lineage (NA, in Morocco, Algeria and 87 Sicily) and a well-known European AT lineage (Tougard et al., 2018). 88 89 In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation. In several cases, morphological and molecular data are 90

The taxonomic status of Eurasian trouts, i.e., all Salmo spp. except Atlantic salmon, Salmo

91 correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018). However, they

sometimes disagree: for example, Salmo marmoratus, considered as very distinct in 92 93 morphology (Delling, 2002), is also characterized by the MA mtDNA and the LDH-C1*(120) allozyme allele. However, MA haplotypes are also found in low frequencies outside the taxon 94 95 (Bernatchez et al., 1992; Snoj et al., 2009; Pustovrh et al., 2011; Tougard et al., 2018) and the 120-allele is rare in Trebuscica River (Slovenia) otherwise pure and isolated S. marmoratus 96 population (Berrebi et al., 2000b). Another example of marker disagreement is illustrated by 97 S. obtusirostris. This species, while fixed for a unique and specific mtDNA haplotype in the 98 Neretva River (Snoj et al., 2002), is fixed for the AD mtDNA lineage in Jadro River 99 population. Other frequent kinds of contradictions have been observed, especially in the 100 101 Balkans with numerous taxa sharing similar AD haplotypes (Sušnik et al., 2004, 2006; Snoj et al., 2010). These kinds of discrepancies may be explained by ancient introgression (Sušnik et 102 103 al., 2007). Another explanation is the Dobzhansky–Muller model which accounts for 104 cytonuclear incompatibilities (Burton and Barreto, 2012). Despite the high number of more or less distinguishable taxa within the genus Salmo, 105 106 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 including both kinds of data and the fact that several tentatively valid taxa are poorly 108 109 described lacking clear diagnoses (Kottelat and Freyhof, 2007). Within the native distribution of Salmo, a large part of its diversity is found in basins of the Tyrrhenian islands, Corsica, 110 Sardinia and Sicily (Berrebi et al., 2019), and especially in Corsica where numerous 111 differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together 112 with several other Mediterranean trouts, are often referred to as Salmo macrostigma (Duméril 113 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr 114

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)

and to Sardinian trout at first by Boulenger (1901) also confirmed by Pomini (1941). Since 117 118 that, Corsican trout have been characterized both for allozymes (Guyomard and Krieg, 1986; Berrebi, 1995), mtDNA (Bernatchez et al., 1992; Berrebi et al., 2019) and microsatellites 119 120 (Berrebi et al., 2007, 2019), showing that they mainly belong to the AD lineage and possess the highly diagnostic allozyme allele LDH-4*(040). Morphological data on Corsican trout is 121 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the 122 123 description of variation in color pattern among populations (Lascaux et al., 2010). In the same way, the non-introgressed Sardinian populations were characterized by only the AD lineage 124 and allele LDH-C1 100/100 (Sabatini et al. 2018). Some authors describe, for the Sardinian 125 126 populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics accompanied by different phenotypes (Sabatini et al., 2011; Zaccara et al., 2015) 127 Regarding distinctiveness of S. macrostigma sensu stricto, Tougard et al. (2018) 128 129 analyzed complete mtDNA sequences from one syntype and one topotypic specimen and concluded they belonged to the NA lineage. In the same study, samples from Corsica and 130 Sardinia were associated to AD, ME, MA or AT lineages. 131 The present study is deliberately "cross-disciplinary", the main focus being to 132 describe and discuss the Salmo diversity irrespective of different views on classification and 133 134 taxonomy. Consequently, the use of different names, e.g. S. marmoratus or S. lourosensis only serve the purpose of pointing out a certain subset of trouts. Both molecular and 135 morphological data are presented and discussed for indigenous Salmo sp. from Corsica and 136 Sardinia, here called Tyrrhenian trout. Regarding comparison to Algerian S. macrostigma, 137

morphological data obtained from museum specimens are also included and discussed in the 138 light of recent molecular findings (Tougard et al., 2018).

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143 **2 Material and methods**

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145 **2.1. Sampling**

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

Comparative material includes different sets of *Salmo* spp. depending on analyses 153 and the question of interest: distinction towards i) Salmo macrostigma from Algeria, ii) 154 Atlantic basin Salmo trutta, iii) Salmo sp. from Spain. Comparative material for morphometry 155 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e., within the 156 157 SL-range of Tyrrhenian trout samples. A description of contemporary and comparative material is given in Table 1, obtained from several museum collections: CMK, Collection of 158 159 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 Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian Academy of 163 Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg, Germany. The 164 165 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 material in addition to that in Table 1 (Delling, unpublished) is included for a broader 167

168 comparison of head length within *Salmo*. A complete list of studied material is provided as169 supplementary information (Table S1).

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171 **2.2. Molecular methods**

DNA was extracted from fin clips using the Chelex/proteinase K protocol described by 172 Estoup et al. (1996). Three to six individuals have been considered by locality in the 173 174 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 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer 176 177 (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of MgCl₂ (25 mM), 10 µl of 5x PCR buffer, 1 U of Taq polymerase (GoTaq® Promega) and about 50 ng of genomic 178 DNA. The conditions for PCR were an initial denaturation (95°C, 5 min) followed by 30 179 180 cycles of strand denaturation (94°C, 1 min), primer annealing (52°C, 1 min) and DNA extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR 181 amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA 182 fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR 183 products were purified and sequenced in both directions to confirm polymorphic sites by the 184 185 Macrogen Company, Seoul, South Korea (https://dna.macrogen.com/) and the platform GenSeq of the Institut des Sciences de l'Evolution de Montpellier (Montpellier, France). 186 The sequences of CR were aligned together with reference haplotypes retrieved from 187 GenBank, using MEGA v5.05 (Tamura et al., 2011). Haplotypes for the new sequences were 188 generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and 189 distribution among populations were evaluated with a median-joining network (Bandelt et al., 190 191 1999) constructed with PopART (Leigh and Bryant, 2015). In order to assign a phylogenetic position to the seven contemporary samples (CAM, SPE, POZ, ESE, CAR, CHA and NIN), 192

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

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196 2.3. Morphology methods

Methodology follows Delling et al. (2000) and Delling (2002). The length of the uppermost 197 gill raker on the lower limb of the first gill arch (right side) was measured in situ using a pair 198 199 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 the present study is head length (HL) measured from tip of the snout to posterior margin of 201 202 the operculum. However, the measurement that quantifies the size of the head more accurately is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig. 203 204 2). Below, the abbreviation HLpp is applied for that measurement.

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

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

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

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

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236 **3 Results**

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238 **3.1. Molecular results**

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

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

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

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255 **3.2. Morphology**

Morphometric data are given in Table 3, and meristic data are summarized in Tables 4 to 6 256 257 including extensive comparative material (Tables 1 and S1). Selected results from ordination by means of PCA are given for analyses focusing on variation between Tyrrhenian trout as a 258 whole towards Atlantic basin S. trutta (Fig. 5). Corresponding character loadings are given in 259 Supplementary Tables S2 and S3. The distinction of Tyrrhenian trout towards S. macrostigma 260 is illustrated with a biplot (Fig. 6) focusing on the major morphological trait of the Tyrrhenian 261 trout, viz. the longer head (HLpp) and slightly shorter caudal peduncle. The two Spanish 262 specimens are included in all analyses and graphs. For discussion related to the comparatively 263 large head in Tyrrhenian trout, HLpp is also presented as box plots in comparison to an 264 extended number of Salmo samples (Fig. 7). 265 266

267 *Variation among Tyrrhenian trout samples*

At first glance (Figs. 8A to 8G), the Tyrrhenian trout resembles Atlantic basin S. trutta, i.e. 268 269 rather strong jaws, numerous red and black spots, black and white edges of fins, most prominent in CAM, CAR, CHJ and SPE. Black spots are sometimes irregularly distributed, 270 271 more or less aggregated along the flanks of the body (SPE, NIN) in contrast to, e.g. CAM having its spots more evenly distributed (Fig. 8A). There are large variations in meristic 272 273 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 remaining samples. 276

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

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

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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)

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

characters that distinguish them best (Fig. 6). The Tyrrhenian trout has longer head (HLpp)

and slightly shorter caudal peduncle compared to *S. macrostigma* (Table 3).

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300

301 **4 Discussion**

302 4.1. Genetic diversity: a strong differentiation pattern

According to results from previous (Tougard et al., 2018; Berrebi et al., 2019) and 303 present studies, the Tyrrhenian trout is mainly characterized by an island specific mtDNA-304 305 radiation within the AD lineage, as well as, to a lesser degree, by other lineages (AT, ME and MA). The recently described NA lineage is also naturally present in Sicily. Thus, within a 306 307 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 study were new (Table 2) despite more than two decades of CR sequencing in Salmo. The 310 presence of the ME lineage in the NIN sample, also observed in Corsica by Tougard et al. 311 312 (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 al., 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred, 314 according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007). 315 316 Moreover, the presence of MA lineage in some isolated Corsican rivers including CAR and the range of distribution of the NA lineage (Morocco, Algeria, Sicily: Tougard et al., 2018; 317

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 (Tougard 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).
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329	4.2. Morphological diversity in light of genetic diversity
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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 salmons (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

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

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

Despite the complex pattern of migrations, hybridizations and genetic radiation in the Tyrrhenian trout history, also giving rise to rather morphologically distinct populations in Corsica and Sardinia (Figs. 8A to 8G), they all share a comparatively large head (Fig. 6 and 7). This contradictory pattern of morphological homogeneity for certain characters opposed to strong differentiation in others take probably account of the genetic data in the search for possible explanations. Turning focus to the overall large head in Tyrrhenian trout, it is 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 ADcs13 found in Ebro is very close to the Tyrrhenian AD haplotypes
389	(Fig. 4).

392 **5 Conclusions**

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

404

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411 **7 References**

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Apostolidis AP, Madeira M-J, Hansen MM, Machordom A. 2008. Genetic structure and
demographic history of brown trout (*Salmo trutta*) populations from the southern
Balkans. *Freshw Biol* 53: 1555-1566.

- Apostolidis AP, Triantaphyllidis C, Kouvatsi A, Economidis PS. 1997. Mitochondrial DNA
 sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout)
 populations. *Mol Ecol* 6: 531-542.
- Aurelle D, Berrebi P. 2001. Genetic structure of brown trout (*Salmo trutta*, L.) populations
 from south-western France: data from mitochondrial control region variability. *Mol Ecol* 10: 1551-1561.
- Bandelt HJ, Forster P, Röhl A. 1999. Median-joining network for inferring intraspecific
 phylogenies. *Mol Biol Evol* 16:37-48
- Barlow, GW. 1961. Causes and significance of morphological variation in fishes. *Syst Zool* 10
 (3): 105-117.
- Bardakçi F, Degerli N, Ozdemir O, Basibuyuk HH. 2006. Phylogeography of the Turkish
 brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. *J Fish Biol* 68:
 36-55.
- Bernatchez L. 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from
 phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA
 variation. *Evolution* 55: 351-379.
- Bernatchez L, Guyomard R, Bonhomme F. 1992. DNA sequence variation of the
 mitochondrial control region among geographically and morphologically remote
 European brown trout *Salmo trutta* populations. *Mol Ecol* 1: 161-173.
- 435 Berrebi P. 1995. Etude génétique des truites de Corse. Rapport final 1995: Université
 436 Montpellier II. 36p. https://data.oreme.org/trout/home.
- 437 Berrebi P. 2015. Three brown trout *Salmo trutta* lineages in Corsica described through
 438 allozyme variation. *J Fish Biol* 86: 60-73.
- Berrebi P, Caputo Barrucchi V, Splendiani A, Muracciole S, Sabatini A, Palmas F, Tougard
 C, Arculeo M, Marić S. 2019. Brown trout (*Salmo trutta* L.) high genetic diversity

- 441 around Tyrrhenian Sea as revealed by nuclear and mitochondrial markers.
 442 *Hydrobiologia* 826: 209-231.
- Berrebi P, Dubois S, Recorbet B, Muracciole S, Mattei J. 2007. Les progrès en génétique
 obtenus lors du LIFE. *Guide de gestion de la truite macrostigma*. 52-60.
 https://data.oreme.org/trout/home.
- Berrebi P, Jesenšek D, Crivelli AJ. 2017. Natural and domestic introgressions in the marble
 trout population of Soca River (Slovenia). *Hydrobiologia* 785: 277-291.
- Berrebi P, Poteaux C, Fissier M, Cattaneo-Berrebi G. 2000a. Stocking impact and allozymic
 diversity in brown trout from Mediterranean southern France. *J Fish Biol* 56(4): 949960.
- Berrebi P, Povz M, Jesenšek D, Crivelli AJ. 2000b. The genetic diversity of native, stocked
 and hybrid populations of marble trout in the Socă river, Slovenia. *Heredity* 85: 277–
 287.
- Boulenger G A. 1901. On the occurrence of *Salmo macrostigma* in Sardinia. *Annals and Magazine of Natural History* (Ser. 7) 8: 14.
- Bookstein FL, Chernoff B, Elder RL, Humphries JM, Smith GR, Strauss RE 1985.
 Morphometrics in evolutionary biology. *Academy of Natural Sciences of Philadelphia*, *Special Publication*, 15. 277pp.
- Burton RS, Barreto FS. 2012. A disproportionate role for mtDNA in Dobzhansky–Muller
 incompatibilities? *Mol Ecol* 21: 4942-4957.
- 461 Cortey M, García-Marín J-L. 2002. Evidence for phylogeographically informative sequence
 462 variation in the mitochondrial control region of Atlantic brown trout. *J Fish Biol*463 60:1058-1063
- 464 Cortey M, Pla C, García-Marín JL. 2004. Historical biogeography of Mediterannean Trout.
 465 The role of allopatry and dispersal events. *Mol Phylogenet Evol* 33: 831-844.

- 466 Cortey M, Vera M, Pla C, García-Marín J-L. 2009. Northern and Southern expansions of
 467 Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. *Biol J Linn Soc*468 97: 904-917.
- 469 Delling B. 2002. Morphological distinction of marble trout, *Salmo marmoratus*, in
 470 comparison to marbled *Salmo trutta* from River Otra, Norway. *Cybium* 26: 283-300.
- 471 Delling B. 2003. Species diversity and phylogeny of *Salmo* with emphasis on Southern trouts
 472 (Teleostei, Salmonidae): *Doctoral Dissertation, Swedish Museum of Natural History*.
- 473 Delling B. 2010. Diversity of western and southern Balkan trouts, with the description of a
 474 new species from the Louros River, Greece (Teleostei: Salmonidae). *Ichthyol Explor*475 *Freshw* 21: 331-344.
- Delling B, Crivelli AJ, Rubin J-F, Berrebi P 2000. Morphological variation in hybrids
 between *Salmo marmoratus* and alien *Salmo* species in the Volarja stream, Soca River
 basin, Slovenia. *J Fish Biol* 57: 1199-1212.
- Delling B, Doadrio I. 2005. Systematics of the trouts endemic to Moroccan lakes, with
 description of a new species (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 16: 4964.
- 482 Doadrio I, Perea S, Yahyaoui A. 2015. Two new species of Atlantic trout (Actinopterygii,
 483 Salmonidae) from Morocco. *Graellsia* 71: e031.
- 484 Duftner N, Weiss S, Medgyesy N, Sturmbauer C. 2003. Enhanced phylogeographic
 485 information about Austrian brown trout populations derived from complete
 486 mitochondrial control region sequences. *J Fish Biol* 62: 427-435.
- 487 Duméril A. 1858. Note sur une truite d'Algérie (*Salar macrostigma*, A. Dum.). *C R Acad Sci*488 *Paris* 47: 160–162

- Estoup A, Largiader CR, Perrot E, Chourrout D. 1996. Rapid one-tube DNA extraction for
 reliable PCR detection of fish polymorphic markers and transgenes. *Mol Mar Biol Biotechnol* 5:295-298.
- Ferguson A, Mason FM. 1981. Allozyme evidence for reproductively isolated sympatric
 populations of brown trout *Salmo trutta* L. in Lough Melvin, Ireland. *J Fish Biol* 18:
 629-642.
- 495 Froese R, Pauly D. 2019. FishBase, The Global Database of Fishes. http://www.fishbase.org.
- 496 García-Marín J-L, Utter FM, Pla C. 1999. Postglacial colonization of brown trout in Europe
 497 based on distribution of allozyme variants. *Heredity* 82: 46-56.
- 498 Gauthier A, Berrebi P. 2007. La colonisation de l'île par différentes souches de truite. *Guide*499 *de gestion de la truite macrostigma*. 4-10. https://data.oreme.org/trout/home.
- Giuffra E, Bernatchez L, Guyomard R. 1994. Mitochondrial control region and protein coding
 genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from
 northern Italy. *Mol Ecol* 3: 161-171.
- Gratton P, Allegrucci G, Gandolfi A, Sbordoni V. 2013. Genetic differentiation and
 hybridization in two naturally occurring sympatric trout *Salmo* spp. forms from a
 small karstic lake. *J Fish Biol* 82: 637-657.
- 506 Guyomard R. 1989. Diversité génétique de la truite commune. *Bull Fr Pêche Piscic* 314: 118507 135.
- Guyomard R, Krieg F. 1986. Mise en évidence d'un flux génique entre populations naturelles
 de truite fario et souche de repeuplement dans deux rivières de Corse. *Bull Fr Pêche Piscic* 303: 134-140.
- 511 Karakousis Y, Triantaphyllidis C. 1990. Genetic structure and differentiation among Greek
 512 brown trout (*Salmo trutta* L.) populations. *Heredity* 64: 297-304.

- Kottelat M, Freyhof J. 2007. Handbook of European freshwater fishes. Publications Kottelat,
 Cornol, Switzerland.
- Lascaux JM, Lagarrigue T, Mennessier JM, Muracciole S. 2010. Analyse de la variabilité de
 la ponctuation et des caractères ornementaux des truites des cours d'eau corses: Etude
 réalisée par ECOGEA dans le cadre du programme de gestion de la truite corse. 31p.
- Leigh, JW, Bryant D. 2015. PopART: Full-feature software for haplotype network
- 519 construction. *Meth Ecol Evol* 6:1110–1116.
- Lerceteau-Köhler E, Schliewen U, Kopun T, Weiss S. 2013. Genetic variation in brown trout
 Salmo trutta across the Danube, Rhine, and Elbe headwaters: a failure of the
 phylogeographic paradigm? *BMC Evol Biol* 13: 176.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA
 polymorphism data. *Bioinformatics* 25: 1451–1452.
- Martinez P, Castro J, Pardo BG, Bouza C, Hermida M, Vilas R. 2009. High Ag-NOR-site
 variation associated to a secondary contact in brown trout from the Iberian Peninsula.
 Genetica 136: 419-427.
- 528 Meraner A, Baric S, Pelster B, Dalla Via J. 2007. Trout (Salmo trutta) mitochondrial DNA
- polymorphism in the centre of the marble trout distribution area. *Hydrobiologia* 579:
 337-349.
- Norden CR. 1961. Comparative osteology of representative salmonid fishes with particular
 reference to the grayling (*Thymallus arcticus*) and its phylogeny. J Fisheries Res
 Board Can 18:679-971.
- Olivari G, Brun G. 1988. Le nombre de caeca pyloriques dans les populations naturelles de
 truites communes *Salmo trutta*, Linné en Corse. *Bull Ecol* 19: 2-3.
- Pankhurst, NW Montgomery, JC. 1994. Uncoupling of visual and somatic growth on the
 rainbow trout *Oncorhynchus mykiss*. *Brain Behav Evol* 44: 149-155.

- 538 Pomini, FP. 1941. Ricerche sul Salmo macrostigma. Boll Pesca Idrobiol Pescicolt 16: 3–36.
- Pustovrh G, Sušnik Bajec S, Snoj A. 2011. Evolutionary relationship between marble trout of
 the northern and the southern Adriatic basin. *Mol Phylogenet Evol* 59: 761-766.
- Roule L. 1933. Le peuplement des cours d'eau de la Corse en poissons. *Bull Fr Pêche Piscic*63: 61-62.
- Sabatini A, Cannas R, Follesa M C, Palmas F, Manunza A, Matta G, A. Pendugiu A, Serra P,
 Cau A. 2011. Genetic characterization and artificial reproduction attempt of endemic
 Sardinian trout *Salmo trutta* L., 1758 (Osteichthyes, Salmonidae): Experiences in
 captivity. *Ital J Zool* 78: 20–26.
- Sabatini A, Podda C, Frau G, Cani M V, Musu A, Serra M, Palmas F. 2018. Restoration of
 native Mediterranean trout *Salmo cettii* Rafinesque, 1810 (Actinopterygii,
 Salmonidae) populations using an electric barrier as mitigation tool. *Europ Zool J*85(1): 137-149
- Sanz N. 2018. Phylogeographic history of brown trout: a review. In: Lobón-Cerviá J and Sanz
 N, eds. Brown trout: biology, ecology and management: John Wiley & Sons Ltd. 1763.
- Snoj A, Glamuzina B, Razpet A, Zablocki J, Bogut I, Lerceteau-Köhler E, Pojskic N, Sušnik
 S. 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. *Hydrobiologia* 651: 199-212.
- Snoj A, Maric S, Sušnik Bajec S, Berrebi P, Janjani S, Schöffmann J. 2011. Phylogeographic
 structure and demographic patterns of brown trout in North-West Africa. *Mol Phylogenet Evol* 61: 203-211.
- Snoj A, Maric S, Berrebi P, Crivelli AJ, Shumka S, Sušnik S. 2009. Genetic architecture of
 trout from Albania as revealed by mtDNA control region variation. *Genet Sel Evol* 41:
 22.

- Snoj A, Melkic E, Sušnik S, Muhamedagic S, Dovc P. 2002. DNA phylogeny supports
 revised classification of *Salmothymus obtusirostris*. *Biol J Linn Soc* 77: 399–411.
- Splendiani A., Palmas F., Sabatini A., Caputo Barucchi V. 2019. The name of the trout:
 considerations on the taxonomic status of the *Salmo trutta* L., 1758 complex
 (Osteichthyes: Salmonidae) in Italy. *Europ Zool J* 86(1): 432-442.
- Stearley RF, Smith GR. 1993. Phylogeny of the pacific trouts and salmons, *Oncorhynchus*,
 and genera of the family Salmonidae. *Trans Am Fisher Soc* 122:1-33.
- Suarez J, Bautista JM, Almodovár A, Machordom A. 2001. Evolution of the mitochondrial
 control region in Palaearctic brown trout (*Salmo trutta*) populations: the
 biogeographical role of the Iberian Peninsula. *Heredity* 87: 198-206.
- Sušnik S, Knizhin I, Snoj A, Weiss S. 2006. Genetic and morphological characterization of a
 Lake Ohrid endemic, *Salmo (Acantholingua) ohridanus* with a comparison to
 sympatric *Salmo trutta*. *J Fish Biol* 68: 2-23.
- Sušnik S, Schöffmann J, Snoj A. 2004. Phylogenetic position of *Salmo (Platysalmo) platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data. J *Fish Biol* 64: 947-960.
- Sušnik S, Weiss S, Tomljanovic T, Delling B, Treer T, Snoj A. 2007. Reticulate evolution:
 ancient introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei: Salmonidae). *Biol J Linn Soc* 90: 139-152.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, *et al.* 2011. MEGA5: Molecular
 evolutionary genetics analysis using maximum likelihood, evolutionary distance, and
 maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
- Tougard C, Justy F, Guinand B, Douzery EJP, Berrebi P. 2018. Salmo macrostigma
 (Teleostei, Salmonidae): nothing more than a brown trout (S. trutta) lineage? J Fish
 Biol 93: 302-310.

- Turan, D, Dogan E, Kaya C, Kanyılmaz M. 2014a. *Salmo kottelati*, a new species of trout
 from Alakır Stream, draining to the Mediterranean in southern Anatolia, Turkey
 (Teleostei, Salmonidae). *ZooKeys* 462: 135–151.
- Turan D, Kalaycı G, Bektaş Y, Kaya C, Bayçelebi E. 2020. A new species of trout from the
 northern drainages of Euphrates River, Turkey (Salmoniformes: Salmonidae). *J. Fish Biol.* online (https://doi.org/10.1111/jfb.14321).
- Turan D, Kottelat M, Bektas Y. 2011. *Salmo tigridis*, a new species of trout from the Tigris
 River, Turkey (Teleostei: Salmonidae). *Zootaxa* 2993: 23-33.
- Turan D, Kottelat M, Engin S. 2009. Two new species of trouts, resident and migratory,
 sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae). *Ichthyol Explor Freshw* 20: 333-364.
- Turan D, Kottelat M, Engin S. 2012. The trouts of the Mediterranean drainages of southern
 Anatolia, Turkey, with description of three new species (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 23: 219-236.
- Turan D, Kottelat M Engin S. 2014b. Two new species of trouts from the Euphrates drainage,
 Turkey (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 24: 275–287.
- Turan D, Kottelat M, Kaya C. 2017. *Salmo munzuricus*, a new species of trout from the
 Euphrates River drainage, Turkey (Teleostei: Salmonidae). *Ichthyol Explor Freshw*28: 55-63.
- 607 Zaccara S, Trasforini S, Antognazza C M, Puzzi C, Robert Britton J, Crosa G. 2015.
- 608 Morphological and genetic characterization of Sardinian trout *Salmo cettii* Rafinesque,
- 609 1810 and their conservation implications. *Hydrobiologia* 760: 205–223.
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612 8. Figures captions

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

615 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 616 617 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 618 maxilla, from premaxilla end to posterior end of maxilla; 7, upper jaw length, from symphysis 619 620 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 depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest ray; 12, 622 623 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 624 to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base of caudal 625 fin; 17, least depth of caudal peduncle; 18, length of upper caudal fin lobe, from base to tip of 626 longest ray; 19, length of middle caudal fin ray, from base to tip of shortest ray; 20, length of 627 628 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 preoperculum (HLpp); 25, prepelvic length, from upper jaw symphysis to origin of pelvic fin; 632 26, preanal length, from upper jaw symphysis to origin of anal fin; 27, interorbital width, 633 634 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.

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.

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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.

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

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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.

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

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

- 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
- 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	
6	Bo Delling ¹ , Andrea Sabatini ² , Stephane Muracciole ³ ,
7	Christelle Tougard ⁴ and Patrick Berrebi ^{4,5,*}
8	
9	¹ Department of Zoology, Swedish Museum of Natural History, 10405 Stockholm, Sweden.
10	² Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, Via Fiorelli 1,
11	Cagliari, Italy.
12	³ Office National des Forêts, Pont de l'Orta, 20250 Corte, France.
13	⁴ ISEM, Université de Montpellier, CNRS, IRD, EPHE, 34095 Montpellier cedex, France.
14	⁵ Present address: Genome-R&D, 697 avenue de Lunel, 34400 Saint-Just, France.
15	* Corresponding author: Patrick.berrebi@laposte.net
16	
17	Short title: Morphology and genetics of Tyrrhenian trout
18	

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

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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.

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

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

67 **1 Introduction**

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70 diversity is most often referred to as brown trout Salmo trutta or "brown trout complex", whereas taxonomic oriented scientists, mainly focusing on morphology, continue to describe 71 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, 2014b, 2017, 2020; Doadrio et al., 2015; Froese and Pauly, 2019). 74 75 The extensive molecular studies of the genus started mainly with allozymes (Ferguson and Mason, 1981; Karakousis and Triantaphyllidis, 1990; García-Marín et al., 1999; Berrebi 76 et al., 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of 77 78 mitochondrial origin (Giuffra et al., 1994; Apostolidis et al., 1997; Aurelle and Berrebi, 2001; Snoj *et al.*, 2011). These studies led to numerous publications describing genetic variation in 79 the genus. One of the most important findings within the taxonomic context is probably the 80 proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex, 81 Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA) 82 (Bernatchez et al., 1992; Bernatchez, 2001). These lineages are augmented by geographically 83 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 AT lineage into two sister clades: a North African lineage (NA, in Morocco, Algeria and 87 Sicily) and a well-known European AT lineage (Tougard et al., 2018). 88 89 In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation. In several cases, morphological and molecular data are 90

The taxonomic status of Eurasian trouts, i.e., all Salmo spp. except Atlantic salmon, Salmo

salar, is revised continuously. Among molecular oriented ichthyologists, this part of Salmo

91 correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018). However, they

sometimes disagree: for example, Salmo marmoratus, considered as very distinct in 92 93 morphology (Delling, 2002), is also characterized by the MA mtDNA and the LDH-C1*(120) allozyme allele. However, MA haplotypes are also found in low frequencies outside the taxon 94 95 (Bernatchez et al., 1992; Snoj et al., 2009; Pustovrh et al., 2011; Tougard et al., 2018) and the 120-allele is rare in Trebuscica River (Slovenia) otherwise pure and isolated S. marmoratus 96 population (Berrebi et al., 2000b). Another example of marker disagreement is illustrated by 97 S. obtusirostris. This species, while fixed for a unique and specific mtDNA haplotype in the 98 Neretva River (Snoj et al., 2002), is fixed for the AD mtDNA lineage in Jadro River 99 population. Other frequent kinds of contradictions have been observed, especially in the 100 101 Balkans with numerous taxa sharing similar AD haplotypes (Sušnik et al., 2004, 2006; Snoj et al., 2010). These kinds of discrepancies may be explained by ancient introgression (Sušnik et 102 103 al., 2007). Another explanation is the Dobzhansky–Muller model which accounts for 104 cytonuclear incompatibilities (Burton and Barreto, 2012). Despite the high number of more or less distinguishable taxa within the genus Salmo, 105 106 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 including both kinds of data and the fact that several tentatively valid taxa are poorly 108 109 described lacking clear diagnoses (Kottelat and Freyhof, 2007). Within the native distribution of Salmo, a large part of its diversity is found in basins of the Tyrrhenian islands, Corsica, 110 Sardinia and Sicily (Berrebi et al., 2019), and especially in Corsica where numerous 111 differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together 112 with several other Mediterranean trouts, are often referred to as Salmo macrostigma (Duméril 113 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr 114

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)

and to Sardinian trout at first by Boulenger (1901) also confirmed by Pomini (1941). Since 117 118 that, Corsican trout have been characterized both for allozymes (Guyomard and Krieg, 1986; Berrebi, 1995), mtDNA (Bernatchez et al., 1992; Berrebi et al., 2019) and microsatellites 119 120 (Berrebi et al., 2007, 2019), showing that they mainly belong to the AD lineage and possess the highly diagnostic allozyme allele LDH-4*(040). Morphological data on Corsican trout is 121 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the 122 123 description of variation in color pattern among populations (Lascaux et al., 2010). In the same way, the **non-**introgressed Sardinian populations were characterized by only the AD lineage 124 and allele LDH-C1 100/100 (Sabatini et al. 2018). Some authors describe, for the Sardinian 125 126 populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics accompanied by different phenotypes (Sabatini et al., 2011; Zaccara et al., 2015) 127 Regarding distinctiveness of S. macrostigma sensu stricto, Tougard et al. (2018) 128 129 analyzed complete mtDNA sequences from one syntype and one topotypic specimen and concluded they belonged to the NA lineage. In the same study, samples from Corsica and 130 Sardinia were associated to AD, ME, MA or AT lineages. 131

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

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141

143 2 Material and methods

144

145 **2.1. Sampling**

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

Comparative material includes different sets of Salmo spp. depending on analyses 153 154 and the question of interest: distinction towards i) Salmo macrostigma from Algeria, ii) Atlantic basin Salmo trutta, iii) Salmo sp. from Spain. Comparative material for morphometry 155 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e., within the 156 157 SL-range of Tyrrhenian trout samples. A description of contemporary and comparative material is given in Table 1, obtained from several museum collections: CMK, Collection of 158 159 Maurice Kottelat, Cornol, Switzerland; BMNH, British Museum of Natural History, London, UK; MHNG, Museum d'Histoire Naturelle, Geneva, Switzerland; MNCN, 160 161 Museo Nacional de Ciencias Naturales, Madrid, Spain; MNHN, Museum National d'Histoire 162 Naturelle, Paris, France; NMW, Naturhistorisches Museum, Wien, Austria; NRM, Swedish Museum of Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian 163 Academy of Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg, 164 Germany. The sample from Spain, MNHN 1920 228-229, consists of two specimens only but 165 is included in the study because their morphology resembles Tyrrhenian trout (see below). 166
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 Estoup et al. (1996). Three to six individuals have been considered by locality in the 173 174 Tyrrhenian region, being or not the exact individuals included in morphological analyses. Partial mtDNA control region (CR) was amplified by PCR using the PST and FST primers 175 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer 176 (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of MgCl₂ (25 mM), 10 µl of 177 5x PCR buffer, 1 U of Tag polymerase (GoTag® Promega) and about 50 ng of genomic 178 179 DNA. The conditions for PCR were an initial denaturation (95°C, 5 min) followed by 30 cycles of strand denaturation (94°C, 1 min), primer annealing (52°C, 1 min) and DNA 180 extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR 181 amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA 182 fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR 183 184 products were purified and sequenced in both directions to confirm polymorphic sites by the Macrogen Company, Seoul, South Korea (https://dna.macrogen.com/) and the platform 185 GenSeq of the Institut des Sciences de l'Evolution de Montpellier (Montpellier, France). 186 The sequences of CR were aligned together with reference haplotypes retrieved from 187 GenBank, using MEGA v5.05 (Tamura et al., 2011). Haplotypes for the new sequences were 188 generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and 189 190 distribution among populations were evaluated with a median-joining network (Bandelt et al., 1999) constructed with PopART (Leigh and Bryant, 2015). In order to assign a phylogenetic 191

192 position to the seven contemporary samples (CAM, SPE, POZ, ESE, CAR, CHA and NIN),

the network included published GenBank sequences of the lineages AT, ME, AD, MA, DA

and NA, all belonging to the brown trout complex.

195

196 **2.3. Morphology methods**

Methodology follows Delling *et al.* (2000) and **Delling** (2002). The length of the uppermost 197 198 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 calliper and rounded to the nearest 0.1 mm (Fig. 2). One important morphometric character in 200 201 the present study is head length (HL) measured from tip of the snout to posterior margin of the operculum. However, the measurement that quantifies the size of the head more accurately 202 is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig. 203 204 2). Below, the abbreviation HLpp is applied for that measurement.

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

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

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

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

234

235

236 **3 Results**

237

238 **3.1. Molecular results**

Among the 38 contemporary specimens from Corsica and Sardinia, 34 CR sequences were
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

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

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

haplotype (ADcr5), while CHA (Corsica) is characterized by two AD haplotypes (ADcr3 and

ADcr6). SPE and ESE shared ADcr3 with CHA.

254

255 **3.2. Morphology**

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

266

267 *Variation among Tyrrhenian trout samples*

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

Four specimens (1 POZ, 3 ESE) were different in the number of epurals in the caudal skeleton, having three instead of two. All *Salmo* except *S. salar* have two, sometimes fused (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 the fins are shared between several populations of Atlantic basin S. trutta and the Tyrrhenian 283 284 trouts. Dark, more or less ocellated, spots on flanks of the body are also common in both. However, some Tyrrhenian trout have their spots aggregated (Figs. 8F, 8G and p. 415 in 285 Kottelat and Freyhof, 2007). The sample from Spain also possesses this uncommon pattern 286 and was therefore especially highlighted in the comparative material. Sparsely or densely 287 distributed, dark spots on caudal fin are also common in Tyrrhenian trout (Figs. 8B and 8G, 288 CAM and SPE, respectively). Spots on caudal fins are rarely found in Atlantic basin S. trutta. 289 290 Multivariate statistics (Fig. 5) indicate distinction but not complete separation of Tyrrhenian trout from Atlantic S. trutta due to differences in vertebral counts and head size. 291

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)

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

characters that distinguish them best (**Fig. 6**). The Tyrrhenian trout has longer head (HLpp)

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

According to results from previous (Tougard et al., 2018; Berrebi et al., 2019) and 303 304 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 MA). The recently described NA lineage is also naturally present in Sicily. Thus, within a 306 307 rather limited and nowadays isolated region in the south center of Salmo distribution, a comparatively high number of mtDNA lineages (four of the five recognizable major lineages) 308 309 occur naturally. It is also striking that a majority of the haplotypes recovered in the present study were new (Table 2) despite more than two decades of CR sequencing in Salmo. The 310 311 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 explained by ancient introgressions evidenced elsewhere using nuclear markers (Berrebi et 313 al., 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred, 314 according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007). 315 Moreover, the presence of MA lineage in some isolated Corsican rivers including CAR and 316

the range of distribution of the NA lineage (Morocco, Algeria, Sicily: Tougard et al., 2018; 317 318 Berrebi et al., 2019) demonstrates the multiple unknown events of migrations, invasions and hybridizations which complicate the trout genetic pattern in the Tyrrhenian region. Finally, 319 320 the presence of several northern AT haplotypes recorded in the Tyrrhenian trout (Tougard et al., 2018; Berrebi et al., 2019) is due to stocking with commercial AT hatchery strains. 321 Nuclear markers (microsatellites) have also shown that the Tyrrhenian trouts exhibit 322 323 exceptionally differentiated genotypes, at a continental-like level, within the two small sampled islands, but especially in Corsica where numerous autochthonous isolated small 324 populations still survive (Berrebi et al., 2019). This strong differentiation among neighboring 325 326 rivers is typical of dry Mediterranean mountainous regions, never frozen by glaciation and providing way for migration (Apostolidis et al., 2008; Berrebi et al., 2019). 327 328 329 4.2. Morphological diversity in light of genetic diversity 330 Regarding the strong morphological diversification between studied populations of 331 332 Tyrrhenian trout, one explanation may involve random effects. Berrebi et al. (2019) showed very low levels of genetic variation within populations in Corsican streams based on 333 334 microsatellites, suggesting small population sizes and repeated bottleneck events. Hypothetically, the frequent (c. 10%), occurrence of three epurals in Tyrrhenian 335 trout compared to c. 0.1 % in comparative material might be a result of genetic drift 336 accelerated by bottlenecks. Three epurals in the caudal skeleton are typical for, e.g. most 337 Pacific trouts and salmons (Oncorhynchus), graylings (Thymallus) and whitefishes 338 (Coregonus) (Norden, 1961; Stearley and Smith, 1993) and appear to be the ancestral state 339 also retained as a polymorphism in S. salar with 12 out of 40 studied specimens having three. 340

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

347 Comparing Tyrrhenian samples to other Salmo spp., it is tempting to search for a pattern connecting certain characters to certain mtDNA lineages. However, ancient 348 introgression in certain populations without strong impacts on morphology seems rather to be 349 350 the "rule" in many salmonids and other taxa (Martinez et al., 2009; Gratton et al., 2013; Lerceteau-Köhler et al., 2013; Berrebi et al., 2017). The CAR sample also possessing the MA 351 352 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 recent hybrids involving S. marmoratus show a variable but, overall, intermediate phenotype 354 355 (Delling, et al., 2000). Prominent black and white leading edges on fins in several Tyrrhenian populations are similar to Atlantic basin S. trutta and could tentatively be regarded as ancient 356 traces of the AT lineage. This pattern is also present in some North African trout, e.g. S. 357 358 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 Sardinia, except AT lineages of hatchery origin. 360

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

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

The two Spanish specimens, referred to as *Salmo* sp. MNHN 1920 228-229, possess a color pattern with irregularly distributed spots, also found in some Tyrrhenian trouts, and are comparatively large headed (**Fig. 6**). They provide an example of what a hypothetical ancestor could have looked like and, if the interpretation of locality information (Ebro basin) is correct, it makes sense as the basin is dominated by AD haplotypes (Cortey *et al.*, 2004). Also, the haplotype ADcs13 found in Ebro is very close to the Tyrrhenian AD haplotypes (**Fig. 4**).

390

392 **5 Conclusions**

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

404

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411 **7 References**

- 413 Apostolidis AP, Madeira M-J, Hansen MM, Machordom A. 2008. Genetic structure and
 414 demographic history of brown trout (*Salmo trutta*) populations from the southern
 415 Balkans. *Freshw Biol* 53: 1555-1566.
- Apostolidis AP, Triantaphyllidis C, Kouvatsi A, Economidis PS. 1997. Mitochondrial DNA
 sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout)
 populations. *Mol Ecol* 6: 531-542.
- Aurelle D, Berrebi P. 2001. Genetic structure of brown trout (*Salmo trutta*, L.) populations
 from south-western France: data from mitochondrial control region variability. *Mol Ecol* 10: 1551-1561.
- Bandelt HJ, Forster P, Röhl A. 1999. Median-joining network for inferring intraspecific
 phylogenies. *Mol Biol Evol* 16:37-48
- Barlow, GW. 1961. Causes and significance of morphological variation in fishes. *Syst Zool* 10
 (3): 105-117.
- Bardakçi F, Degerli N, Ozdemir O, Basibuyuk HH. 2006. Phylogeography of the Turkish
 brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. *J Fish Biol* 68:
 36-55.
- Bernatchez L. 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from
 phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA
 variation. *Evolution* 55: 351-379.
- Bernatchez L, Guyomard R, Bonhomme F. 1992. DNA sequence variation of the
 mitochondrial control region among geographically and morphologically remote
 European brown trout *Salmo trutta* populations. *Mol Ecol* 1: 161-173.
- 435 Berrebi P. 1995. Etude génétique des truites de Corse. Rapport final 1995: Université
 436 Montpellier II. 36p. https://data.oreme.org/trout/home.

- 437 Berrebi P. 2015. Three brown trout *Salmo trutta* lineages in Corsica described through
 438 allozyme variation. *J Fish Biol* 86: 60-73.
- Berrebi P, Caputo Barrucchi V, Splendiani A, Muracciole S, Sabatini A, Palmas F, Tougard
 C, Arculeo M, Marić S. 2019. Brown trout (*Salmo trutta* L.) high genetic diversity
 around Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. *Hydrobiologia* 826: 209-231.
- Berrebi P, Dubois S, Recorbet B, Muracciole S, Mattei J. 2007. Les progrès en génétique
 obtenus lors du LIFE. *Guide de gestion de la truite macrostigma*. 52-60.
 https://data.oreme.org/trout/home.
- Berrebi P, Jesenšek D, Crivelli AJ. 2017. Natural and domestic introgressions in the marble
 trout population of Soca River (Slovenia). *Hydrobiologia* 785: 277-291.
- Berrebi P, Poteaux C, Fissier M, Cattaneo-Berrebi G. 2000a. Stocking impact and allozymic
 diversity in brown trout from Mediterranean southern France. *J Fish Biol* 56(4): 949960.
- Berrebi P, Povz M, Jesenšek D, Crivelli AJ. 2000b. The genetic diversity of native, stocked
 and hybrid populations of marble trout in the Socă river, Slovenia. *Heredity* 85: 277–
 287.
- Boulenger G A. 1901. On the occurrence of *Salmo macrostigma* in Sardinia. *Annals and Magazine of Natural History* (Ser. 7) 8: 14.
- Bookstein FL, Chernoff B, Elder RL, Humphries JM, Smith GR, Strauss RE 1985.
 Morphometrics in evolutionary biology. *Academy of Natural Sciences of Philadelphia, Special Publication*, 15. 277pp.
- Burton RS, Barreto FS. 2012. A disproportionate role for mtDNA in Dobzhansky–Muller
 incompatibilities? *Mol Ecol* 21: 4942-4957.

- 461 Cortey M, García-Marín J-L. 2002. Evidence for phylogeographically informative sequence
 462 variation in the mitochondrial control region of Atlantic brown trout. *J Fish Biol*463 60:1058-1063
- 464 Cortey M, Pla C, García-Marín JL. 2004. Historical biogeography of Mediterannean Trout.
 465 The role of allopatry and dispersal events. *Mol Phylogenet Evol* 33: 831-844.
- 466 Cortey M, Vera M, Pla C, García-Marín J-L. 2009. Northern and Southern expansions of
 467 Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. *Biol J Linn Soc*468 97: 904-917.
- 469 Delling B. 2002. Morphological distinction of marble trout, *Salmo marmoratus*, in
 470 comparison to marbled *Salmo trutta* from River Otra, Norway. *Cybium* 26: 283-300.
- 471 Delling B. 2003. Species diversity and phylogeny of *Salmo* with emphasis on Southern trouts
 472 (Teleostei, Salmonidae): *Doctoral Dissertation, Swedish Museum of Natural History*.
- 473 Delling B. 2010. Diversity of western and southern Balkan trouts, with the description of a
 474 new species from the Louros River, Greece (Teleostei: Salmonidae). *Ichthyol Explor*475 *Freshw* 21: 331-344.
- Delling B, Crivelli AJ, Rubin J-F, Berrebi P 2000. Morphological variation in hybrids
 between *Salmo marmoratus* and alien *Salmo* species in the Volarja stream, Soca River
 basin, Slovenia. *J Fish Biol* 57: 1199-1212.
- Delling B, Doadrio I. 2005. Systematics of the trouts endemic to Moroccan lakes, with
 description of a new species (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 16: 4964.
- 482 Doadrio I, Perea S, Yahyaoui A. 2015. Two new species of Atlantic trout (Actinopterygii,
 483 Salmonidae) from Morocco. *Graellsia* 71: e031.

- 484 Duftner N, Weiss S, Medgyesy N, Sturmbauer C. 2003. Enhanced phylogeographic
 485 information about Austrian brown trout populations derived from complete
 486 mitochondrial control region sequences. *J Fish Biol* 62: 427-435.
- 487 Duméril A. 1858. Note sur une truite d'Algérie (*Salar macrostigma*, A. Dum.). *C R Acad Sci*488 *Paris* 47: 160–162
- Estoup A, Largiader CR, Perrot E, Chourrout D. 1996. Rapid one-tube DNA extraction for
 reliable PCR detection of fish polymorphic markers and transgenes. *Mol Mar Biol Biotechnol* 5:295-298.
- Ferguson A, Mason FM. 1981. Allozyme evidence for reproductively isolated sympatric
 populations of brown trout *Salmo trutta* L. in Lough Melvin, Ireland. *J Fish Biol* 18:
 629-642.
- 495 Froese R, Pauly D. 2019. FishBase, The Global Database of Fishes. http://www.fishbase.org.
- 496 García-Marín J-L, Utter FM, Pla C. 1999. Postglacial colonization of brown trout in Europe
 497 based on distribution of allozyme variants. *Heredity* 82: 46-56.
- 498 Gauthier A, Berrebi P. 2007. La colonisation de l'île par différentes souches de truite. *Guide*499 *de gestion de la truite macrostigma*. 4-10. https://data.oreme.org/trout/home.
- Giuffra E, Bernatchez L, Guyomard R. 1994. Mitochondrial control region and protein coding
 genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from
 northern Italy. *Mol Ecol* 3: 161-171.
- Gratton P, Allegrucci G, Gandolfi A, Sbordoni V. 2013. Genetic differentiation and
 hybridization in two naturally occurring sympatric trout *Salmo* spp. forms from a
 small karstic lake. *J Fish Biol* 82: 637-657.
- Guyomard R. 1989. Diversité génétique de la truite commune. *Bull Fr Pêche Piscic* 314: 118135.

- Guyomard R, Krieg F. 1986. Mise en évidence d'un flux génique entre populations naturelles
 de truite fario et souche de repeuplement dans deux rivières de Corse. *Bull Fr Pêche Piscic* 303: 134-140.
- 511 Karakousis Y, Triantaphyllidis C. 1990. Genetic structure and differentiation among Greek
 512 brown trout (*Salmo trutta* L.) populations. *Heredity* 64: 297-304.
- 513 Kottelat M, Freyhof J. 2007. Handbook of European freshwater fishes. Publications Kottelat,
 514 Cornol, Switzerland.
- Lascaux JM, Lagarrigue T, Mennessier JM, Muracciole S. 2010. Analyse de la variabilité de
 la ponctuation et des caractères ornementaux des truites des cours d'eau corses: Etude
 réalisée par ECOGEA dans le cadre du programme de gestion de la truite corse. 31p.
- Leigh, JW, Bryant D. 2015. PopART: Full-feature software for haplotype network
- 519 construction. *Meth Ecol Evol* 6:1110–1116.
- Lerceteau-Köhler E, Schliewen U, Kopun T, Weiss S. 2013. Genetic variation in brown trout
 Salmo trutta across the Danube, Rhine, and Elbe headwaters: a failure of the
 phylogeographic paradigm? *BMC Evol Biol* 13: 176.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA
 polymorphism data. *Bioinformatics* 25: 1451–1452.
- Martinez P, Castro J, Pardo BG, Bouza C, Hermida M, Vilas R. 2009. High Ag-NOR-site
 variation associated to a secondary contact in brown trout from the Iberian Peninsula. *Genetica* 136: 419-427.
- Meraner A, Baric S, Pelster B, Dalla Via J. 2007. Trout (*Salmo trutta*) mitochondrial DNA
 polymorphism in the centre of the marble trout distribution area. *Hydrobiologia* 579:
 337-349.

- Norden CR. 1961. Comparative osteology of representative salmonid fishes with particular
 reference to the grayling (*Thymallus arcticus*) and its phylogeny. J Fisheries Res
 Board Can 18:679-971.
- Olivari G, Brun G. 1988. Le nombre de caeca pyloriques dans les populations naturelles de
 truites communes *Salmo trutta*, Linné en Corse. *Bull Ecol* 19: 2-3.
- Pankhurst, NW Montgomery, JC. 1994. Uncoupling of visual and somatic growth on the
 rainbow trout *Oncorhynchus mykiss*. *Brain Behav Evol* 44: 149-155.
- 538 Pomini, **FP**. 1941. Ricerche sul Salmo macrostigma. Boll Pesca Idrobiol Pescicolt 16: 3–36.
- Pustovrh G, Sušnik Bajec S, Snoj A. 2011. Evolutionary relationship between marble trout of
 the northern and the southern Adriatic basin. *Mol Phylogenet Evol* 59: 761-766.
- 541 Roule L. 1933. Le peuplement des cours d'eau de la Corse en poissons. *Bull Fr Pêche Piscic*542 63: 61-62.
- Sabatini A, Cannas R, Follesa M C, Palmas F, Manunza A, Matta G, A. Pendugiu A, Serra P,
- Cau A. 2011. Genetic characterization and artificial reproduction attempt of endemic
 Sardinian trout *Salmo trutta* L., 1758 (Osteichthyes, Salmonidae): Experiences in
 captivity. *Ital J Zool* 78: 20–26.
- Sabatini A, Podda C, Frau G, Cani M V, Musu A, Serra M, Palmas F. 2018. Restoration of
 native Mediterranean trout *Salmo cettii* Rafinesque, 1810 (Actinopterygii,
 Salmonidae) populations using an electric barrier as mitigation tool. *Europ Zool J*85(1): 137-149
- Sanz N. 2018. Phylogeographic history of brown trout: a review. In: Lobón-Cerviá J and
 Sanz N, eds. Brown trout: biology, ecology and management: John Wiley & Sons Ltd.
 17-63.

- Snoj A, Glamuzina B, Razpet A, Zablocki J, Bogut I, Lerceteau-Köhler E, Pojskic N, Sušnik
 S. 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. *Hydrobiologia* 651: 199-212.
- Snoj A, Maric S, Sušnik Bajec S, Berrebi P, Janjani S, Schöffmann J. 2011. Phylogeographic
 structure and demographic patterns of brown trout in North-West Africa. *Mol Phylogenet Evol* 61: 203-211.
- Snoj A, Maric S, Berrebi P, Crivelli AJ, Shumka S, Sušnik S. 2009. Genetic architecture of
 trout from Albania as revealed by mtDNA control region variation. *Genet Sel Evol* 41:
 22.
- Snoj A, Melkic E, Sušnik S, Muhamedagic S, Dovc P. 2002. DNA phylogeny supports
 revised classification of *Salmothymus obtusirostris*. *Biol J Linn Soc* 77: 399–411.
- Splendiani A., Palmas F., Sabatini A., Caputo Barucchi V. 2019. The name of the trout:
 considerations on the taxonomic status of the *Salmo trutta* L., 1758 complex
 (Osteichthyes: Salmonidae) in Italy. *Europ Zool J* 86(1): 432-442.
- Stearley RF, Smith GR. 1993. Phylogeny of the pacific trouts and salmons, *Oncorhynchus*,
 and genera of the family Salmonidae. *Trans Am Fisher Soc* 122:1-33.
- Suarez J, Bautista JM, Almodovár A, Machordom A. 2001. Evolution of the mitochondrial
 control region in Palaearctic brown trout (*Salmo trutta*) populations: the
 biogeographical role of the Iberian Peninsula. *Heredity* 87: 198-206.
- Sušnik S, Knizhin I, Snoj A, Weiss S. 2006. Genetic and morphological characterization of a
 Lake Ohrid endemic, *Salmo (Acantholingua) ohridanus* with a comparison to
 sympatric *Salmo trutta*. *J Fish Biol* 68: 2-23.
- Sušnik S, Schöffmann J, Snoj A. 2004. Phylogenetic position of *Salmo (Platysalmo) platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data. J *Fish Biol* 64: 947-960.

- Sušnik S, Weiss S, Tomljanovic T, Delling B, Treer T, Snoj A. 2007. Reticulate evolution:
 ancient introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei: Salmonidae). *Biol J Linn Soc* 90: 139-152.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, *et al.* 2011. MEGA5: Molecular
 evolutionary genetics analysis using maximum likelihood, evolutionary distance, and
 maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
- Tougard C, Justy F, Guinand B, Douzery EJP, Berrebi P. 2018. Salmo macrostigma
 (Teleostei, Salmonidae): nothing more than a brown trout (S. trutta) lineage? J Fish
 Biol 93: 302-310.
- Turan, D, Dogan E, Kaya C, Kanyılmaz M. 2014a. *Salmo kottelati*, a new species of trout
 from Alakır Stream, draining to the Mediterranean in southern Anatolia, Turkey
 (Teleostei, Salmonidae). *ZooKeys* 462: 135–151.
- Turan D, Kalaycı G, Bektaş Y, Kaya C, Bayçelebi E. 2020. A new species of trout from
 the northern drainages of Euphrates River, Turkey (Salmoniformes:
 Salmonidae). J. Fish Biol. online (https://doi.org/10.1111/jfb.14321).
- Turan D, Kottelat M, Bektas Y. 2011. *Salmo tigridis*, a new species of trout from the Tigris
 River, Turkey (Teleostei: Salmonidae). *Zootaxa* 2993: 23-33.
- Turan D, Kottelat M, Engin S. 2009. Two new species of trouts, resident and migratory,
 sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae). *Ichthyol Explor Freshw* 20: 333-364.
- Turan D, Kottelat M, Engin S. 2012. The trouts of the Mediterranean drainages of southern
 Anatolia, Turkey, with description of three new species (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 23: 219-236.
- Turan D, Kottelat M Engin S. 2014b. Two new species of trouts from the Euphrates drainage,
 Turkey (Teleostei: Salmonidae). *Ichthyol Explor Freshw* 24: 275–287.

604	Turan D, Kottelat M, Kaya C. 2017. Salmo munzuricus, a new species of trout from the
605	Euphrates River drainage, Turkey (Teleostei: Salmonidae). Ichthyol Explor Freshw
606	28: 55-63.
607	Zaccara S, Trasforini S, Antognazza C M, Puzzi C, Robert Britton J, Crosa G. 2015.
608	Morphological and genetic characterization of Sardinian trout Salmo cettii Rafinesque,

609 1810 and their conservation implications. *Hydrobiologia* 760: 205–223.

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612 8. Figures captions

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

615 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 616 617 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 618 maxilla, from **premaxilla** end to posterior end of maxilla; 7, upper jaw length, from 619 620 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, body depth, at level of origin of dorsal fin; 11, dorsal fin length, from base to tip of longest 622 623 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 624 first ray to tip of longest ray; 16, caudal peduncle length, from end of anal fin to middle base 625 626 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; 627 628 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 posterior margin of preoperculum (HLpp); 25, prepelvic length, from upper jaw symphysis to 632 origin of pelvic fin; 26, preanal length, from upper jaw symphysis to origin of anal fin; 27, 633 634 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.

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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.

641

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.

646

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

649

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.

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

- **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,
- 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 preopreculum 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.



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



C. *Salmo* sp. CHJ, NRM62573, 136.6 mm SL; Chjuvone 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. © S. Muracciole

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	Ν	Museum	Reference	Remarks
			capture		Collection		
Salmo 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	
Salmo 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	
СНЈ	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	
Salmo sp. Spain **		Spain	1920	2	MNHN	this study	
Salmo macrostigma *	El Abaich River	Algeria	1866-1907	9	MNHN, BMNH, NMW	Delling and Doadrio (2005)	Syntype included
Salmo pallaryi *	Lake Sidi Ali	Morocco	1927-1936	17	MNHN, BMNH, NRM	Delling and Doadrio (2005)	Syntypes included
Salmo pellegrini *	Tensift River	Morocco	1931	1	NMW	Delling and Doadrio (2005)	Syntype
Salmo akairos*	Lake Ifni	Morocco	1995	10	NRM, MNCN	Delling and Doadrio (2005)	Holotype and paratypes
Salmo trutta	Details in reference	European Atlantic basin	1827-1999	243		Delling (2002)	Numerous samples from several countries and collections
Salmo salar	Details in reference	European Atlantic basin	1882-1998	40		Delling (2002)	Numerous samples from several countries and collections
Salmo marmoratus	Po, Adige, Soca and Neretva Rivers	Italy, Slovenia and Bosnia and Herzegovina	1823-1998	77	NRM, ZISP, MNHN, BMNH,	Delling (2002)	

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

Haplotypes	Accession Number	References / Samples	Locality of first observation
ATcs14	EF530476	Cortey et al. (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 et al. (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 et al. (2003)	Austria (5 Danubian rivers)
Da1b	AY185569	Duftner <i>et al.</i> (2003)	Austria (Lake Gossenköllesee)
Da2	AY185570	Duftner et al. (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 et al., unpubl.	Croatia (Plitvica R.)
Da22	AY185573	Duftner et al. (2003)	Austria (2 Danubian rivers)
Da23a	AY185574	Duftner <i>et al.</i> (2003)	Austria (Kleiner Kamp R.)
Da23b	AY185575	Duftner et al. (2003)	Austria (Lohnbach R.)
Da24	AY185576	Duftner <i>et al</i> . (2003)	Austria (Waldaist R.)
Da26	DQ841194	Meraner et al. (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)

Table 2. Distribution of the haplotypes involved in this study

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)

•		Tyrr	henian	Salmo			Saln	ю тасі	rostigm		Salmo trutta				
	Ν	min	max	mean	SD	Ν	min	max	mean	SD	Ν	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 3. Morphometry of *Salmo* spp. Number of studied specimens (N) for certain measurements varies due to condition of preserved specimens.

	scales from base of adipose												left side						
					fir	n to l	later	al lii	ne						br	anch	ioste	gals	
	11 12 13 14 15 16 17 18 19 20 21 22 23												8	9	10	11	12	13	
Salmo spp. Sardinia						3	2	4								5	3	2	
CAM					2	1	3		2							4	4		
Salmo 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			
Salmo sp. Spain						1	1										2		
Salmo macrostigma						5	3									2	6	1	
Salmo pallaryi							1	4	4	5	3						3	3	11
Salmo pellegrini					1												1		
Salmo akairos		1	2	5	2												1	4	5
Salmo trutta				8	55	90	61	23	4	1					4	40	111	80	8
Salmo salar	2	5	8	15	9		1									9	16	15	
Salmo marmoratus			4	8	33	24	6	2							1	7	29	35	5
Salmo carpio			1	2	5	4	1								2	5	2	4	1
Salmo ischchan					1	6	5	4	4	4					1	8	12	3	
Salmo letnica				2	5	4											7	3	1
Salmo platycephalus						1											1		
Salmo obtusirostris		3	9	7												3	8	8	
Salmo ohridanus	6	6	1												4	9			
Salmo lourosensis						3	4									5	2		
Salmo peristericus					4	6	2								2	9	1		
Salmo dentex					2	2	1										1	2	2

Table 4. Frequency distribution of scale counts from base of adipose fin to lateral line and left side branchiostegal counts in *Salmo* spp.
						vert	ebra	ae					ca	uda	l fin	up	per	proc	curre	ent
																ra	iys			
	52	53	54	55	56	57	58	59	60	61	62	63	10	11	12	13	14	15	16	17
Salmo spp. Sardinia			1			2	1	1							1	3	1	1		
CAM						1	6		1							4	4			
Salmo 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		
Salmo sp. Spain							1	1									1		1	
Salmo macrostigma						4	3									5	2			
Salmo pallaryi					3	13	2	1									4	11	4	
Salmo pellegrini			1													1				
Salmo akairos					6	3	1								1	3	5	1		
Salmo platycephalus								3								1	2			
Salmo obtusirostris					1	4	9							1	8	5				
Salmo ohridanus		1	3	3	1								1	7						
Salmo trutta					1	15	48	69	38	15	1				4	31	60	72	28	
Salmo salar							1	16	15	7		1	1	8	31					
Salmo marmoratus								5	14	42	15	1		1	30	32	10	5		
Salmo carpio								5	7	2						2	7	5		
Salmo ischchan			2	6	12	2								2	7	9	3	1		
Salmo letnica					1	3	1	3							1	6	1			
Salmo lourosensis						2	4	1							5	2				
Salmo peristericus							3	9							4	6	1	1		
Salmo dentex								2	3							2	2	1		

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

			to	otal 1	num	ber (of gi	ll ra	kers	on	first	arch	
	13	14	15	16	17	18	19	20	21	22	23	24	≥25
Salmo spp. Sardinia			2	1	1	4			1				
CAM					3	5							
Salmo spp. Corsica		1	2	1	3	2	1						
SPE					2	3							
POZ						3	1	1					
ESE					3	1	1						
CAR						2	3						
СНЈ					1	1	3						
NIN				2	2	1							
Salmo sp. Spain					1	1							
Salmo macrostigma				1	2	3	2	1					
Salmo pallaryi							2	6	6	1	2		
Salmo pellegrini									1				
Salmo akairos								4	3	1	2		
Salmo platycephalus									1	1			1
Salmo obtusirostris												2	17
Salmo ohridanus					2	4	5	2					
Salmo trutta		1	14	46	99	54	25	4					
Salmo salar						4	12	18	5	1			
Salmo marmoratus	1	4	12	18	28	14							
Salmo carpio				2	6	5							
Salmo ischchan						6	8	7	3				
Salmo letnica							2	6	1	1			1
Salmo lourosensis						2	2	2	1				
Salmo peristericus				3	5	3		1					
Salmo dentex							3	1	1				

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

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

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

Table S3. Character	padings 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