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To cite this version:

Bo Delling, Andrea Sabatini, Stéphane Muracciole, Christelle Tougard, Patrick Berrebi. Morphologic and genetic characterisation of Corsican and Sardinian trout with comments on Salmo taxonomy. Knowledge and Management of Aquatic Ecosystems, 2020, 421, pp.1-16. 10.1051/kmae/2020013 . hal-02566709

HAL Id: hal-02566709
https://hal.umontpellier.fr/hal-02566709

Submitted on 7 May 2020

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

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Short title: Morphology and genetics of Tyrrhenian trout

Abstract - Both morphological and molecular data are presented and discussed for indigenous *Salmo* sp. from Corsica and Sardinia, here called Tyrrhenian trout. For comparison, morphological data obtained from museum specimens, including the Algerian *S. 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 characters, 12 were obtained by means of X-ray. One important morphometric character in the 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 relatively large head compared to Atlantic trout and to *S. macrostigma*. On the contrary, other 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 variation of Tyrrhenian trout even exceeds that of the extensive comparative material. Each trout has been genetically characterized. New haplotypes from Tyrrhenian trout were discovered, belonging to three mitochondrial lineages viz. Adriatic, marble and Mediterranean, however, Adriatic haplotypes are dominant. Mixing morphological and genetic data, observed morphology lacks any obvious correlation to mitochondrial lineages and it is concluded that Tyrrhenian trout show no particular affinity to *S. macrostigma* from Algeria.

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

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

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Résumé - La présente étude détaille et discute les données morphologiques et moléculaires 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 compris S. *macrostigma* d'Algérie, sont discutées à la lumière des découvertes moléculaires récentes et nouvelles. Au total, 29 mesures et 20 caractères méristiques ont été considérés pour chaque spécimen. Parmi ces caractères méristiques, 12 ont été obtenus au moyen de 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 particulièrement stable chez toutes les truites tyrrhéniennes, qui ont montré une tête 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 des nageoires, la profondeur du corps ou le nombre d'hypuraux dans le squelette caudal sont 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 caractérisée et de nouveaux haplotypes de truite tyrrhénienne ont été découverts, appartenant à trois lignées mitochondriales à savoir les lignées adriatique, marbrée et méditerranéenne, les haplotypes adriatiques étant dominants. En combinant les données morphologiques et 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. macrostigma* d'Algérie.

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

1 Introduction

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* 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 new species and recognizes at least 50 distinct taxa (Delling and Doadrio, 2005; Sušnik *et al.*, 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).

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 *et al.*, 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of 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 the genus. One of the most important findings within the taxonomic context is probably the proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex, Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA) (Bernatchez *et al.*, 1992; Bernatchez, 2001). These lineages are augmented by geographically more limited lineages such as the Duero (DU) lineage (Suarez *et al.*, 2001) and the Dades trout (Snoj *et al.*, 2011), both close to AT, and the Tigris (TI) lineage (Bardakçi *et al.*, 2006) 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 Sicily) and a well-known European AT lineage (Tougard *et al.*, 2018).

In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation. In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018). However, they sometimes disagree: for example, *Salmo marmoratus*, considered as very distinct in 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 (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* population (Berrebi *et al.*, 2000b). Another example of marker disagreement is illustrated by *S. obtusirostris*. This species, while fixed for a unique and specific mtDNA haplotype in the Neretva River (Snoj *et al.*, 2002), is fixed for the AD mtDNA lineage in Jadro River population. Other frequent kinds of contradictions have been observed, especially in the 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 al.*, 2007). Another explanation is the Dobzhansky–Muller model which accounts for cytonuclear incompatibilities (Burton and Barreto, 2012).

Despite the high number of more or less distinguishable taxa within the genus *Salmo*, large portions of its populations are not easily referred with accuracy to any existing taxon (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 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, Sardinia and Sicily (Berrebi *et al.*, 2019), and especially in Corsica where numerous differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together with several other Mediterranean trouts, are often referred to as *Salmo macrostigma* (Duméril 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr marks retained in adults (Duméril, 1858). This is a common feature in many *Salmo* spp. and 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 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 (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 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the 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 and allele LDH-C1 100/100 (Sabatini *et al.* 2018). Some authors describe, for the Sardinian populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics accompanied by different phenotypes (Sabatini *et al.*, 2011; Zaccara *et al.*, 2015)

Regarding distinctiveness of *S. macrostigma sensu stricto,* Tougard *et al.* (2018) 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 Sardinia were associated to AD, ME, MA or AT lineages.

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

2 Material and methods

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 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 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e., within the 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 Maurice Kottelat, Cornol, Switzerland; BMNH, British Museum of Natural History, London, UK; MHNG, Museum d´Histoire Naturelle, Geneva, Switzerland; MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain; MNHN, Museum National d'Histoire Naturelle, Paris, France; NMW, Naturhistorisches Museum, Wien, Austria; NRM, Swedish Museum of Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg, Germany. The sample from Spain, MNHN 1920 228-229, consists of two specimens only but is included in 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 comparison of head length within *Salmo*. A complete list of studied material is provided as supplementary information (Table S1).

2.2. Molecular methods

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 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 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of $MgCl_2$ (25 mM), 10 µl of 5x PCR buffer, 1 U of Taq polymerase (GoTaq® Promega) and about 50 ng of genomic 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 extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR 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 GenSeq of the Institut des Sciences de l’Evolution de Montpellier (Montpellier, France).

The sequences of CR were aligned together with reference haplotypes retrieved from GenBank, using MEGA v5.05 (Tamura *et al.*, 2011). Haplotypes for the new sequences were generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and 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 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.

2.3. Morphology methods

Methodology follows Delling *et al.* (2000) and Delling (2002). The length of the uppermost gill raker on the lower limb of the first gill arch (right side) was measured *in situ* using a pair 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 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 is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig. 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 rooted counts were used as an ordination method (Bookstein *et al.*, 1985). Some informative 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 specimens. PCAs were performed using SYSTAT 13. Colour pattern descriptions are most 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 were also considered. In preserved material, light spots are interpreted as red spots based on 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.

3 Results

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.

3.2. Morphology

Morphometric data are given in Table 3, and meristic data are summarized in Tables 4 to 6 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 whole towards Atlantic basin *S. trutta* (Fig. 5). Corresponding character loadings are given in Supplementary Tables S2 and S3. The distinction of Tyrrhenian trout towards *S. macrostigma* is illustrated with a biplot (Fig. 6) focusing on the major morphological trait of the Tyrrhenian trout, viz. the longer head (HLpp) and slightly shorter caudal peduncle. The two Spanish specimens are included in all analyses and graphs. For discussion related to the comparatively large head in Tyrrhenian trout, HLpp is also presented as box plots in comparison to an extended number of *Salmo* samples (Fig. 7).

Variation among Tyrrhenian trout samples

At first glance (Figs. 8A to 8G), the Tyrrhenian trout resembles Atlantic basin *S. trutta*, i.e. 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, 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 characters between different populations and the range of variation sometimes exceeds that of 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 remaining samples.

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).

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

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 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 Kottelat and Freyhof, 2007). The sample from Spain also possesses this uncommon pattern and was therefore especially highlighted in the comparative material. Sparsely or densely distributed, dark spots on caudal fin are also common in Tyrrhenian trout (Figs. 8B and 8G, CAM and SPE, respectively). Spots on caudal fins are rarely found in Atlantic basin *S. trutta*. 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.

Distinction of Tyrrhenian trout samples towards S. macrostigma

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. 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).

4 Discussion

4.1. Genetic diversity: a strong differentiation pattern

According to results from previous (Tougard *et al.*, 2018; Berrebi *et al.*, 2019) and present studies, the Tyrrhenian trout is mainly characterized by an island specific mtDNA-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 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) 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 presence of the ME lineage in the NIN sample, also observed in Corsica by Tougard *et al.* (2018) and in other Tyrrhenian samples not included in the morphological analyses, is explained by ancient introgressions evidenced elsewhere using nuclear markers (Berrebi *et al.*, 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred, according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007). 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;

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, 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.

Nuclear markers (microsatellites) have also shown that the Tyrrhenian trouts exhibit exceptionally differentiated genotypes, at a continental-like level, within the two small sampled islands, but especially in Corsica where numerous autochthonous isolated small populations still survive (Berrebi *et al.*, 2019). This strong differentiation among neighboring 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).

4.2. Morphological diversity in light of genetic diversity

Regarding the strong morphological diversification between studied populations of 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 microsatellites, suggesting small population sizes and repeated bottleneck events.

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

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.

Comparing Tyrrhenian samples to other *Salmo* spp., it is tempting to search for a pattern connecting certain characters to certain mtDNA lineages. However, ancient 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; Lerceteau-Köhler *et al.*, 2013; Berrebi *et al.*, 2017). The CAR sample also possessing the MA 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 recent hybrids involving *S. marmoratus* show a variable but, overall, intermediate phenotype (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 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.*, 2018). However, neither NA nor AT lineages have so far been reported from Corsica and 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

(Fig. 7). It should be noted that sample sizes vary considerably (Fig. 7) but several of them 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 Morocco, *S. akairos* and *S. pallaryi,* together with *S. marmoratus* and *S. dentex* inhabiting 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 it is fully possible that the large head is a result of a founder effect during ancient 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 growing rainbow trout, *Oncorhynchus mykiss*. Thus, the comparatively large head in 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, also for *O. mykiss* that retarded growth results in larger eyes. The Tyrrhenian trout possesses 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.

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).

5 Conclusions

Data presented herein suggest that within the rather unresolved *Salmo* complex in the 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 studied to survey the distribution of this morphology. It would be a large step forward if this 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. This long-term work already began with several recent molecular papers (Sanz, 2018; 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 data (Tougard *et al.*, 2018), that they show no particular affinity to *S. macrostigma* once described from Algeria.

6 Acknowledgements

We thank Douglas Jones at the Institute of Freshwater Research, Drottningholm, for improving the English. This work was realized with the support of LabEx CeMEB, an ANR "*Investissements d'avenir*" program (ANR-10-LABX-04-01).

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8. Figures captions

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.

Fig. 7. Box plot of premaxilla to preoperculum length as % of SL, totally 518 specimens 116-208 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 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. Turkey and Mediterranean are samples scattered in the regions. Boxes represent median value +/- 25% of the observations, and whiskers the inner fences. Asterisks are outside or far outside values.

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 mm SL; Camboni Sardinia. **B.** CAR, NRM 62571, 167.6 mm SL; Carnevale 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 Corsica. **F.** SPE, NRM61812, 148.1 mm SL Speloncellu Corsica. **G.** NIN, Lake Ninu Corsica © S. Muracciole

RESEARCH PAPER

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

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Short title: Morphology and genetics of Tyrrhenian trout

Abstract - Both morphological and molecular data are presented and discussed for indigenous *Salmo* **sp**. from Corsica and Sardinia, here called Tyrrhenian trout. For comparison, morphological data obtained from museum specimens, including the Algerian *S. 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 characters, 12 were obtained by means of X-ray. One important morphometric character in the 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 relatively large head compared to Atlantic trout and to *S. macrostigma*. On the contrary, other 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 variation of Tyrrhenian trout even exceeds that of the extensive comparative material. Each trout has been genetically characterized. New haplotypes from Tyrrhenian trout were discovered, belonging to three mitochondrial lineages viz. Adriatic, marble and Mediterranean, however, Adriatic haplotypes are dominant. Mixing morphological and genetic data, observed morphology lacks any obvious correlation to mitochondrial lineages and it is concluded that Tyrrhenian trout show no particular affinity to *S. macrostigma* from Algeria.

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

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

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Résumé - La présente étude détaille et discute les données morphologiques et moléculaires 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 compris S. *macrostigma* d'Algérie, sont discutées à la lumière des découvertes moléculaires récentes et nouvelles. Au total, 29 mesures et 20 caractères méristiques ont été **considérés pour** chaque spécimen. Parmi ces caractères méristiques, 12 ont été obtenus au moyen de 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 particulièrement stable chez toutes les truites tyrrhéniennes, qui ont montré une tête 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 des nageoires, la profondeur du corps ou le nombre d'hypuraux dans le squelette caudal sont 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 caractérisée et de nouveaux haplotypes de truite tyrrhénienne ont été découverts, appartenant à trois lignées mitochondriales à savoir les **lignées adriatique, marbrée et méditerranéenne**, les haplotypes adriatiques étant dominants. En combinant les données morphologiques et 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. macrostigma* d'Algérie.

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

1 Introduction

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* 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 new species and recognizes **at least** 50 distinct taxa (Delling and Doadrio, 2005; Sušnik *et al.*, 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**).

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 *et al.*, 2000a; Berrebi, 2015), later on shifting focus towards DNA sequencing mainly of 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 the genus. One of the most important findings within the taxonomic context is probably the proposed five main mitochondrial DNA (mtDNA) lineages within the brown trout complex, Atlantic (AT), Mediterranean (ME), Adriatic (AD), marble (MA) and Danubian (DA) (Bernatchez *et al.*, 1992; Bernatchez, 2001). These lineages are augmented by geographically more limited lineages such as the Duero (DU) lineage (Suarez *et al.*, 2001) and the Dades trout (Snoj *et al.*, 2011), both close to AT, and the Tigris (TI) lineage (Bardakçi *et al.*, 2006) 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 Sicily) and a well-known European AT lineage (Tougard *et al.*, 2018).

In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation. **In several cases, morphological and molecular data are correlated, strengthening hypotheses on taxa delimitation (Sanz, 2018).** However, they sometimes disagree: for example, *Salmo marmoratus*, considered as very distinct in 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 (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* population (Berrebi *et al.*, 2000b). Another example of marker disagreement is illustrated by *S. obtusirostris*. This species, while fixed for a unique and specific mtDNA haplotype in the Neretva River (Snoj *et al.*, 2002), is fixed for the AD mtDNA lineage in Jadro River population. Other frequent kinds of contradictions have been observed, especially in the 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 al.*, 2007). Another explanation is the Dobzhansky–Muller model which accounts for cytonuclear incompatibilities (Burton and Barreto, 2012).

Despite the high number of more or less distinguishable taxa within the genus *Salmo*, large portions of its populations are not easily referred with accuracy to any existing taxon (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 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, Sardinia and Sicily (Berrebi *et al.*, 2019), and especially in Corsica where numerous differentiated indigenous populations still survive. Trout from Corsica and Sardinia, together with several other Mediterranean trouts, are often referred to as *Salmo macrostigma* (Duméril 1858) – a species originally described from Algeria. The name *macrostigma* refers to the parr marks retained in adults (Duméril, 1858). This is a common feature in many *Salmo* spp. and 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 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 (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 so far restricted to pyloric caeca counts (Olivari and Brun, 1988; Guyomard, 1989) and the 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 and allele LDH-C1 100/100 (Sabatini *et al.* 2018). Some authors describe, for the Sardinian populations, different haplotypes (Ad1, Ad2, Tyrrh1) with highly polymorphic characteristics accompanied by different phenotypes (Sabatini *et al.*, 2011; Zaccara *et al.*, 2015)

Regarding distinctiveness of *S. macrostigma* ***sensu stricto***, Tougard *et al.* (2018) 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 Sardinia were associated to AD, ME, MA or AT lineages.

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

2 Material and methods

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 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 is restricted to specimens within standard length (SL) - range (116-208 mm), i.e**.,** within the 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 Maurice Kottelat, Cornol, Switzerland;** BMNH, British Museum of Natural History, London, UK**; MHNG, Museum d´Histoire Naturelle, Geneva, Switzerland**; MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain; MNHN, Museum National d'Histoire Naturelle, Paris, France; NMW, Naturhistorisches Museum, Wien, Austria; NRM, Swedish Museum of Natural History, Stockholm, Sweden; ZISP, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum für Hamburg, Germany**.** The sample from Spain, MNHN 1920 228-229, consists of two specimens only but is included in 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 comparison of head length within *Salmo*. **A complete list of studied material is provided as supplementary information (Table S1).**

2.2. Molecular methods

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 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 (Cortey and García-Marín, 2002). Each 50 µl reaction included 0.4 µM of each primer (Eurofins MWG Operon), 0.2 mM of dNTP (2 mM each), 2 mM of $MgCl_2$ (25 mM), 10 µl of 5x PCR buffer, 1 U of Taq polymerase (GoTaq® Promega) and about 50 ng of genomic 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 extension (72°C, 1 min), then followed by a final extension (72°C, 5 min). All PCR amplifications were performed in Eppendorf Mastercycler thermocyclers. The amplified DNA fragments were run on a 0.8% agarose gel to verify the efficiency of amplification. The PCR 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 GenSeq of the Institut des Sciences de l’Evolution de Montpellier (Montpellier, France).

The sequences of CR were aligned together with reference haplotypes retrieved from GenBank, using MEGA v5.05 (Tamura *et al.*, 2011). Haplotypes for the new sequences were generated with DnaSP v5.10.1b (Librado and Rozas, 2009). Haplotype relationships and 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 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.

2.3. Morphology methods

Methodology follows Delling *et al.* (2000**)** and **Delling (**2002). The length of the uppermost gill raker on the lower limb of the first gill arch (right side) was measured *in situ* using a pair 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 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 is the distance from the premaxilla to the posterior margin of the preoperculum (No. 24 in Fig. 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 rooted counts were used as an ordination method (Bookstein *et al.*, 1985). Some informative 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 specimens. PCAs were **performed** using SYSTAT 13. Colour pattern descriptions are most 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 were also considered. In preserved material, light spots are interpreted as red spots based on 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.

3 Results

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.

3.2. Morphology

Morphometric data are given in Table 3, and meristic data are summarized in Tables 4 to 6 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 whole towards Atlantic basin *S. trutta* (**Fig. 5**). Corresponding character loadings are given in Supplementary **Tables S2 and S3.** The distinction of Tyrrhenian trout towards *S. macrostigma* is illustrated with a biplot **(Fig. 6)** focusing on the major morphological trait of the Tyrrhenian trout, viz. the longer head (HLpp) and slightly shorter caudal peduncle. The two Spanish specimens are included in all analyses and graphs. For discussion related to the comparatively large head in Tyrrhenian trout, HLpp is also presented as box plots in comparison to an extended number of *Salmo* samples **(Fig. 7)**.

Variation among Tyrrhenian trout samples

At first glance (**Figs. 8A to 8G**), the Tyrrhenian trout resembles Atlantic basin *S. trutta,* i.e. 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, 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 characters between different populations and the range of variation sometimes exceeds that of 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 remaining samples.

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)**.

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

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 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 Kottelat and Freyhof, 2007). The sample from Spain also possesses this uncommon pattern and was therefore especially highlighted in the comparative material. Sparsely or densely distributed, dark spots on caudal fin are also common in Tyrrhenian trout (**Figs. 8B and 8G**, CAM and SPE, respectively). Spots on caudal fins are rarely found in Atlantic basin *S. trutta*. 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.

Distinction of Tyrrhenian trout samples towards S. macrostigma

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. 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).

4 Discussion

4.1. Genetic diversity: a strong differentiation pattern

According to results from previous (Tougard *et al.*, 2018; Berrebi *et al.*, 2019) and present studies, the Tyrrhenian trout is mainly characterized by an island specific mtDNA-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 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) 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 presence of the ME lineage in the NIN sample, also observed in Corsica by Tougard *et al.* (2018) and in other Tyrrhenian samples not included in the morphological analyses, is explained by ancient introgressions evidenced elsewhere using nuclear markers (Berrebi *et al.*, 2007; Berrebi, 2015). It shows that possible secondary contacts must have occurred, according to the post-glacial invasion of Corsica hypothesis (Gauthier and Berrebi, 2007). 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; 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, 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.

Nuclear markers (microsatellites) have also shown that the Tyrrhenian trouts exhibit exceptionally differentiated genotypes, at a continental-like level, within the two small sampled islands, but especially in Corsica where numerous autochthonous isolated small populations still survive (Berrebi *et al.*, 2019). This strong differentiation among neighboring 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).

4.2. Morphological diversity in light of genetic diversity

Regarding the strong morphological diversification between studied populations of 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 microsatellites, suggesting small population sizes and repeated bottleneck events.

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

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.

Comparing Tyrrhenian samples to other *Salmo* spp., it is tempting to search for a pattern connecting certain characters to certain mtDNA lineages. However, ancient 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; Lerceteau-Köhler *et al.*, 2013; Berrebi *et al.*, 2017). The CAR sample also possessing the MA 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 recent hybrids involving *S. marmoratus* show a variable but, overall, intermediate phenotype (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 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.*, 2018). However, neither NA nor AT lineages have so far been reported from Corsica and 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 (**Fig. 7**). It should be noted that sample sizes vary considerably (**Fig. 7**) but several of them 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 Morocco, *S. akairos* and *S. pallaryi,* together with *S. marmoratus* and *S. dentex* inhabiting 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 it is fully possible that the large head is a result of a founder effect during ancient 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 growing rainbow trout, *Oncorhynchus mykiss*. Thus, the comparatively large head in 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, also for *O. mykiss* that retarded growth results in larger eyes. The Tyrrhenian trout possesses 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.

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**).

5 Conclusions

Data presented herein suggest that within the rather unresolved *Salmo* complex in the 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 studied to survey the distribution of this morphology. It would be a large step forward if this 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. This long-term work already began with several recent molecular papers (Sanz, 2018; 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 data (Tougard *et al.*, 2018), that they show no particular affinity to *S. macrostigma* once described from Algeria.

6 Acknowledgements

We thank Douglas Jones at the Institute of Freshwater Research, Drottningholm, for improving the English. This work was realized with the support of LabEx CeMEB, an ANR "***Investissements d'avenir***" program (ANR-10-LABX-04-01).

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8. Figures captions

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.

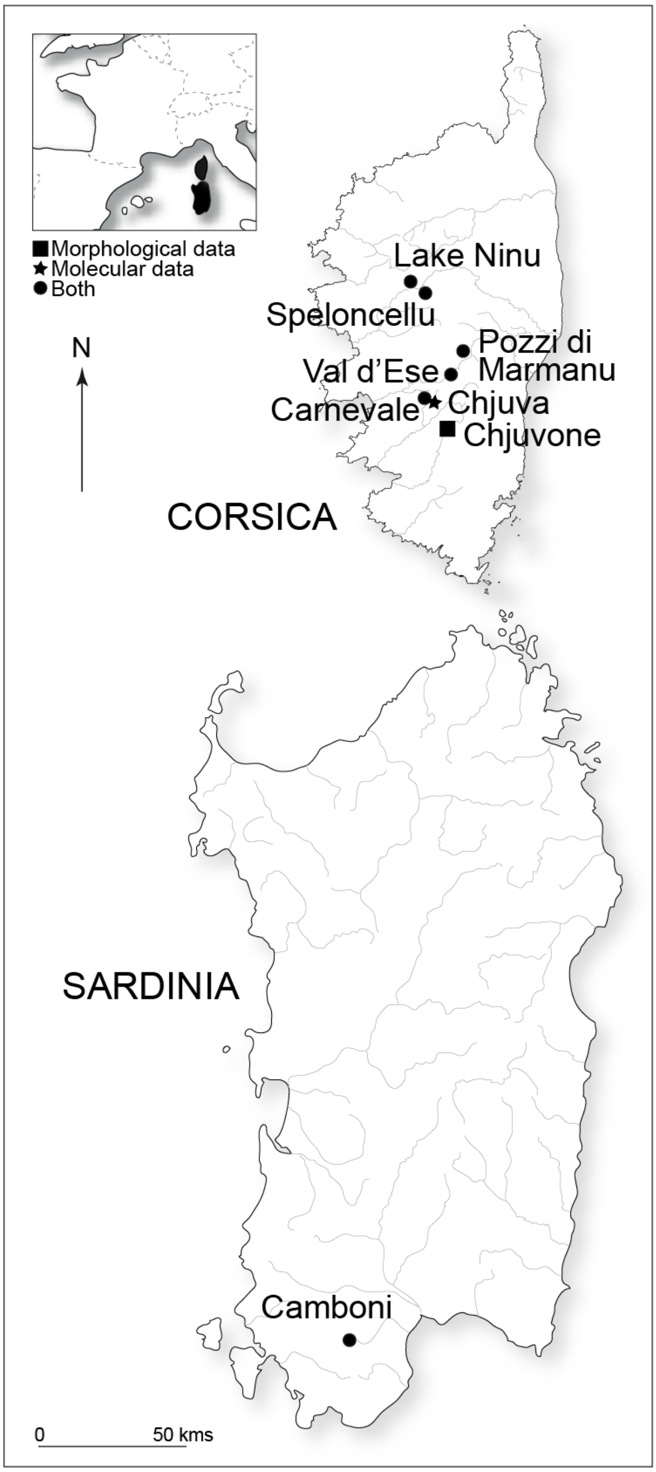
Fig. 4. Median-joining network of control region haplotypes of some trout samples (new Tyrrhenian haplotypes and AT, AD, DA, MA, ME and NA haplotypes from GenBank). Haplotypes are indicated by numbers as given in Table 2. Black circles are for nodes, and hatch marks are for mutation steps.

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.

Fig. 7. Box plot of premaxilla to preoperculum length as % of SL, totally 518 specimens 116-208 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 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**. Turkey and Mediterranean are samples scattered in the regions. Boxes represent median value +/- 25% of the observations, and whiskers the inner fences. Asterisks are outside or far outside values.

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 mm SL; Camboni Sardinia. B. CAR, NRM 62571, 167.6 mm SL; Carnevale 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 Corsica. F. SPE, NRM61812, 148.1 mm SL Speloncellu Corsica. G. NIN, Lake Ninu Corsica © S. Muracciole



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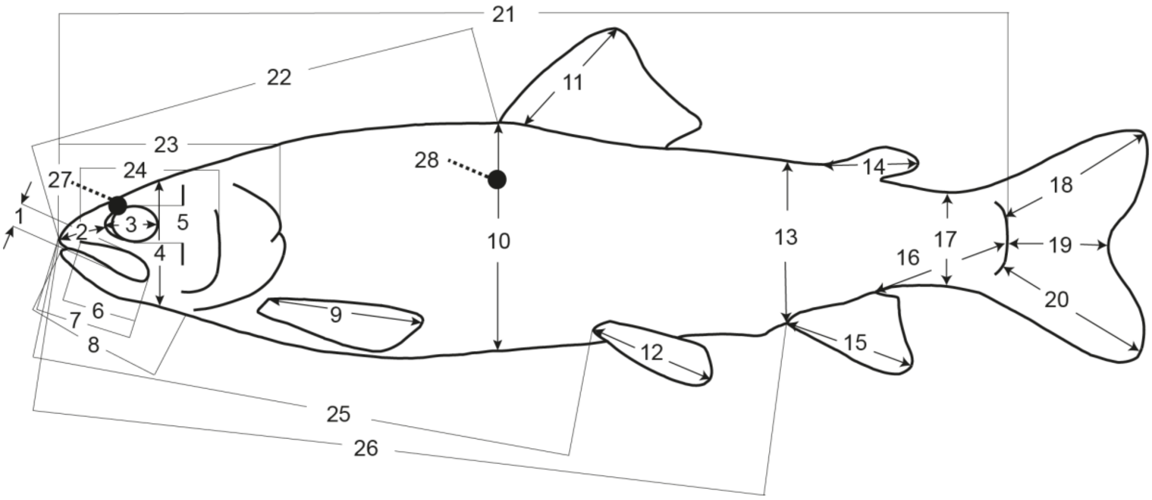

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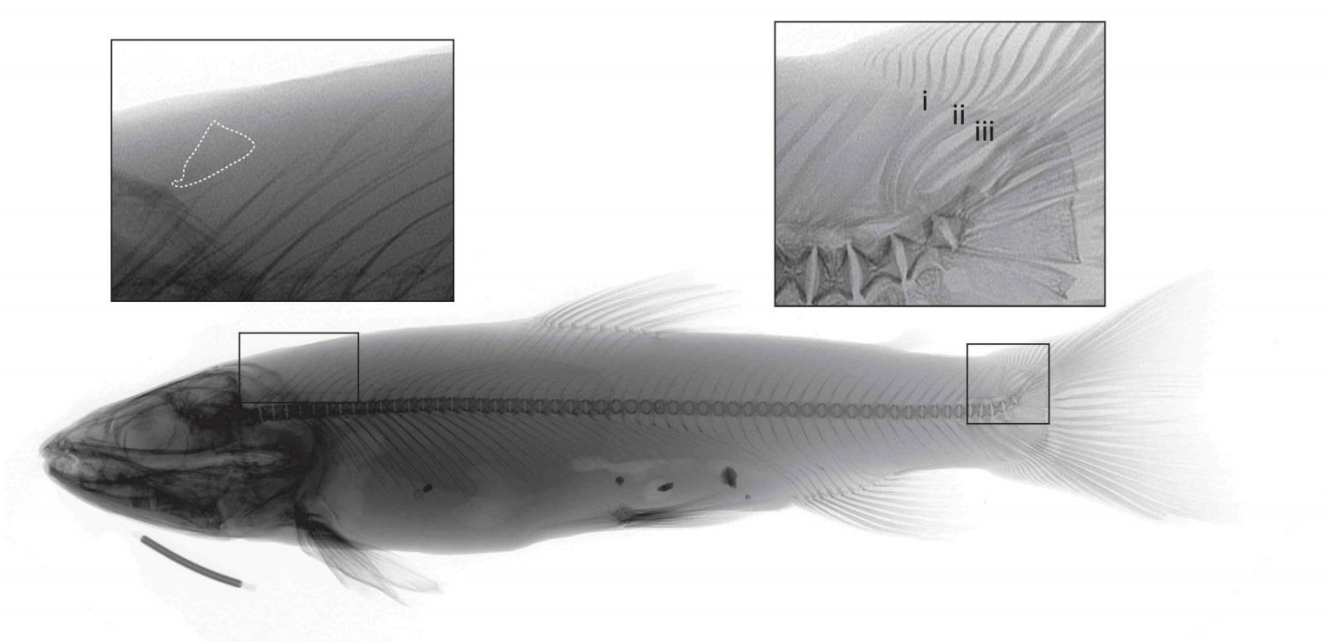

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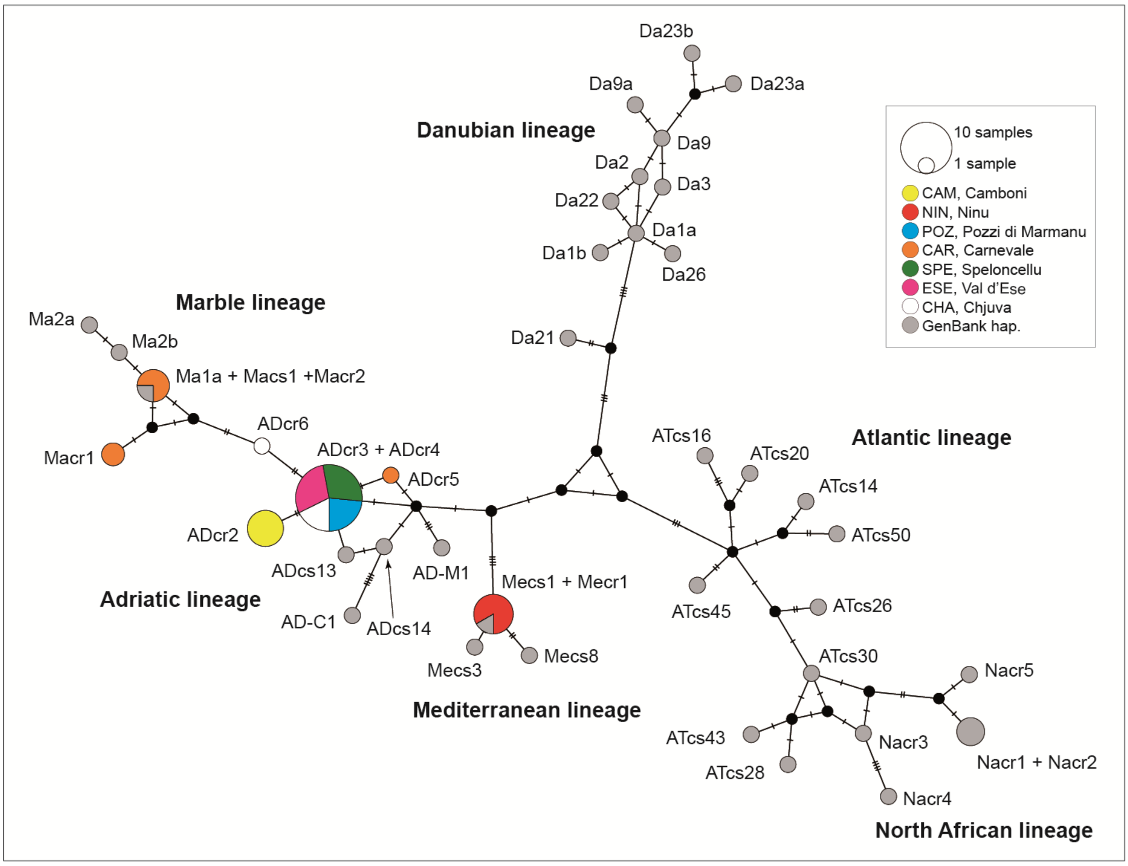

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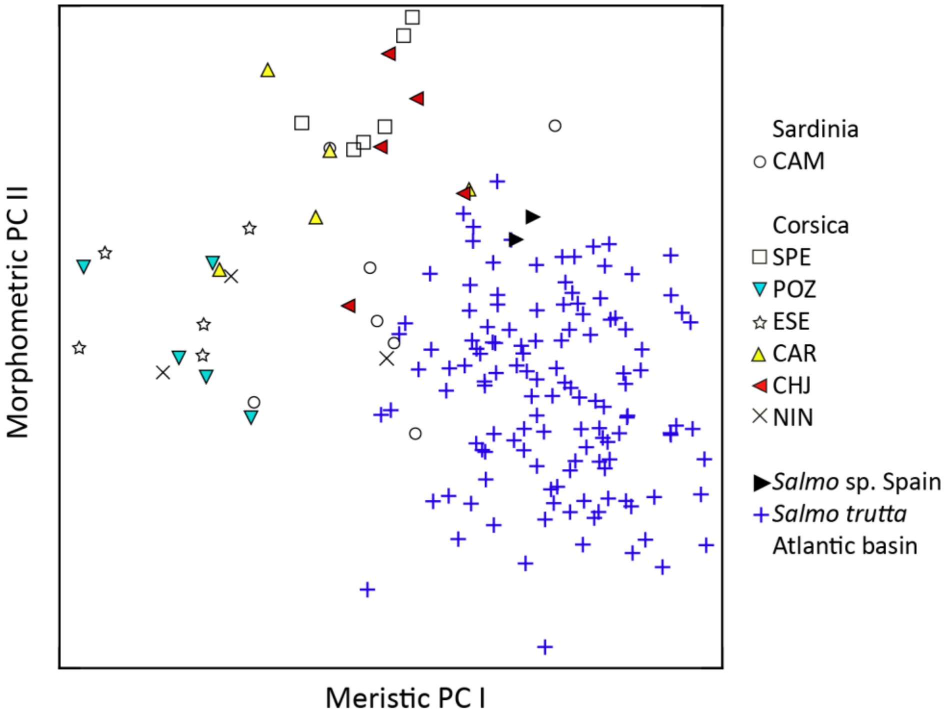

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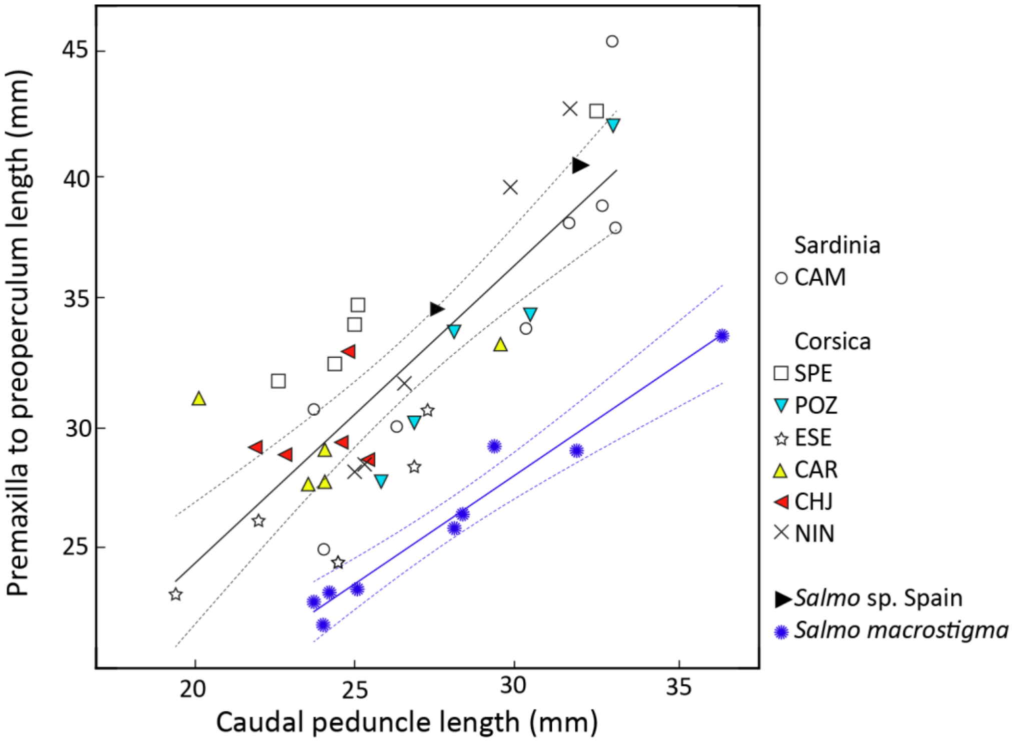

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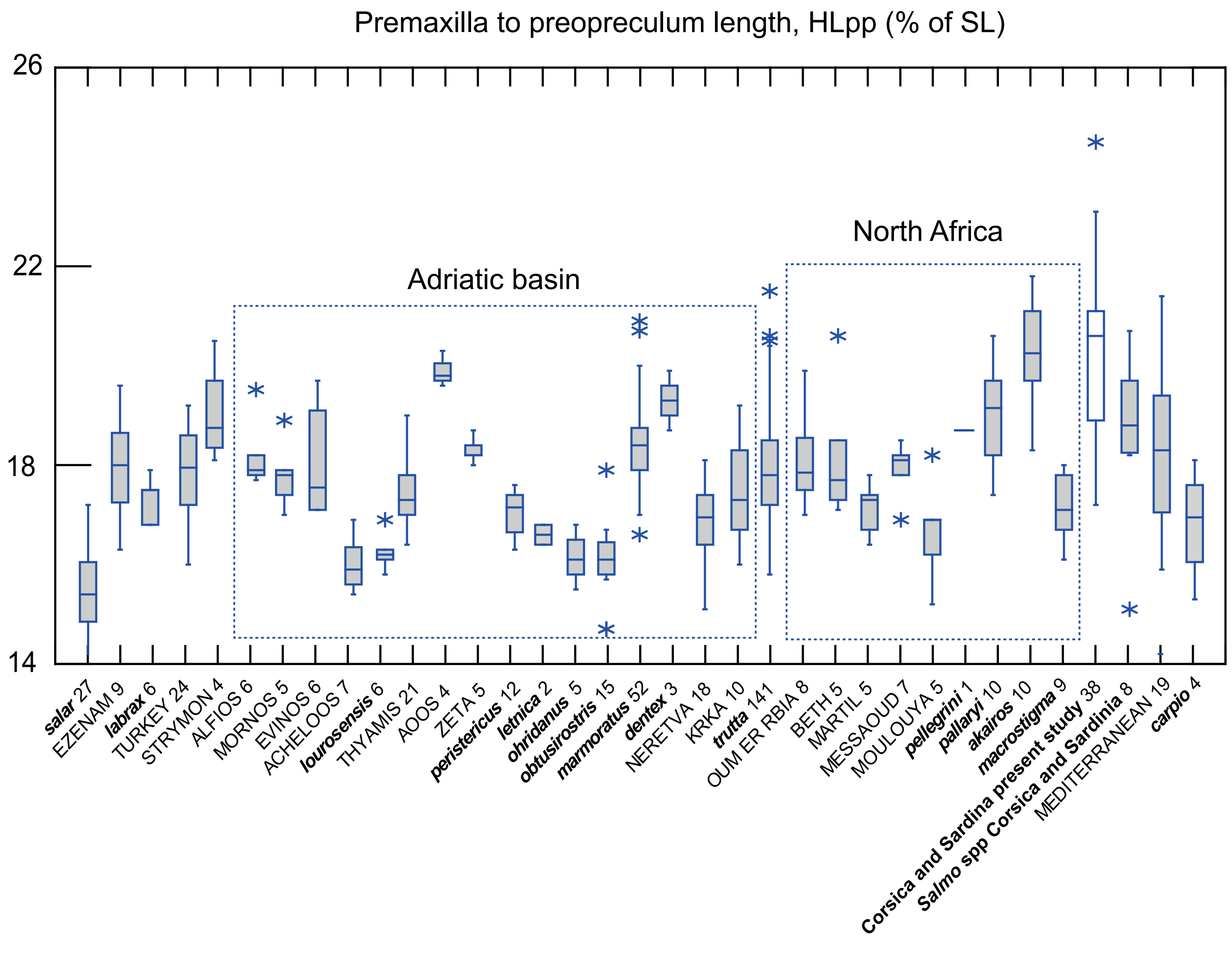
Premaxilla to preopreculum length, HLpp (% of SL)
26
22
18
14
Adriatic basin
North Africa
salar 27
EZENAM 9
labrax 6
TURKEY 24
STRYMON 4
ALFIOS 6
MORNOS 5
EVINOS 6
ACHELOOS 7
lourosensis 6
THYAMIS 21
AOOS 4
ZETA 5
peristericus 12
letnica 2
ohridanus 5
obtusirostris 15
marmoratus 52
dentex 3
NERETVA 18
KRKA 10
trutta 141
OUM ER RBIA 8
BETH 5
MARTIL 5
MESSAOUD 7
MOULOUYA 5
pellegrini 1
pallaryi 10
akairos 10
macrostigma 9
Corsica and Sardina present study 38
Salmo spp Corsica and Sardinia 8
MEDITERRANEAN 19
carpio 4

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

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

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

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

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

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

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

G. *Salmo* sp. NIN, Lake Ninu Corsica. © 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 capture**	**N**	**Museum Collection**	**Reference**	**Remarks**
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	
CHJ	Chjuvone	Corsica	2012	5	NRM 62573	this study	Morphology only
CHA	Chjuva	Corsica	2004	5		this study	Genetics only
NIN	Lake Ninu	Corsica	2013	5	NRM 65092	this study	
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.

Table 2. Distribution of the haplotypes involved in this study

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

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

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

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

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

	scales from base of adipose fin to lateral line													left side branchiostegals					
	11	12	13	14	15	16	17	18	19	20	21	22	23	8	9	10	11	12	13
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 5. Frequency distribution of vertebral counts and caudal fin upper procurrent rays in *Salmo* spp.

	vertebrae												caudal fin upper procurrent rays							
	52	53	54	55	56	57	58	59	60	61	62	63	10	11	12	13	14	15	16	17
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 6. Frequency distribution of gill raker counts in *Salmo* spp.

	total number of gill rakers on first arch												
	13	14	15	16	17	18	19	20	21	22	23	24	≥25
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						
CHJ					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				

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 pistericus
NRM 42538, 60784

Salmo letnica
MHCH 2573.92
MNHN 1977-0262
NMW 65650
ZMH 791, 9182
ZISP39456

Salmo ohridanus
CMK17387
MNHN1924-0227, one uncatalogued specimen
ZISP 39455
ZMH 790, 1461

Salmo obtusirostris
MHCH 608.66
MNHN A6037, A7589, 1904-0032
NRM46364
ZISP39451
ZMH10743

Salmo marmoratus

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

Salmo dentex

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

NERETVA

NRM 46358, 46359, 46361, 46362, 46370

KRKA

NMW 65860, 65915, 65930

Salmo trutta (Atlantic basin, not listed above)

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

OUM ER RBIA

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

BETH

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

MARTIL

BMNH 1887.12.23:4-10

MESSAOUD

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

MOULOYA

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

Salmo pellegrini

NMW 19546 (SYNTYPE)

Salmo pallaryi

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

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

Salmo akairos

MNCN 115018-029, 115022 (HOLOTYPE)

Salmo sp. (Corsica and Sardinia)

BMNH 1901.6.4:1-6, 1909.2.25:14
MHCH 730.22
MNHN 1896-0005, 1896-0006, 1896-0007, 1896-0008, 1896-0009, 1896-0010, 1896-0011
NMW66115
ZMH4302

MEDITERRANEAN

BMNH 1877.1.6:3-4, 1887.1.6:1-2, 1901.8.6:6-7, 1940.2.10:1-3
MHCH 52.82
MNHN 0000-0002, 0000-2575, 0000-3646, 0000-3652, 0000-3653, 000-3354, 1960-0347
nrm7190
ZMH4221

Salmo carpio

NMW 59704, 65957
NRM 28000 (NEOTYPE), 41539
ZISP 40513, 40514, 48207

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

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

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

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