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 The name of the trout: considerations on the taxonomic status of Italian populations of brown trout (*Salmo trutta* L., 1758 complex) (Osteichthyes, Salmonidae)

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Abstract

The systematic status of the Italian brown trout in the Salmo trutta L., 1758 complex (including marble, Mediterranean and lacustrine trout), has long been - and is still today - subject of controversies among ichthyologists. The specific name and the taxonomic rank changed several times in the last years, and the natural occurrence of this salmonid fish in some Italian areas was debated due to spread of alien strains. The main difficulty with the taxonomy of the Italian trout stems from the impossibility of disentangling it "on paper" or, even worse, trying to face this systematic issue considering only a very limited (local/national scale) part of the brown trout range. The taxonomy of the Italian trout population is inextricably linked to the necessity of clarifying first phylogeny and phylogeography in an overall Mediterranean context. The opportunity of a non "self-referential" taxonomy is even more fundamental for a vulnerable salmonid like the Italian brown trout, for which there is a very conflicting management problem related to sport fishing and, at the same time, the urgent need for effective conservation measures. It is however necessary to emphasize that conservation is independent from taxonomy but must start from the level of the local population. In fact, management units need stability and they cannot, therefore, coincide with entities - the Linnean species - requiring continuous taxonomic revisions. Modern molecular methods are the best tools for defining these units of management and conservation in an evolutionary perspective.

Key words: Italian brown trout, taxonomy, evolution, speciation, phylogeography, restocking, management, conservation.

An historical overview on taxonomy of the Italian brown trout (including Mediterranean, marble, and lacustrine trout)

The salmonid fish genus *Salmo* Linnaeus, 1758 harbours in Italy different phenotypes living in different freshwater habitats, like i) mountain streams along south-western Alps, Apennines and main islands (the Mediterranean trout, characterized by medium-small size and more or less numerous dark and/or reddish spots on flanks superimposed on vertical Parr-marks), ii) upper and lower reaches of the Po river basin (the marble trout, bigger in size and with irregular brown lines forming a marbled pattern) and iii) the lacustrine trout (the "carpione") from Garda (up to 500 mm in standard length with uniform, silver coloration and few dark spots) and Fibreno (small size, with large reddish or dark brown ocellated spots on flanks, superimposed on Parr-marks) Lakes (Figure 1). These forms are collectively attributed to the brown trout of the *Salmo trutta* Linnaeus, 1758 complex (see Caputo et al. 2009; Splendiani et al. 2019), to which a systematic review was recently devoted, with reference to Mediterranean (Europe and North Africa) river basins (Lobon-Cerviá et al. 2018).

A paragraph in the work of Lobon-Cerviá et al. (2018) focused on the systematic status of the Italian brown trout, long since - and still today - subject of controversies among ichthyologists. In fact, the specific name and the taxonomic rank attributed to populations living along the Alpine and Apennine chains and in the major islands (Corsica, Sardinia and Sicily) changed several times in the last years (see Kottelat 1997; Kottelat & Freyhof 2009; Bianco & Delmastro 2011; Zerunian 2013; Bianco 2014) and the natural occurrence of this salmonid fish in some Italian areas was also questioned (see Gandolfi et al. 1991). The controversial autochthony of brown trout represents the clear consequence of a long-lasting history of introduction of domestic-strains of Atlantic provenance belonging to the nominal species Salmo trutta L., 1758, starting between the second half of the nineteenth century and the early twentieth century (e.g., Bettoni 1895; Figure 2) and still persisting despite European and Italian laws contrary to the spread of alien stocks (see UZI, 2018). Indeed, already in the early 50s, Sommani (1951) did not include the populations of central Italy in his zoogeographic revision, because, in his opinion, they were too compromised by the spread of Atlantic hatchery trout. In this work, the Author consider the Mediterranean trout of Italy belonging to the nominal form, Salmo trutta L., 1758, with the exception of Sicilian, Corsica, Sardinian and south Latium populations, attributed to Salmo macrostigma (Dúmeril, 1858), due to the typical phenotype characterized by large and sparse dark spots on the body sides (see Duchi 2018) (Figure 1C). The occurrence in Sardinia of a trout species originally described by Dúmeril for Algeria was reported at first by Boulenger (1901), and the "macrostigma trout" was later recognized as typical of the circum-Mediterranean countries (e.g., Tortonese, 1954). The Adriatic trout described by Pomini (1941) as Salmo ghigii on specimens from a tributary (Sagittario River) belonging to the Aterno-Pescara river basin (central-eastern Italy)

was successively considered by Sommani a synonym of *Salmo trutta*. In this case, Sommani argued that the morphological variability observed at intra-basin level was often greater than that subsisting between different basins, so it was not possible to distinguish clearly two taxa of trout comparing the Tyrrhenian and Adriatic-Jonian slopes of the Italian Peninsula.

The zoogeographic picture described by Sommani (1951) was substantially taken up by Tortonese (1970) which, however, does not recognize as valid the status of species for S. macrostigma because in Sardinia, as already pointed out by Pomini (1940), there was a certain chromatic variability that would not allow to clearly distinguish the macrostigma trout from the typical Salmo trutta "not even at the level of subspecies" (Figure 1D; Figure 3). In more recent years, Gandolfi & Zerunian (1987), Gandolfi et al. (1991) and Zerunian (2004) attribute the status of semi-species to macrostigma and consider it potentially distributed along the Tyrrhenian side of the Italian peninsula and in its major islands. In addition, Zerunian & Gandolfi (1990) raise a lacustrine population living in a small karstic lake in southern Latium (Fibreno lake) to the rank of species, Salmo fibreni, distinct from the macrostigma trout. The taxonomic scenario for the Apennine and insular Italian trout was profoundly revised by Kottelat (1997) and Kottelat & Freyhof (2007), according to which Salmo macrostigma should be restricted to Maghreb populations, so the correct name for insular and Apennine Tyrrhenian Mediterranean trout would be Salmo cettii Rafinesque Schmaltz 1810, originally used by the Franco-German zoologist to describe trout living in rivers of eastern Sicily ("fiumi del val demonte e val di Noto", Rafinesque Schmaltz 1810, p. 55). On the other hand, the Authors recognize specific rank also for the Mediterranean trout populations living along the Adriatic side of Apennine (up to the Vomano river, in Abruzzi region) and in the upper reaches of the Alpine streams belonging to the Po plan basin, for which they propose the name Salmo cenerinus Chiereghini, 1847. However, Bianco & Delmastro (2011) and Bianco (2014) considered cenerinus as a junior synonym for Salmo marmoratus Cuvier, 1829, so suggested to use the name Salmo farioides Karaman, 1938 originally established for the Krka river and several others Dalmatian rivers. To corroborate the same specific status of the trout of the two sides of the Adriatic Sea, Bianco (2014) argued that "these basins include numerous primary or primary-like representatives of the Padany-Venetian district: Padogobius bonelli, Alburnus alborella, Squalius squalus, Pomatoschistus canestrini, and Barbus plebejus. Also, for palaeogeographic history, dispersal events occurred during the last Würmian glaciation, when the extended Po basin reached the meso-Adriatic ditch in the central Adriatic Sea, joining rivers of the two Adriatic slopes".

As for northern Italy, the historical picture is completed considering two other taxa of the genus *Salmo*, namely the marble trout, *S. marmoratus* Cuvier, 1829 (Figure 1B) and the lacustrine form, *S. carpio* L., 1758 (Figure 1A). The first one was long time synonymized with *Salmo trutta* (e.g., Festa

1892), but it was considered as a valid taxon since 30s of the twentieth century and the only native trout present in the medium and lower river courses in the Po plan and upper Adriatic basins (Gridelli 1935; Pomini 1937; Sommani 1960). According to Tortonese (1970) "marmoratus" would represent instead a subspecies within *Salmo trutta*, allowing for the possibility of fertile crossings with the nominal form. Also *S. carpio*, endemic of Lake of Garda, was considered a subspecies by Tortonese (1970) "for the close affinities with *Salmo trutta* with which is interbreeding".

The most recent overall review of Lobon-Cerviá et al. (2018), only based on distributional and bibliographic data, attribute to *Salmo cettii* Rafinesque Schmaltz 1810 all the Mediterranea trout populations living in peninsular and insular Italy, in addition to recognizing a full species status to the marble trout (*S. marmoratus* Cuvier, 1829) and the two lacustrine endemics (*S. carpio* L., 1758 and *S. fibreni* Zerunian & Gandolfi 1990).

Italian trout biodiversity enters the molecular systematics era

The taxonomy of Italian populations of the brown trout was significantly influenced by the "molecular revolution", started in the early 90s, with the pioneering works of Patarnello et al. (1994) and Giuffra et al. (1994, 1996). The first one was based on direct sequencing of segments of the cytochrome b and 16S rRNA mitochondrial genes on Italian samples of marmoratus, macrostigma, carpio, fibreni and trutta samples from Italy and north Europe (Ireland). This work failed at evidencing significant differences among the compared taxa, with a possible exception, due to subtle differences, between trutta and marmoratus. The problem with this study was probably related with both the low sample size and the molecular markers used, with a scarce capacity of resolution among trout genomes. In fact, a more exhaustive picture was obtained in the almost contemporary work of Giuffra et al. (1994) due to a combined sequence/RFLP analysis on coding (ATPase subunit VI and Cytochrome b) and non-coding (control region or D-loop) regions of mitochondrial DNA. This approach was already used in the seminal work of Bernatchez et al. (1992; see also Bernatchez 2001) to distinguish five European phylogenetic lineages, namely Atlantic (AT), Adriatic (AD), Danubian (DA), Marmoratus (MA) and Mediterranean (ME) (Figure 4). Three of them are widespread in native Italian trout populations, namely MA, mostly linked to lowland marble trout, AD and ME found in mountain Mediterranean trout. In fact, Giuffra et al. (1994) describe that all marmoratus populations were monophyletic in origin and represented a distinct evolutionary lineage among the north Italian trout populations examined and demonstrated the large spread of AT strain in Po plan drainages. However, the origin of the phenotypically distinct Salmo carpio was not associated with any phylogenetically distinct branching but included four mtDNA lineages (AD, ME, MA and AT, the

latter, however, considered absent by Gratton et al. 2014 and Meraner & Gandolfi 2017), thus suggesting a recent post-glacial origin by mixing of allopatrically evolved genetic strains. In a subsequent work, Giuffra et al. (1996), based on allozyme loci and mtDNA approach, deepened the question of the endemic Po plain Salmo, confirming the possible hybrid origin of S. carpio, resulted in rather recent post-glacial times by introgressive hybridization between Mediterranean and marble trout. Concerning these latter forms, the Authors suggested a process of incipient parapatric speciation, driven by pre-zygotic barriers and adaptation to different habitats (lower and upper parts of the rivers, respectively). According to Giuffra et al. (1996), these delicate evolutionary processes, still ongoing, would be at risk due to massive stocking with fish-farm trout originating from the Atlantic drainages that have already introgressed many wild trout populations and could act as 'genetic bridges', favouring gene flow between the two species. However, contributions of Splendiani et al. (2006, 2007) underlined the role of possible ancient natural contacts in shaping the current genetic makeup of brown trout in central Italy, with unique marmoratus-like genotypes harboured in Mediterranean trout-like phenotypes along Adriatic Apennine streams. This would be a consequence of expansion to the south of the Po plain in the Glacial maxima, favouring paleo-introgression phenomena between marble trout and Apennine Mediterranean trout. The same would apply for the occurrence of MA haplotypes in native trout from Greece (Apostolidis et al. 1997), Dalmatia (Bernatchez 2001) and Albania (Snoj et al. 2009), that would represent the southernmost offshoots of marble trout in consequence of Pleistocene glaciations. What are more difficult to explain are the MA haplotypes found in Corsica (Lerceteau-Köhler et al. 2013), but in this case it is possible to invoke the role of the Apennines as a semi-permeable barrier permitting crossing of MA and other Adriatic lineages on the Tyrrhenian side of Italy, including Corsica (Bianco, 1990, 1994). However, a different interpretation by Gandolfi & Meraner (2017) proposed that MA lineage would be already established long before divergence among the major mitochondrial clades begun, which would only recently be fixed due to drift in Northern marble trout populations.

The paper of Gratton et al. (2014) was the first attempt to face taxonomy and evolution of the genus *Salmo* in Italy with a wider multilocus Bayesian approach including mtDNA control region, 11 microsatellite loci (non-coding nuclear DNA) and 8 nuclear genomic fragments (mostly intronic sequences). This work analyzed over 500 trout individuals belonging to the different Italian *Salmo* taxa, namely *marmoratus*, *carpio*, *cenerinus*, *cettii* and *fibreni*. However, also in this case, only two main evolutionary lineages seem to emerge from the study, namely *Salmo marmoratus* and a "peninsular" lineage. The lake Garda endemic *S. carpio* would be mostly derived from an ancestral population genetically close to the current "peninsular" lineage with a very limited contribution, if any, from a marble ancestry. Alternatively, the presence of both AD and MA mtDNA haplotypes

within genome of *S. carpio* could be the result of an ancestral polymorphism within 'peninsular' brown trout, thus pointing to the non-private character of MA mtDNA haplotypes for the marble trout taxon (see also Meraner & Gandolfi 2017). The presumed species occupying the two sides of the Apennines, namely *S. cettii* and *S. fibreni* in the Tyrrhenian and *S. cenerinus/farioides* in the Adriatic slope, would be indeed no more than two evolutionary lines separated very recently, after the LGM (last glacial maximum, ca 18,000 years ago), probably representing a single species. Unfortunately, the paper of Gratton et al. (2014) used for comparative purpose only domestic trout belonging to Atlantic drainages, without considering Balkan, Rhone basin, Iberian or Maghreb samples, where probably populations related to the Italian ones are present. Trout from the Italian major islands (Corsica, Sardinia and Sicily) are also not analyzed, and these limitations make the work of Gratton et al. (2014) rather weak for wider taxonomic purpose. However, other useful contributions have shed light on the trout biodiversity of the principal Italian islands.

The native trout inhabiting Corsica, Sardinia and Sicily were classified at first as "Salmo macrostigma", for the presence of specimens with sparse and large black dots on the body sides, although Tortonese (1970) observed that "Sardinian and Corsican trout are more variable in ornamentation, also showing red and brownish spots" (Figure 1D, 3). Mitochondrial DNA molecular studies of different Authors indicated that these insular populations harbour indeed very different genetic lineages. Corsican and Sardinian specimens are characterized by AD, MA and ME haplotypes (Lerceteau-Köhler et al. 2013; Zaccara et al. 2015; Sabatini et al. 2011, 2018; Berrebi et al. 2019), while Sicilian ones are the only Italian trout having haplotypes belonging to the southern AT or African sub-clade (Scöffmann et al. 2007; Snoj et al. 2011; Fruciano et al. 2014; Tougard et al. 2018) (Figure 4). Thus, on the base of these consistent molecular data, the name Salmo cettii Rafinesque Schmaltz 1810 is useless for designating Tyrrhenian and insular (e.g., Kottelat & Freyhof, 2007) or even all Italian Mediterranean trout (see Rondinini et al. 2013; Lóbon-Cerviá et al. 2018). In fact, the species "cettii" was described on specimens from rivers of eastern Sicily (see above), phylogenetically linked to Maghreb trout populations (see also Duchi, 2018). Therefore, Salmo cettii Rafinesque Schmaltz 1810 should be considered as a senior synonym of Salmo macrostigma (Dúmeril, 1858), and eventually be used for the trout belonging to the southern Atlantic clade (e.g., Cortey et al. 2009; Snoij et al. 2011), assuming North-African and Sicilian trout populations are worthy of taxonomic distinction (but see Tougard et al. 2018).

Another significant study examined the possibility to use ancient DNA (aDNA) for systematic and phylogeographic purpose (Splendiani et al. 2017). In this paper a partial sequence of D-loop was obtained from a trout collection deposited at the Zoological Museum "La Specola" of the Florence University. The trout specimens were collected by the former Director of the Museum, Professor

 Adolfo Targioni Tozzetti (1823-1902) that in the 1880s was commissioned by the Italian Government to evaluate the distribution and abundance of the Italian trout for possible exploitation just after the Italian unification (Bettoni 1895). The analysis of 17 specimens coming from different Italian localities (peninsular and the two major islands, namely Corsica and Sardinia, including Salmo marmoratus, S. cettii and S. carpio, Figure 1) indicated, very interestingly, that probably in the second half of the nineteenth century allochthonous trout belonging to the nominal form Salmo trutta L., 1758 (AT lineage from norther Atlantic drainages, sensu Bernatchez 2001) was not yet widespread within the Italian rivers. This observation is in line with the history of restocking, which massively started between the end of the nineteenth century and the beginning of the twentieth century (see Bettoni 1895; Tortonese 1970; Figure 2). Another interesting finding is that, in addition to marble trout (MA lineage), in North-Western Italy genetic lineages referred to as native Mediterranean trout (namely AD and ME) are also detected in historical samples. The autochthony of these evolutionary lineages was thus confirmed in this area of Italy, contrary to what was claimed in a publication of the Italian association of freshwater ichthyologists (AIIAD, 2014; see also Forneris et al. 2011). In fact, giving credit to local rumours, this paper considers that the Mediterranean trout was introduced in Piedmont by the Queen Elena of Savoy (1873-1952) - passionate angler (see Siccardi 1996) - in the first half of the twentieth century. On the other hand, a clear description of native trout phenotypes was provided by Festa (1892) and Casalis (1833, 1852) for specimens collected in the central-western Alps well before the "restocking era". In populations from Corsica, Sardinia and Latium, formerly attributed to Salmo macrostigma (Figure 1C), aDNA revealed the presence of AD and ME lineages, also observed in other museum specimens from Tyrrhenian Apennine side, and currently classified as Salmo cettii (see above). Similar findings were recently obtained by Fabiani et al. (2017) for the "macrostigma" Latium population, harbouring indeed AD and ME haplotypes. As for S. carpio, a single specimen belonged to AD lineage, thus confirming that this lacustrine trout does not represent a peculiar evolutionary line, but probably no more than an ecotype of the Lake Garda (Splendiani et al. 2017).

Lastly, the huge spread of AT genetic lineages belonging to the nominal form *Salmo trutta* L., 1758, due to restocking, was repeatedly emphasized as the main threat to the survival of native trout biodiversity and the consequent impossibility for taxonomic clarification (e.g., Nonnis Marzano et al. 2003; Caputo et al. 2004; Caputo et al. 2009; Bianco, 1991, 2006; Splendiani et al. 2019). In particular, Splendiani et al. (2013, 2016a) focused on the role of biotic and abiotic factors promoting the spreading of alien domestic trout strain in Italy, with particular reference to the geologic substrate of river basins and the ice Alpine cap during glacial maxima. The role of permeable rock (determining suitable habitats) is of strong importance to determine the resilience of native trout to introgression

of alien strains, while the expansion of glacial cap on the Alps explains well the reason for the presence of only alien brown trout in the central-east Alps, in consequence of introduction in recent times of AT strains after extirpation of native trout, due to the destructive action of the Alpine ice sheet.

Concluding remarks

The overall picture that emerges from the works published so far on the taxonomy and evolution of Italian trout is unfortunately still controversial and not conclusive. For instance, the last paper in order of time which purported to clarify the taxonomy of the Italian trout (Lobón-Cerviá et al. 2018), actually did not even notice the impossibility of using the specific name Salmo cetti Rafinesque Schmaltz 1810 on a national scale, being it a senior synonym for S. macrostigma Dúmeril, 1858 (see above). In our opinion, the main difficulty with taxonomy of Italian trout is that it is impossible to disentangle it "on paper" or facing the question only at local/national scale. The taxonomy of Italian trout population is inextricably linked to the necessity of clarifying first phylogeny and phylogeography in an overall context, with the help of powerful molecular tools available today (e.g., Next Generation Sequencing). The necessity of a non "self-referential" taxonomy is all the more fundamental for a fish like the brown trout, for which there is a tremendous conflicting interest between biological conservation and sport fishing management (e.g., UZI, 2008). In fact, due to European and local law restrictions in the use of alien stocks for supportive breeding (see Council of the European Communities 1993; UZI, 2018), there is a great pressure by angling associations to have domestic "autochthonous" trout to bypass these limitations. In this context, the taxonomic confusion about native Italian trout represents an ad hoc opportunity for the trade in presumed native stocks produced by trout farmers and/or fishing associations. In the next years, this latter practice will probably represent a further threat for the conservation of the native genetic diversity of brown trout populations from the Italian Peninsula. First, based on the analysis of recent stocking records (years 2008-2018), domestic Mediterranean stocks of brown trout have been used irrespective of their geographic origin. For example, the waters courses of the Provinces of Como, Sondrio, Lecco and Bergamo (central Alps) are yearly stocked with the same Mediterranean trout coming from a presumed native Apennine strain (Splendiani et al. in preparation). The use of wild animals of different Italian provenances for breeding in captivity can favour translocation phenomena, as in the case illustrated by Splendiani et al. (2019), with the observation of a Tyrrhenian haplotype in a hatchery producing "native Mediterranean" trout for restocking purpose on the Adriatic slope of the Apennines. Furthermore, this presumed native trout stock was actually a mix between Atlantic and

native trout. The production and spreading in nature of this kind of trout, fraudulently being passed off as native, will lead to the future impossibility to delineate the phylogeographic history of the original populations, and therefore will represent an obstacle to describing a reliable taxonomic picture of the Italian native trout. Added to this is the still huge and illegal use of Atlantic domestic trout that are poured into the Italian rivers in tons of specimens every year for stocking (Splendiani et al. in preparation). In this context, the paradoxical disinterest of the main Italian environmental associations is a real pity, as they give priority to conservation of the "most charismatic" homeothermic vertebrates (see Fenoglio et al. 2018; Tiberti 2018) regardless of the freshwater fish so severely threatened with extinction (e.g., Zerunian 2002; Rondinini et al. 2013). It is equally paradoxical that for the Italian Ministry of the Environment major angling associations are recognized as environmental associations: in a sense, entrusting the trout to the fishermen is like "having the fox guard the henhouse"!

To conclude, it is increasingly clear that conservation strategies cannot be merely based on the Linnean species, as there is the risk of useless taxonomic inflation (e.g., Isaac et al. 2004). In fact, since in the case of the trout the identification of the management units can only start at the river basin or sub-basin level, giving a species name to each of these units would make the species list disproportionate (e.g., https://www.fishbase.de/Nomenclature/). Second, management units need stability and they cannot, therefore, coincide with entities - the Linnean species - requiring continuous taxonomic revisions (Mace 2004). It seems, therefore, necessary to emphasize once again that conservation should be taxonomy independent. On the contrary, the conservation and even the management of the native trout biodiversity must start from the level of the local population, considering genetic structure even at micro-geographical scale (see Laikre et al. 1999; Sanz 2017; Berrebi et al. 2019; Splendiani et al. 2019). Modern molecular methods represent the indispensable rationale for defining these units of management and conservation in an evolutionary perspective.

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Legend to figures

Figure 1. Some museum specimens used for an ancient DNA study (Splendiani et al. 2017). A, Lake Garda carpione (collected in 1877); B, marble trout from Po basin (collected in 1876); C, Mediterranean trout from Ninfa (Southern Latium, collected in 1897), "macrostigma" phenotype; D, Mediterranean trout from Corsica (Tartagina forest, collected in 1892), with brownish and reddish dots phenotype. Numbers indicate the collection number of the Zoological Museum "La Specola" of the Florence University.

Figure 2. Results of restocking with hatchery trout in central Apennines rivers (Adriatic slopes of Italy, province of Pesaro-Urbino) in the first years of Twentieth century (Società Ittiofila di Cagli, 1905).

Figure 3. Three different phenotypes observed in Sardinia. A, phenotype with low number of black spots with a pale halo and high number of red spots (Riu Piras); B, phenotype with high number of black spots without halo and high mean diameter of black and red spots (Riu Litteras and Riu Furittu); C, phenotype with high number of black spots with a pale halo and low number of red spots (Riu Flumineddu).

Figure 4. A, Maximum likelihood tree for genus *Salmo* based on 980 bp of the mtDNA control region (from Snoj et al. 2011). The purple box indicates Southern AT-clade (including Maghreb and Sicily haplotypes). B, approximate distribution of the main lineages (AD, Adriatic; AT, Atlantic; DA, Danubian; MA, Marmoratus; Me, Mediterraneus, according to Bernatchez, 2001. ME and AD are widely admixed in Mediterranea basin; MA fixed in but not exclusive to marbled trout, see text). Asterisks indicate haplotypes belonging to the Southern AT-clade obtained from ancient DNA (* from Algeria specimens, Tougard et al. 2018; ** from Southern Italy specimens, Splendiani et al. 2016b).

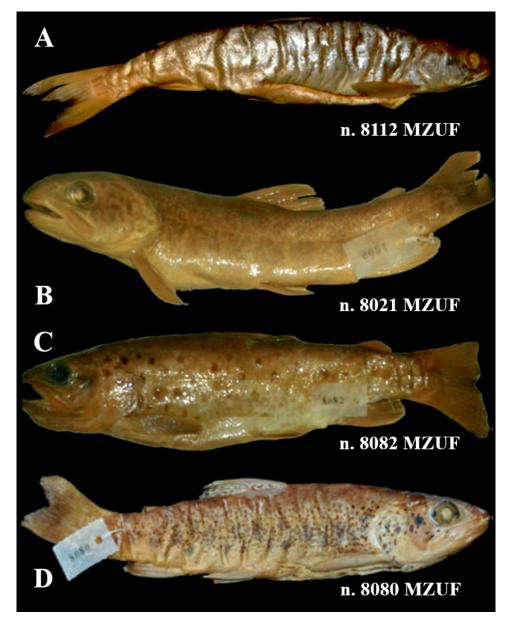


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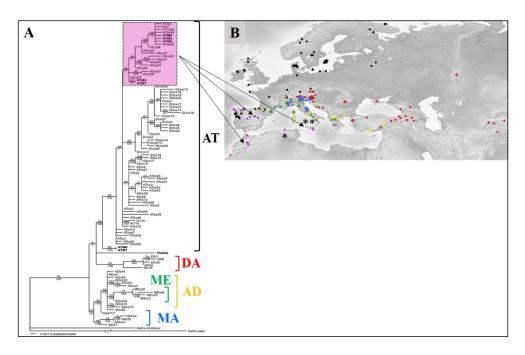


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