Salmo macrostigma (Teleostei, Salmonidae): Nothing more than a brown trout (S. trutta) lineage?

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Salmo macrostigma (Teleostei, Salmonidae): nothing more than a brown trout (*S. trutta*) lineage?

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We examined specimens of the macrostigma trout *Salmo macrostigma*, which refers to big black spots on the flanks, to assess whether it is an example of taxonomic inflation within the brown trout *Salmo trutta* complex. Using new specimens, publicly available data and a mitogenomic protocol to amplify the control and cytochrome *b* regions of the mitochondrial genome from degraded museum samples, including one syntype specimen, the present study shows that the macrostigma trout is not a valid species. Our results suggest the occurrence of a distinct evolutionary lineage of *S. trutta* in North Africa and Sicily. The name of the North African lineage is proposed for this lineage, which was found to be sister to the Atlantic lineage of brown trout, *S. trutta*.

KEYWORDS

Conservation, mtDNA, North Africa, Sicily, syntype, taxonomic status

1 | INTRODUCTION

All biological conservation is based on taxonomy and the central unit in taxonomy is the species. Since the advent of Linnaean binomial taxonomy, taxonomists have been describing, naming and classifying species, mainly based on morphological characters. During the past 3 decades, numbers of described species have increased in part due to the widespread use of advanced technologies, such as molecular phylogenetics, DNA barcoding, computed tomography, geographical information systems and various internet tools (Bickford *et al*., 2007; Padial *et al*., 2010; Zachos *et al.*, 2013). The increased availability and selective use of these data have led to reinterpretations of species limits based only on changes in species concept rather than new discoveries (*e.g.* cryptic species). This process is known as 'taxonomic inflation' (Isaac, Mallet, & Mace, 2004; Zachos *et al*., 2013) and it has resulted in over-splitting in which numerous known subspecies are elevated to the species rank (Isaac *et al*., 2004; Zachos *et al*., 2013). Species elevated in this way have often been found to be invalid, with numerous subspecies boundaries based on discontinuities in the geographic distribution of phenotypic traits poorly aligning with described molecular lineages (Burbrink *et al*., 2000; Phillimore & Owens, 2006; Newman & Rissler, 2011; Prie *et al*., 2012; Deichmann *et al*., 2017). However, effective conservation and management rely on the proper definition of operational units (*e.g.* evolutionarily significant units, ESU; Moritz, 1994). Such units require that variation related to species status be appropriately assessed and considered before relevant taxonomic decisions are made (Mace, 2004; Sites & Marshall 2004). To gain support, operational conservation and management units also need congruent molecular and morphological patterns (Fraser & Bernatchez, 2001; Agapow *et al*., 2004; Sites & Marshall, 2004).

In the brown trout *Salmo trutta* L. 1758, natural diversity is notably characterized by complex patterns of phenotypically distinct geographic forms and considerable life-history variation (Bernatchez *et al*., 1992; Elliott, 1994; Kottelat & Freyhof, 2007; Sanz, 2017). In addition to *S. trutta* and *Salmo salar* L. 1758, studies of biological variation during the last century have led to the description of several *Salmo* L. 1758 species, often having very restricted geographical ranges (Berrebi *et al*., 2013; Clavero *et al*., 2017; Ninua *et al*., 2018). Depending on the source, the number of *Salmo* species ranges from around 25 (Kottelat & Freyhof, 2007; IUCN, 2016) to nearly 50 (Froese & Pauly, 2018). Among these, a dozen were described during the past decade (Turan *et al*., 2009; Delling, 2010; Turan *et al*., 2011, 2012, 2014), such as *Salmo akairos* Delling & Doadrio 2005 and *Salmo viridis* Doadrio, Perea, & Yahyaoui 2015 described from Morocco. As illustrated by the numerous references cited above, which are only a snapshot of the available literature, research in this area is very active and molecular data have already begun to show that some species are not valid [Sanz (2017) provides a detailed list and references]. Apart from *S. salar* and few other valid species [*e.g. Salmo obtusirostris* (Heckel 1851); Snoj *et al.*, 2002], this diversity is subsumed within the *Salmo trutta* species complex (Sanz, 2017). Hence, this complex represents an interesting case of possible taxonomic inflation that has confounding taxonomic ramifications, hampers the understanding of the species' evolutionary history and impedes the development of appropriate strategies to protect natural trout diversity (Bernatchez *et al.*, 1992; Antunes *et al.*, 2001; Fumagalli *et al.*, 2002; Snoj *et al.*, 2010; Crête-Lafrenière *et al.*, 2012; Gratton *et al.*, 2013, Ninua *et al.*, 2018). Furthermore, to our knowledge, no molecular phylogenetic study within this complex has included type specimens (*e.g.* holotype, isotype, syntype) to check the validity of taxa, even though these are the only objective link to the Linnean binomial (Mutanen *et al.*, 2015).

Considered to be distributed in southern Eurasia and North Africa (Tortonese, 1954; Geldiay, 1968; Geldiay & Balik, 1988; Kottelat & Freyhof, 2007), *Salmo macrostigma* (Duméril 1858) represents one interesting case study to test for taxonomic inflation within the *Salmo* genus. The term macrostigma was used by Duméril (1858) to describe a trout with big black spots on the flanks named *Salar macrostigma*, found in the Oued-el-Abaïch 40 km west of Collo in Algeria. According to Duméril (1858), two identical specimens, here considered syntypes, were brought to Paris, France, one for the collections of the National Museum of Natural History (MNHN) and the other for the French Zoological Society. The term macrostigma has subsequently been used at both the species (*Salmo macrostigma*) and subspecies (*S. t. macrostigma*) level to characterize other trout native to North Africa (Morocco: Vivier, 1948; Azeroual *et al.*, 2000; Abba *et al.*, 2010; Lbadaoui *et al.*, 2011), Turkey (Togan *et al.*, 1995; Bardakci *et al.*, 2006; Kara *et al.*, 2010) and the Middle East (Berg, 1949; Derzhavin, 1929; Segherloo *et al.*, 2012). Macrostigma phenotypes have also been reported in Italy (including Sardinia and Sicily; Boulenger, 1901; Mola, 1928; Gandolfi *et al.*, 1991; Patarnello *et al.*, 1994; Massidda, 1995; Nonnis Marzano *et al.*, 2003; Ciuffardi & Arillo, 2006; Querci *et al.*, 2013), Albania (Rakaj & Flloko, 1995; Cullaj *et al.*, 2005), Greece (Kattoulas, 1972; Karakousis & Triantaphyllidis, 1990) and Corsica, France, (Guyomard, 1989; Roche & Mattei, 1997; Gauthier & Berrebi, 2007). A macrostigma phenotype might be observed in *Salmo cettii* Raffinesque 1810, inhabiting Sicily (Schöffmann *et al.*, 2007; Kottelat & Freyhof, 2007) and Duchi (2018) rightly reported that confusion still exists regarding how to distinguish *S. macrostigma* from *S. cettii*. Indeed, distribution of the macrostigma trout remains controversial and some authors assert that it should be exclusively used for Algerian populations (Zouakh, 2009; Turan *et al.*, 2011). Thus, the use of macrostigma for populations beyond Algeria and evaluation of the taxonomic status of *S. macrostigma* itself within or outside the *S. trutta* complex required genetic comparison

of the Algerian macrostigma syntypes with European, Asian and other North African trout populations.

Mitochondrial DNA markers are valued for phylogenetic reconstructions using both museum and fresh samples because nucleic acids degrade over time and mitochondrial genomes (mtDNA) are available at much higher copy numbers per cell compared with single-copy nuclear DNA (Höss, 2000; Wandeler *et al.*, 2003) and because mtDNA has reliable barcoding properties across vertebrates, including fishes (Allio *et al.*, 2017). Despite its uniparental inheritance, mtDNA remains an important marker in studies of the *S. trutta* complex partly because so much data are already available from previous salmonid studies (Bernatchez *et al.*, 1992; Snoj *et al.*, 2002; Verspoor *et al.*, 2002; Sušnik *et al.*, 2006; Crête-Lafrenière *et al.*, 2012) and partly because a well-supported mtDNA-based hypothesis of phylogenetic relationships is available (Bernatchez, 2001; Cortey *et al.*, 2004; Vera *et al.*, 2010; Snoj *et al.*, 2011; Sanz, 2017).

In this study, traditional mtDNA marker analyses were combined with a mitogenomic approach used to obtain sequence data from the damaged DNA of museum samples labelled as *S. macrostigma,* including the syntype catalogued at National natural history museum, Paris (MNHN). The aim of the present study was to characterize: phylogenetic relationships of this syntype with other *Salmo* mtDNA lineages or species; the taxonomic status of *S. macrostigma* and whether this name refers to a distinct phylogenetic lineage; the limits of the geographic distribution of this lineage.

2 | MATERIALS AND METHODS

2.1 | Sampling

Fin or DNA samples were obtained for 67 specimens belonging to European, Moroccan and Turkish populations of *S. trutta* (Supporting Information Table S1). To assess the phylogenetic position of the macrostigma trout, a small piece of muscle from the MNHN syntype specimen (MNHN_IC_A_7585) and three other museum samples attributed to the macrostigma trout (MNHN_IC_0000_1909_C, MNHN_IC_1977_272_A and NMW_67984) were analysed (Supporting Information Table S1).

2.2 | DNA extraction and sequencing

Total genomic DNA from 67 specimens was extracted from 96% ethanol-preserved fin tissue following standard procedures (Sambrook, Fritsch, & Maniatis, 1989). The entire control region (CR) and cytochrome *b* (*cytb*) gene were amplified by PCR with the following primer sets, respectively: *Str-DL1F* (5'-GCACCGACTACACTATCATT-3') and StrDL1R (5'-TTTATATGTTTGATTGAGA-3') designed for this study; SalmoCBF (5'-CATAATTCCTGCCCGGACTCTAACC-3') and *Salmo-CBR* (5'-TTTAACCTCCGATCTCCGGATTACA-3') corresponding to the *cytb* primers mentioned in Crête-Lafrenière *et al.* (2012). Direct sequencing was carried out in both directions at the technical facilities of the genotyping and sequencing platform of the Institute for Evolutionary Sciences Montpellier (ISEM).

For the museum samples, DNA retrieval was optimized using a protocol recently developed by Tilak *et al.* (2015) to build and sequence shotgun Illumina (www.illumina.com) libraries from small quantities of degraded genomic DNA. First, DNA extraction with blank controls was performed in the ADN dégradé platform of ISEM dedicated to damaged DNA experiments, with the DNeasy Blood and Tissue kit (QIAGEN; www.qiagen.com) following the recommendations of the manufacturer, except decreasing the elution volume to 100 µl.

The Illumina library preparation procedure thus followed the recommendations of Tilak *et al.* (2015) for blunt-end repair, adapter ligation, adapter fill-in and indexing PCR steps. An equimolar mix including a library for each museum sample was handed over to GATC-Biotech (www.eurofinsgenomics.eu) for the sequencing step on a single Illumina HiSeq2000 lane. Mapping of short reads (≤ 100 bp) and annotation of the complete mitogenomes were carried out against a reference genome of *S. trutta* (JQ390057) using Geneious 7 (Kearse *et al.*, 2012). Reads were mapped as follows: a minimum of 24 contiguous nucleotides matched the reference genome; a 5% maximum of mismatches per read; a minimum of 95% overlap similarity with the reference genome; a 3% indel maximum not exceeding a gap size of 10 nucleotides.

2.3 | Quality control of mitogenomes

To evaluate the potential presence of nuclear pseudogenes in the assemblies, each annotated mitogenome was carefully inspected by eye to detect abnormal boundaries, frameshifts and premature stop codons in protein coding genes. Three other quality controls recommended by Botero-Castro *et al.* (2016) were also performed: detailed information on the origin of the samples used for mitogenome sequencing and the museum voucher of each sample was provided to ensure taxonomic identification and easier cross-referencing; a CR and *cytb* barcoding identification of the specimens through a phylogenetic tree based on the closest available sequences was done to avoid specimen misidentification; a phylogenetic analysis of the new mitogenomes in the context of closely related species was conducted to get a clear depiction of the evolutionary affinities of the new mitogenomes and their degree of divergence compared with close relatives.

2.4 | Phylogenetic analyses and genetic distances

Mitogenomes of the four museum samples and of several GenBank *S. trutta* (4) and *S. salar* (2) were aligned using the Geneious aligner with default parameters. Control region and *cytb* sequences were extracted from these mitogenomes and aligned by hand using MEGA 6.06 (Tamura *et al.*, 2013) with newly produced and GenBank sequences obtained for additional specimens (Supporting Information Table S1).

Phylogenetic analyses were performed on a dataset including 46 new sequences of the control region. As mtDNA lineages of *S. trutta* were previously identified from CR sequences, 71 GenBank sequences were added to identify these lineages in our dataset. Sequences belonged to eight main recognized lineages of *S. trutta*: Atlantic; Danubian; Adriatic; Marbled or *S.* (*trutta*) *marmoratus* Cuvier 1829; Mediterranean; Duero; Tigris; Dades (MacCrimmon & Marshall, 1968; Elliott, 1989; Bernatchez *et al.*, 1992; Suárez *et al.*, 2001; Cortey *et al.*, 2004, 2009; Vera *et al.*, 2010; Snoj *et al.*, 2011; Özen, 2013). Thus, a total of 117 CR sequences were used for phylogenetic reconstructions. Phylogenetic analyses were also based on a dataset including 70 new and 49 GenBank sequences of *cytb* (*i.e.* 119 *cytb* sequences in total). The two datasets were concatenated in a single dataset when sequences for both markers were available for the same individual. This concatenated dataset included 89 new and GenBank sequences (Supporting Information Table S1). According to the *cytb* phylogeny from Crête-Lafrenière *et al.* (2012), these separated and concatenated datasets were completed with GenBank sequences of *S. obtusirostris* and *Salmo ohridanus* Steindachner 1892. Finally, the tree was rooted by designating *S. salar,* as an outgroup (Supporting Information Table S1).

Phylogenetic reconstructions were conducted on the mitogenomic dataset as well as on both separate and concatenated CR and *cytb* datasets through the technical facilities of the

platform Montpellier bioinformatics biodiversity at ISEM. Phylogenetic trees were reconstructed using a maximum-likelihood approach (ML) in the program PhyML 3.0 (Guindon *et al.*, 2010) and Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Best-fitting models of sequence evolution were identified for each marker dataset as well as the concatenated dataset using MrModeltest 2.3 (Nylander, 2004). A generalized time reversible (GTR) model (Yang, 1994) with a proportion of invariable sites (*I*) and a gamma distribution (*G*) was selected for the concatenated dataset in ML and the mitogenomic dataset in both ML and BI. A mixed model was recommended for a concatenated BI analysis in which the CR region and *cytb* codon positions were separately partitioned: HKY (Hasegawa *et al.*, 1985) + I + G for CR; K80 (Kimura, 1980) for the first codon position of *cytb*; GTR + I + G for the second and third positions of the *cytb*. Nodal robustness was estimated by bootstrap percentage values (BP) after 1000 pseudo-replicates under ML optimization. Five independent runs of five Markov chain Monte-Carlo chains were simultaneously carried out for 5 000 000 generations under BI optimization. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consensus of trees sampled every 100 generations after a burn-in stage of 25 000 generations.

Intra- and interlineage genetic distances were estimated by the uncorrected *p*-distance on the separate and concatenated datasets with MEGA.

3 | RESULTS

New sequences were deposited in the European Nucleotide Archive under the accession numbers LT617521–LT617587 for *cytb*, LT617588–LT617629 for CR and LT617630–LT617633 for the mitogenomes (Supporting Information Table S1). Alignments of the complete CR and *cytb* sequences were 1019 and 1140 nucleotides long with 108 and 128

phylogenetically informative sites, respectively. Alignment of the mitogenomes comprised 16 683 positions with 907 phylogenetically informative sites (Supporting Information Table S2).

3.1 | TREE TOPOLOGIES

Phylogenetic analyses generated similar tree topologies in BI and ML (Figure 1 and Supporting Information Figures S1–S3 with PP and BP values). The monophyly of *S. trutta* was supported with high PP (1.00) values in the concatenated tree (Figure 1) and with high PP (1.00) and BP (100%) values in the mitogenome tree (Supporting Information Figure S3). In the other analyses, *S. trutta* formed a clade with *S. ohridanus* and *S. obtusirostris* (PP = 1.00; BP = 100%). The two latter species were always highly supported (PP = 1.00; 98% < BP < 100%) but were included in *S. trutta* in the CR and *cytb* gene trees (Supporting Information Figures S1, S2).

In the CR gene tree (Supporting Information Figure S2), the Mediterranean, Marbled, Danubian and Turkish mtDNA lineages were moderately to highly supported (0.93 < PP < 0.99; 57% < BP < 95%), whereas the Atlantic, Duero, Sicilian and Moroccan specimens were clustered together with weak support (PP ≤ 0.80; BP ≤ 50%). In the *cytb* gene tree (Supporting Information Figure S1), the Atlantic, Mediterranean, Dades, Danubian and Duero lineages had moderate to high support values (PP = 1.00; 71% < BP < 95%), whereas the Adriatic, Marbled and Turkish lineages were spread out in the tree. In the tree obtained from the concatenated dataset, all mitochondrial lineages were moderately to highly supported (0.99 < PP < 1.00; 55% < BP < 100%). In the separate and concatenated trees, relationships between these lineages remained unresolved.

3.2 | Genetic distances

Genetic distances from the separate and concatenated datasets were estimated within and between *S. trutta* lineages, *S. obtusirostris*, *S. ohridanus and S. salar* (Tables 1 and Supporting Information Tables S3, S4). In *S. trutta*, intragroup distances were as follows: 0.0 ± 0.0% to 0.6 ± 0.1% for *cytb*; 0.0 ± 0.0% to 0.4 ± 0.1% for CR (but distances rose to 5.4 ± 0.5% for the group including Atlantic, North African, Sicilian and Duero specimens); 0.1 ± 0.0% to 0.5 ± 0.1% for the concatenated dataset. The intergroup distances were as follows: 0.6 ± 0.2% to 1.7 ± 0.3% for *cytb*; 0.7 ± 0.2% to 1.3 ± 0.2% for CR (but 6.2 ± 0.5% for the group including Atlantic, North African, Sicilian and Duero specimens with the Danubian lineage); 0.5 ± 0.1% to 1.6 ± 0.3% for concatenated dataset. From the concatenated dataset, genetic distances between *S. trutta* and its closest related species were: 1.7 ± 0.2% with *S. obtusirostris* (2.1 ± 0.4% and 3.9 ± 0.4% for *cytb* and CR, respectively), 2.6 ± 0.3% with *S. ohridanus* (2.4 ± 0.4% and 5.6 ± 0.5% for *cytb* and CR, respectively) and 5.7 ± 0.5% with *S. salar* (5.9 ± 0.6% and 8.0 ± 0.7% for *cytb* and CR, respectively).

3.3 | Position of the macrostigma museum specimens

It clearly appears from the concatenated dataset that the three macrostigma MNHN samples originating from Northern Africa (Algeria and Morocco; Supporting Information Table S1) form a clade with new and GenBank specimens from Morocco and Sicily (PP = 0.99; BP = 55%) (Figure 1). This clade is closely related to the Atlantic lineage of *S. trutta* (PP = 1.00; BP = 76%; Figure 1) and not at all to the Dades lineage, endemic to Morocco (Snoj *et al.*, 2011). The last museum sample attributed to a macrostigma trout (NMW 67984, originating from Albania; Supporting Information Table S1) clustered with the *S. obtusirostris* specimens

from Bosnia and Herzegovina and Croatia in all trees (Figures 1and Supporting Information Figures S1, S2).

4 | DISCUSSION

The term macrostigma is widely used to phenotypically describe peri-Mediterranean trout samples from North Africa, Turkey, Middle East and Southern Europe. However, no phylogenetic study focused on this phenotype has previously been performed. Its recognition as a valid species may contribute to taxonomic inflation because morphological and meristic characters that strongly vary within the *S. trutta* complex are being erroneously used to diagnose an invalid taxon. Results showed that the existence of a single *S.* (*t.*) *macrostigma* (sub)species covering this very wide geographic area is unlikely as the four macrostigma trout considered in this study did not cluster together. No specimen from the Balkans, Turkey, Iran or France, where macrostigma is thought also to be distributed, was found to cluster with the MNHN syntype or with other macrostigma specimens considered in this study. They were all found to cluster within recognized *S. trutta* lineages (*e.g.* Danubian for Iranian samples; Adriatic for Albanian samples; Atlantic, Adriatic, Marbled and Mediterranean for Corsican samples).

The macrostigma syntype from MNHN and two other macrostigma trouts clustered with specimens from Moroccan and Sicilian populations that were grouped in a well-supported clade in the concatenated tree (Figure 1). The geographical origin of these three museum specimens matched with the location from where *S. macrostigma* was originally described (Oued-el-Abaïch, Algeria; Duméril, 1858). However, results did not support the existence of a monophyletic *S. macrostigma* restricted to North Africa, but rather showed that these specimens with alternative phenotypes are included in the *S. trutta* complex. Individuals

of this well-supported Siculo-North African clade are distributed in four more-or-less geographically restricted groups with unresolved relationships (Figure 1) and with low intergroup genetic distances (0.2 to 0.6% for *cytb* and 0.2 to 0.5% for CR in Doadrio *et al.*, 2015). Such distances compared with the intergroup genetic distances between *S. trutta* and other species considered (*S. obtusirostris*, *S. ohridanus and S. salar*, always > 2% for *cytb*, > 3.5% for CR) do not support the definition of valid *Salmo* species. According to Snoj *et al.* (2011), the evolutionary legacy of *Salmo* diversification in Northern Africa is probably the result of several waves of colonisation in North Africa during the Pleistocene. It may have occurred that *S. trutta* mtDNA haplotypes replaced undetected macrostigma, and possibly other (*e.g.* similarity of cytochrome oxidase I haplotypes among Mediterranean trout; Figure S1 in Geiger *et al*., 2014), mtDNA haplotypes during this process. However, samples that grouped with the three macrostigma trout are all Sicilian (T250009, T25010 and T25011) and Moroccan samples from Lake Ifni (I1, MNCN85764 and MNCN85765) in the concatenated tree (Figure 1). This suggests that at least one wave of mtDNA replacement would had to have been strong enough and relatively uniform throughout the range covered by those samples, from western Morocco (Lake Ifni) to Sicily. Additionally, some authors have reported that the so-called *S. macrostigma* is restricted to Mediterranean drainages of Morocco and Algeria (Delling & Doadrio, 2005; Zouakh, 2009; Doadrio *et al.*, 2015), while Lake Ifni is located in the Atlantic slope of Morocco. Thus, a multi-replacement scenario over a wide North African geographic area does not appear to be very parsimonious. This has to be investigated further but, at this stage, the North African *S. macrostigma* appears to be more of a myth than a well-supported reality. Nuclear data are necessary to address the relevance of *S. macrostigma* and other described *Salmo* species as valid species. Phylogenomic methods to delimit taxon boundaries are challenging but have greatly improved in the past few years. Such methods can distinguish between structure associated with intraspecific variation and

introgression from that resulting from speciation (Wagner *et al.*, 2013; Mutanen *et al.*, 2015; Zarza *et al.*, 2016; Baumsteiger *et al.*, 2017) and sometimes allow for a deep taxonomic reassessment of evolutionary units in challenging taxa (Papakostas *et al.*, 2016), or even reconciliation of morphological and molecular taxonomies in integrative studies (Dejaco *et al.*, 2016; Morard *et al.*, 2016). The *Salmo* genus is certainly one interesting case to consider because large scale integrative taxonomic studies are currently lacking. Consideration of type specimens certainly remains crucial (Mutanen *et al.*, 2015; Schultz *et al.*, 2015). New techniques may facilitate the retrieval of more complete mitochondrial (van der Valk *et al.*, 2017) and nuclear data (*e.g.* Grandjean *et al.*, 2017) from museum specimens.

Conversely, it is clear from Figure 1 that North African macrostigma specimens and other samples probably belong to a previously undescribed North African mtDNA lineage in *S. trutta*, with the well-known Atlantic lineage as its sister clade. This confirms previous results based on partial and complete CR that have already underlined the close phylogenetic relationships between native Moroccan and Sicilian populations (Schöffmann *et al.*, 2007; Snoj *et al.*, 2011; Fruciano *et al.*, 2014).

However, if *S. macrostigma* is not a valid species based on our mtDNA data, native populations of the so-called North African lineage are currently threatened by human activities (*e.g.* increased water use, environmental degradation due to deforestation) and by introgression with domestic *S. trutta* from hatcheries (Snoj *et al.*, 2011; Fruciano *et al.*, 2014). To manage native biodiversity better, Zachos *et al.* (2013) particularly discouraged both species-splitting based on gene trees inferred from mtDNA and phenetic analyses aimed at diagnosability. These authors recommend the use of concepts such as ESUs (considering the spatial distribution of the genetic diversity; Moritz, 1994; Almodóvar *et al.*, 2006), or alternatives [*e.g.* operational conservation units (OCU), reflecting interaction with socio-economic issues (Dodson *et al.*, 1998; Machordom *et al.*, 2000)], which highlight

intraspecific diversity without promoting taxonomic inflation and the arbitrary concept of subspecies (Freudenstein *et al.*, 2017). The North African lineage described in this study and probably more local groups within this lineage certainly must be managed in a more adaptive and integrated evolutionary framework, such as with ESUs, to be more effective (Fraser & Bernatchez, 2001).

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Supporting Information

Supporting Information may be found in the online version of this paper

FIGURE 1 Bayesian phylogenetic tree reconstructed from a concatenated alignment of the mitochondrial control and cytochrome *b* regions. Sample labels are given in Table S1. Museum samples are in bold. *, Macrostigma MNHN syntype; **, the accession number of the mitogenome used as a reference for read mapping. Numbers at nodes are for posterior probabilities (≥ 0.80) and bootstrap percentages (≥ 50%). –, Nodes weakly supported in either the maximum likelihood or Bayesian inference analyses. Lineage or species names are indicated on the right.

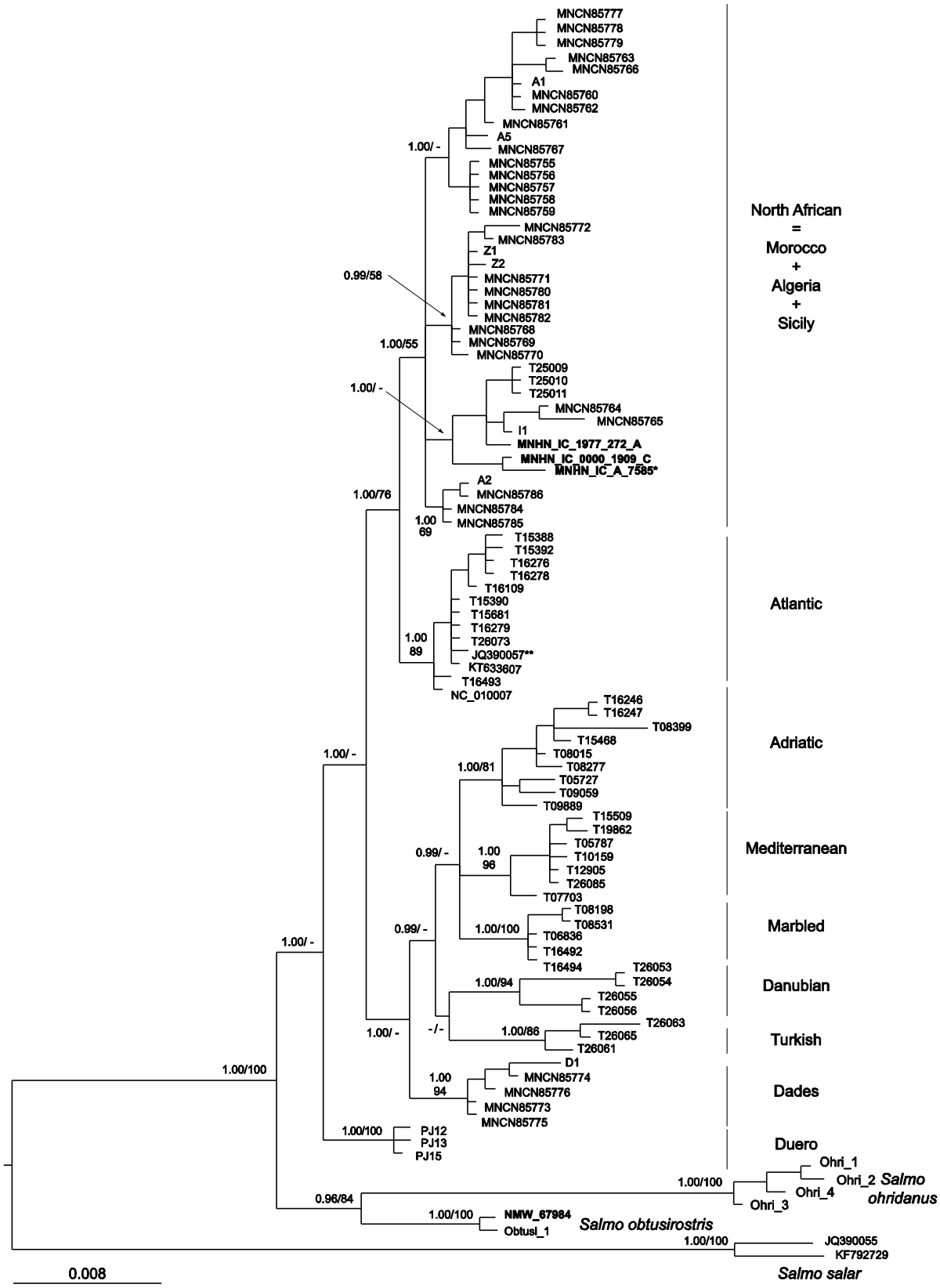

TABLE 1 Genetic distances between lineages and the most closely related species of *Salmo trutta* based on the concatenated dataset. Genetic distances (with S.E. in brackets) in the diagonal are intragroup distances (in bold), while the genetic distances below the diagonal (with their S.E. above the diagonal) are intergroup distances

	1	2	3	4	5	6	7	8	9	10	11	12
Adriatic	**0.004 (0.001)**	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.005
Atlantic	0.011	**0.001 (0.000)**	0.002	0.002	0.002	0.001	0.002	0.002	0.002	0.003	0.003	0.005
Dades	0.009	0.009	**0.002 (0.001)**	0.002	0.002	0.002	0.002	0.001	0.002	0.003	0.003	0.005
Danubian	0.012	0.013	0.011	**0.005 (0.001)**	0.003	0.002	0.002	0.002	0.002	0.004	0.003	0.005
Duero	0.011	0.009	0.011	0.016	**0.001**	0.002	0.002	0.002	0.002	0.003	0.002	0.005

					(0.000)							
North African	0.011	0.005	0.009	0.013	0.010	**0.003**	0.002	0.002	0.002	0.003	0.002	0.005
						(0.001)						
Marbled	0.008	0.011	0.008	0.012	0.013	0.010	**0.001**	0.002	0.002	0.004	0.003	0.005
							(0.000)					
Mediterranean	0.009	0.011	0.007	0.012	0.013	0.011	0.008	**0.001**	0.002	0.004	0.003	0.005
								(0.000)				
Turkish	0.009	0.011	0.010	0.012	0.013	0.011	0.010	0.011	**0.003**	0.004	0.003	0.005
									(0.001)			
S. obtusirostris	0.018	0.016	0.018	0.021	0.016	0.016	0.016	0.019	0.018	**0.001**	0.003	0.005
										(0.001)		
S. ohridanus	0.028	0.029	0.022	0.026	0.031	0.024	0.027	0.029	0.026	0.027	**0.002**	0.005
											(0.001)	
S. salar	0.058	0.059	0.055	0.059	0.059	0.057	0.058	0.058	0.056	0.068	0.061	**0.008**
												(0.002)