Will the genomics revolution finally solve the Salmo systematics?

Christelle Tougard

To cite this version:

Christelle Tougard. Will the genomics revolution finally solve the Salmo systematics?. Hydrobiologia, 2022, 849, pp.2209-2224. 10.1007/s10750-022-04872-2 . hal-03630657

Will the genomics revolution finally solve the *Salmo* systematics?

Christelle Tougard

ISEM, Université de Montpellier, CNRS, IRD, EPHE, Montpellier, France.

corresponding author: Institut des Sciences de l'Evolution de Montpellier, Université de Montpellier, Place Eugène Bataillon, CC065, 34095 Montpellier cedex 05, France; christelle.tougard@umontpellier.fr

ORCID: 0000-0002-6525-0698

Abstract

On one hand, studies on *Salmo* biological variations during the last centuries have led to the morphological description of several *Salmo* species (>50). On the other hand, *Salmo trutta* is seen as a polymorphic species, i.e. including populations with different morphotypes and ecotypes, subdivided into nine genetically divergent evolutionary lineages. For 30 years, phylogeographic and phylogenetic investigations tried to solve the *Salmo* systematic problem using the mitochondrial control region, sometimes combined with other mitochondrial (e.g. protein coding region or rRNA genes) or nuclear (e.g. allozymes or microsatellites) markers. With the advent of high throughput next-generation sequencing, complete mitogenomes were made available for *Salmo* phylogenetic studies. Even better, complete genome or chromosomes with annotations as well as genes of interest can now be loaded from public database. However, in the genomic era, some challenges still need to be addressed such as an appropriate taxon sampling or the identification of orthologous genes before having an accurate phylogeny. In the present review, I examine how traditional molecular markers contributed to our knowledge of trout systematics, and what we can expect from the genomics revolution.

Keywords: mitochondrial DNA, molecular phylogeny, next-generation sequencing technologies, nuclear DNA, *Salmo trutta*

Context

Salmonidae are the focus of much scientific attention, in particular the subfamily Salmoninae, with, at least, three species (*Onchorhynchus mykiss* (Walbaum, 1792), *Salmo salar* Linnaeus, 1758, and *Salmo trutta* Linnaeus, 1758) among the ten most studied fishes (Hutching, 2014; Birnie-Gauvin et al., 2019). Despite this attention, some issues remain, however, unresolved, such as *Salmo* systematics. Problems started nearly 300 years ago when Linnaeus first classified *Salmo trutta* under different common and Latin names (Linnaeus, 1758). Because of complex patterns of phenotypically distinct geographic forms and considerable life-history strategies (Bernatchez et al., 1992; Elliott, 1994; Kottelat & Freyhof, 2007; Sanz, 2018), studies of *Salmo* biological variations during the last centuries have led to the morphological description of several *Salmo* species (or morphospecies; Cronquist, 1978), from around 30 (Kottelat & Freyhof, 2007; IUCN, 2022) to more than 50 (Behnke, 1986; Froese & Pauly, 2021) depending on the source. Molecular data have, however, refuted the validity of some species, such as *S. dentex* (Heckel, 1851) (Snoj et al., 2010) or *S. platycephalus* Behnke, 1968 (Sušnik et al., 2004), whereas they confirmed the reclassification of others in the genus *Salmo*, such as *S. obtusirostris* (Heckel, 1851) (Snoj et al., 2002) and *S. ohridanus* Steindachner, 1892 (Phillips et al., 2000; Sušnik et al., 2006). Indeed, most of the described (morpho)species have close phylogenetic relationships with *S. trutta*, and, for this reason, several taxonomic and phylogenetic studies consider *S. trutta* as a complex of species, except *S. salar*, *S. obtusirostris* and *S. ohridanus* (e.g. Patarnello et al., 1994; Lo Brutto et al., 2010; Vera et al., 2011; Meraner et al., 2013; Gratton et al., 2014; Tošić et al., 2014; Splendiani et al., 2017). On the other hand, *Salmo trutta* is also seen as a polymorphic species, i.e. including populations with different morphotypes and ecotypes, subdivided into several genetically divergent evolutionary lineages (Bernatchez et al., 1992; Bernatchez, 2001; Suárez et al., 2001; Sušnik et al., 2005; Bardakci et al., 2006; Vera et al., 2010; Snoj et al., 2011; Tougard et al., 2018). To avoid confusion, the term “trout” is here used to refer to both the *S. trutta* species complex and the evolutionary lineages.

There is no phylogenetic study based on morphological characters and including all *Salmo* species. Attempts of molecular phylogeny were restricted to a limited number of *Salmo* species, while relationships between *S. trutta* evolutionary lineages are not fully resolved (e.g. Bernatchez, 2001; Cortey et al., 2004; Pustovrh et al., 2014; Sanz, 2018; Tougard et al., 2018; Guinand et al., 2021; Hashemzadeh Segherloo et al., 2021). In all cases, *Salmo* is a monophyletic genus with *S. salar* as the first offshoot of this group (Crespi & Fulton, 2004; Crête-Lafrenière et al., 2012; Shedko et al., 2013; Horreo, 2017; Lecaudey et al., 2018). From the molecular dating, this genus originated during the Miocene, between 16 and 10 Myr (Crête-Lafrenière et al., 2012; Shedko et al., 2013; Horreo, 2017; Lecaudey et al., 2018). In most phylogenetic studies, *S. obtusirostris* is the sister species of *S. ohridanus*, except in Pustovrh et al. (2014) where *S. obtusirostris* was found to be the sister species of *S. trutta* and *S. marmoratus* Cuvier, 1829. The clade including *S. obtusirostris*, *S. ohridanus* and trout is dated back to the Pliocene, around 5-4 Myr (Crête-Lafrenière et al., 2012; Pustovrh et al., 2014; Lecaudey et al., 2018), while the trout age was estimated between 4 and 0.40 Myr (Crête-Lafrenière et al., 2012; Pustovrh et al., 2014; Horreo, 2017; Lecaudey et al., 2018). This is more or less concordant with the fossil record since the oldest fossil remains of *Salmo*, found in Croatia, dated from the Middle or Upper Miocene (13 – 5.33 Myr; Gorjanović-Kramberger, 1891; Anđelković,1989), and those of *S. trutta*, found in Caucasus, dated from the Pliocene (~ 2 Myr) (Vladimirov, 1946, 1948). Several hypotheses were proposed in trying to understand the trout evolutionary history during the Pleistocene, and how it was impacted by the Quaternary climatic changes (e.g. Hamilton et al., 1989; García-Marín et al., 1999; Sanz et al., 2000; Bernatchez, 2001; Antunes et al., 2002; Cortey et al., 2004; McKeown et al., 2010; Snoj et al., 2011; Splendiani et al., 2016). However, since phylogenetic relationships between evolutionary lineages are poorly resolved, and no phylogeny includes all *Salmo* species, this history can only be partial at best.

Paradoxically, *S. trutta,* considered as one of the world's invasive species in countries (at least 24) where it was introduced, is imperiled in much of its native distribution, i.e. in Eurasia and North Africa (Budy et al., 2013; Elliott, 2018). Among the 36 *Salmo* species listed in the International Union for Conservation of Nature, 18 are threatened or endangered, and one (*S. pallaryi* Pellegrin, 1924) considered extinct (Crivelli, 2006; IUCN 2022). Trout provides recreation and food to million of people and plays important roles in ecosystem functioning, but it is extremely sensitive to habitat disturbances induced by human activities (e.g. overfishing, pollution, dams, deforestation, agriculture, grazing, and mining) and climate change (Muhlfeld et al., 2018). Native trout diversity is also threatened by the massive stocking of domesticated strains that facilitates the spread of pathogens and parasites. By hydridization of native populations with specimens from hatcheries, stocking erodes genetic diversity or eliminates original local adaptations (García-Marín et al., 1998; Lobón-Cerviá, 2018). Even if the genetic diversity of *Salmo* populations is relatively well understand, notably at the intra-basin level, concrete conservation strategies should take into account practices that do not erode and/or modify this natural diversity. Efforts to protect native trout morphological, ecological and genetic diversity should rely on appropriate local and/or global conservation and management strategies, and systematics is essential to biodiversity conservation by helping to set conservation priorities (Wheeler, 2001). However, what must be done in the case of a messy systematics as in the trout case?

Indeed, systematics discrepancies hamper our understanding of trout evolutionary history, and impede the development of appropriate strategies to protect natural diversity of native trout (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; Hashemzadeh Segherloo et al., 2021). Trout systematics thus needs urgently revision/clarification. This systematics was mostly investigated from a genetic standpoint, and the use of genetics in trout systematics has a long history (Fig. 1). In the present review, I examine how traditional molecular markers, and notably mitochondrial DNA (mtDNA), contributed to our knowledge of trout systematics, and what we can expect from the genomics revolution.

PAST: mitochondrial DNA rise

Until the end of the 1980s, genetic discrimination of trout relied mostly on protein electrophoretic (allozyme) analyses (e.g. Ryman & Ståhl, 1980; Ferguson & Mason, 1981; Vuorinen & Piironen, 1984; Guyomard & Krieg, 1986; Skaala & Nævdal, 1989). First investigations on trout systematics were performed with these markers, and refuted the classification of Greek trout populations into five subspecies (Karakoukis & Triantaphyllidis, 1990). Despite their usefulness in the delineation of genetically distinct salmonid populations, allozymes displayed a limited ability to identify existing genetic diversity by detecting only a part of amino acid substitutions and examining only coding regions of the genome (Hynes et al., 1989). Genetic diversity was thus studied in a population genetics context (genetic similarities between populations) rather than in a phylogenetic view (relationships among or within species with inference of their evolutionary history).

By the early 1990s, two molecular markers were proposed as new diagnostic markers to genetically differentiate salmonid species and populations: the eye-specific lactate dehydrogenase *Ldh-5* nuclear locus (Hamilton et al., 1989), and the mitochondrial DNA (Hynes et al., 1989). The *Ldh-5* locus, now called *LDH-C1** locus, is polymorphic for two co-dominant alleles, *Ldh-5 (100)* or *LDH-C1*100* and *Ldh5 (90)* or *LDH-C1*90*. The "*100"* or "ancestral" allele is present in several species of Salmonidae including southern European trout populations, while the "*90"* or "modern" allele is fixed for north-western European and hatchery trout populations (Hamilton et al., 1989). Initially proposed to differentiate "ancestral" from "modern" populations in phylogeographic studies, this marker is today mostly used to differentiate natural populations from domesticated Atlantic strains or to detect and characterize introgression patterns between these populations (Berrebi et al., 2019; Rossi et al., 2019; Splendiani et al., 2019; Vera et al., 2019; Kanjuh et al., 2020). Oleinik et al. (2017) also tested this locus as phylogenetic marker in a study including 11 salmonid genera. If the genus *Salmo* was monophyletic, this locus failed to solve phylogenetic relationships between *S. obtusirostris*, *S. ohridanus* and *S. trutta*. Indeed, the most used molecular marker to date in trout phylogeographic and phylogenetic studies (116 out of 137; Table S1) is mtDNA (Fig. 1). Compared to nuclear DNA (nucDNA), mtDNA is a fast evolving and easy-to-isolate genome with a small size, simple constitution, and uniparental inheritance avoiding recombination and allowing matrilineal phylogeny inferences and molecular dating (Hynes et al., 1989; Meyer, 1993). Comparison of mtDNA variations can be realised by restriction fragment length polymorphism (RFLP) analyses of mitochondrial fragments and/or by nucleotide sequences of mitochondrial partial or complete genes (e.g. Hansen & Loeschcke, 1996; Osinov & Bernatchez, 1996; Machordom et al., 2000; Weiss et al., 2000; Aurelle & Berrebi, 2001; Bernatchez, 2001; Sell & Spirkovski, 2004; Bardakci et al., 2006; Splendiani et al., 2006; Apostolidis et al., 2008; Cortey et al., 2009; Griffiths et al., 2009; McKeown et al., 2010; Schenekar et al., 2014).

Craze for mitochondrial markers, and especially the control region (CR), is particularly linked to a study focused on the phylogeography of European *S. trutta* populations (Bernatchez et al., 1992). Based on two CR fragments (310+330 base pairs, bp), this study was the first assessment of phylogenetic relationships between widely remote European populations (151 individuals from 24 populations). Identified haplotypes were distributed in five major mitochondrial evolutionary lineages: Adriatic (AD), Atlantic (AT), Danubian (DA), *marmoratus* or Marble (MA) and Mediterranean (ME) (see Fig. 2 for their distribution). These genetic lineages were not congruent with ecological and morphological differentiation (Bernatchez et al., 1992). These preliminary results were later confirmed by Bernatchez (2001) in a study based on 1794 individuals from 174 populations. Bernatchez (2001) suggested that both Pleistocene glaciations and biological factors impacted the distribution of these major lineages as well as trout population structure. Since then, others evolutionary lineages, with a more restricted distribution (Fig. 2), were highlighted: the Duero lineage (DU) endemic of the north-western Iberian Peninsula (Cortey et al., 2009; Vera et al., 2010, 2015); the Tigris lineage (TI) endemic of the Çatak river in Turkey (Sušnik et al., 2005; Bardakci et al., 2006); the Dades lineage endemic of the Drâa basin in Morocco (Snoj et al., 2011); the North-African lineage (NA) found in Morocco, Algeria, Sicily and maybe in Spain (Tougard et al., 2018; Splendiani et al., 2019). Mitochondrial DNA was also a valuable marker to estimate past trout genetic diversity from museum samples (Giuffra et al., 1994; Lahnsteiner & Jagsch, 2005; Splendiani et al., 2017) or even archeological remains (Splendiani et al., 2016), notably because nucleic acids degrade over time, and mtDNA is available in much higher copy numbers per cell compared with single-copy nucDNA (Höss, 2000; Wandeler et al., 2003).

The mitochondrial control region is thus considered as a reference to identify trout lineages or species (Table S2). However, this highly variable marker does not allow, alone, to get resolved trout phylogenetic relationships. Other mitochondrial markers, such as protein coding (cytochrome *b*, or cytochrome *c* oxidase, ATPase and NADH dehydrogenase subunits) and/or rRNA (12S and 16S) genes, have been proposed as alternatives (e.g.

Giuffra et al., 1994; Patarnello et al., 1994; Machordom et al., 2000; Dudu et al., 2010; Lo Brutto et al., 2010; Dudu et al., 2011; Crête-Lafrenière et al., 2012; Shedko et al., 2013; Rezaei, 2015; Turan et al., 2020). Some phylogenetic studies have involved also nucDNA such as allozymes (e.g. Bernatchez & Osinov, 1995; Riffel et al., 1995; Apostolidis et al., 1996; Antunes et al., 1999; Bouza et al., 2001; Cagigas et al., 2002), microsatellites (e.g. Sušnik et al., 2007; Snoj et al., 2011; Kohout et al., 2013; Gratton et al., 2014; Marić et al., 2017) or nuclear genes (e.g. *transferin* in Antunes et al., 2002; internal transcribed spacer 1 or ITS1 in Presa et al., 2002; Turan et al., 2009; Vera et al., 2011; C intron of growth hormone 2 or GH2C in Razpet et al., 2007; somatolactin in Snoj et al., 2010; recombination activating gene 1 or RAG1 in Shedko et al., 2012). For both mitochondrial and nuclear alternatives, few studies were, however, fully freed from the CR reference. Even the genomic study of Hashemzadeh Segherloo et al. (2021) based on 15,169 single nucleotide polymorphisms markers (SNPs) includes CR sequences. Recently, Pustovrh et al. (2011a, 2014) and Snoj et al. (2021) performed phylogenetic analyses based exclusively on nuclear loci. These studies contradicted mtDNA observations. Among the 7000 to 8000 bp of the final alignments, few variations are, however, informative sites (70, 196 and 286, respectively), and this low number of information could provide insufficient phylogenetic signal, and thus false phylogenetic inference (Philippe & Douzery, 1994; Wiens, 2003; Phillipe et al., 2004) as suggested, for instance, by the lack of resolution at several nodes of the trees and the trichotomy linking *S. obtusirostris, S. marmoratus* and *S. trutta* in Pustovrh et al. (2011a, 2014).

Over the years, molecular diagnostic protocols were designed to allow rapid trout identification, either from SNPs (CR SNPs in Apostolodis et al., 2007; nucDNA SNPs in Pustovrh et al., 2011b; Casanova et al., 2022) or pyrosequencing assay (Keller et al., 2010). Both approaches did not receive, however, the expected success. As illustrated by the numerous references cited above, genetic trout identification is mostly done through mtDNA or nucDNA sequences, and the combination or concatenation of these markers provided better inferences on trout systematics as in the following examples: inclusion of *Acantholingua ohridana* (cytochrome *b*, ITS1 and GH2C sequences; Phillips et al., 2000) and *Salmothymus obtusirostris* (CR, cytochrome *b* and *LDH-C1** sequences; Snoj et al., 2002) in the genus *Salmo* (*S. ohridanus and S. obtusirostris*, respectively); reclassification of *Salmo* (*Platysalmo*) *platycephalus* in a lower taxonomic category (CR, cytochrome *b* and ITS1 sequences; Sušnik et al., 2004).

With the advent of high throughput next-generation sequencing (NGS), complete mitochondrial genomes (or mitogenomes) were made available for *Salmo* phylogenetic studies (Horreo, 2017). Compared to *S. salar* (Hurst et al., 1999), it is very recently that first *S. trutta* mitogenomes were published. One (KT634053) was obtained in a more classical way with amplification by PCR and Sanger sequencing of overlapping mtDNA fragments (Li et al., 2016), while the other (KT633607) was obtained through shotgun libraries subjected to Illumina Miseq sequencing (Sahoo et al., 2016). Since then, mitogenomes were produced for several trouts (*S. macrostigma* (Duméril, 1858), *S. ischchan* Kessler, 1877, *S. trutta fario* Linnaeus, 1858 and *S. trutta caspius* Kessler, 1877) from modern (Rezaei & Akhshabi, 2017; Rezaei et al., 2017) or museum (Levin et al., 2018, 2022; Nedoluzhko et al., 2018a, 2018b; Tougard et al., 2018) samples. More than any other technology, NGS has also made thousand of loci and SNPs available through the whole trout genome. For instance, Restriction-site Associated DNA sequencing markers (RADseq) or double digest RADseq (ddRADseq) were recently used to screen for genetic variations in Atlantic and Mediterranean populations (Leitwein et al., 2016, 2018; Saint-Pé et al., 2019), while RADseq and Genotyping-by-Sequencing methods were recently used to investigate phylogenetic relationships among Salmoninae and some *Salmo* species, respectively (Lecaudey et al., 2018; Hashemzadeh Segherloo et al., 2021).

PRESENT: new hope through next-generation sequencing

Despite 30 years of trout phylogeographic and phylogenetic investigations, no consensus was found between morphological and genetic approaches about trout systematics. In fact, no molecular phylogeny has yet included specimens from all trout (morpho)species and/or covering the whole geographic distribution of *S. trutta* evolutionary lineages. Some studies underlined, however, relationships between one of the nine trout lineages from CR - sometimes combined with other mtDNA or nucDNA markers - with some trout (morpho)species (e.g. Lo Brutto et al., 2010; Kalayci et al., 2018; Tougard et al., 2018; Hashemzadeh Segherloo et al., 2021; Table S2). Moreover, it should be noted that higher morphological diversity is associated to southern lineages compared to the AT lineage, but this diversity is not congruent with genetic diversity known for each evolutionary lineage. The AT and DA lineages have an extended distribution (Fig. 2) associated to a higher haplotype diversity (Sanz, 2018) compared to the AD, Dades, DU, MA, ME, TI and NA lineages. This unequally distributed diversity could be one of the reasons why phylogenetic relationships between lineages remain poorly solved. However, it seems more likely that currently used mtDNA (control region or cytochrome *b*) and/or nucDNA markers are not sufficiently informative. Informative sites in these markers represent a weak proportion of the genetic variation, compared, for instance, to the whole mitochondrial genome (Fig. 3).

As already mentioned, NGS technologies are promising to study trout phylogeny. Through these technologies, the whole annotated genome of *Salmo trutta* was recently made available from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genome/31807). Complete genome or chromosomes with annotations as well as genes of interest can now be loaded, and used to identify more appropriate markers (e.g. Sass et al., 2016; Hughes et al., 2018; Cloutier et al., 2019; Hansen et al., 2021) to get an accurate trout phylogeny, and to understand morphological character evolution such as colour pattern, a key character in trout description and identification.

FUTURE: challenges to face

Without clear phylogenetic consensus, it is unrealistic to draw conclusions on the trout evolutionary history. It seems that genomics methods can provide qualitatively and quantitatively new types of information that cannot be achieved using traditional methods, and our ability to reconstruct evolutionary histories from (phylo)genomic analyses will thus help us to define conservation units. However, if technical processes of NGS technologies are increasingly better mastered, practical and analytical issues remain challenging such as taxon sampling, identification of orthologous genes, bioinformatics, and data storage.

Sampling choice is a crucial step in phylogenetic studies. First, stocking programs may conceal *Salmo* natural genetic patterns. In Morocco, for instance, *Salmo* populations with higher levels of genetic diversity were those affected by stocking programs (Perea et al., 2020). Then, it may lead to contradictory evolutionary assumptions as in the studies of Doadrio et al. (2015) and Tougard et al. (2018) focused on Moroccan populations. In Doadrio et al. (2015), specimens only from Moroccan populations were taken into account, and no type specimen was included in the molecular dataset, while Tougard et al. (2018) included in their molecular dataset sequences from Doadrio et al. (2015) and specimens belonging to the nine evolutionary lineages, valid species (*S. orhidanus and S. obtusirostris*), as well as one syntype of *S. macrostigma*. Conclusions of both studies were not congruent: Doadrio et al. (2015) considered populations of the Lake Isli and the Drâa basin as distinct species (*S. viridis* Doadrio et al., 2015 and *S. multipunctata* Doadrio et al., 2015, respectively); Tougard et al. (2018) identified a new evolutionary lineage (NA) including specimens from Algeria (notably *S. macrostigma* syntype), Sicily and all Moroccan populations, except the Drâa basin population that was already considered a different lineage (Dades) by Snoj et al. (2011). Therefore, in phylogenetic studies, especially those discussing specific status, and for more relevant comparisons, it would be recommended to include, in the molecular dataset, sequences of specimens from the evolutionary lineages, some genetically valid species such as *S. obtusirostris* and *S. ohridanus* and/or type specimens (e.g. holotype, syntype, lectotype) to check the taxa validity, and thus to strengthen phylogenetic and/or systematic inferences.

A diversity of NGS technologies exists to produce data suitable for phylogenomic studies (e.g. Díaz-Arce et al., 2016; Dong et al., 2016; Hughes et al., 2018; Parhi et al., 2019). One main question then arises: will it be possible to easily identify orthologous genes since the common ancestor of all Salmonidae experienced a whole genome duplication no later than 88 Myr, resulting in an autotetraploid genome (Moghadam et al., 2011; Alexandrou et al., 2013; Macqueen & Johnston, 2014)? Two recently released databases, PhyloFish database (http://phylofish.sigenae.org/index.html; Pasquier et al., 2016) and SalmoBase (https://salmobase.org/; Samy et al., 2017) constitute promising genomic tools for this challenging key task. The PhyloFish database is a resource offering the possibilty to analyze gene expression after gene duplication for 23 ray-finned fish species, including six salmonids (*Thymallus thymallus* (Linnaeus, 1758), *Coregonus lavaretus* (Linnaeus, 1758), *C. clupeaformis* (Mitchill, 1818), *S. trutta*, *Oncorhynchus mykiss*, *Salvelinus fontinalis* (Mitchill, 1814)) (Pasquier et al., 2016), while SalmoBase is an online database to access, visualize and download genomic data of salmonids (*S. salar*, *S. trutta*, *O. mykiss*, *O. kisutch* (Walbaum, 1792), *Salvelinus alpinus* (Linnaeus, 1758)) (Samy et al., 2017). Guidelines for *de novo* or graph-based inference methods for orthologous gene identification were also proposed, and several pipelines are available for automating this procedure (see Kapli et al., 2020).

Phylogenomic reconstruction methods are of two kinds: methods based on whole-genome features (such as SNPs), and methods based on primary sequences (such as mitogenomes). First methods are, however, sensitive to hidden paralogy, horizontal gene transfert or tree reconstruction artifacts (Delsuc et al., 2005; Philippe et al., 2005). For these reasons, the second methods, having already a substantial methodological background, are thus currently preferred and recommended (Delsuc et al., 2005; Philippe et al., 2005; Kapli et al., 2020). These latter phylogenomic methods have, however, also their own sets of problems related to phylogenomic dataset construction and inference. Although NGS technologies have greatly improved sequence quality, they also led to the amplification of data errors that are manually intractable because of the amount of data generated (Philippe et al., 2017). Despite the development of numerous custom scripts, no consensus has yet been found regarding the quality controls that should be included in phylogenomic dataset construction (Philippe et al., 2017). A special care should be then brought to the data assembly to avoid frameshift errors (sequencing or annotation errors) for protein-coding genes, and/or contaminant sequences (commensals/symbionts, parasites, gut contents in animals, environmental sources or experimental errors) (Philippe et al., 2017; Kapli et al., 2020). Contaminants can be identified and excluded based on the GC content of sequences, read coverage and taxonomy of sequence similarity matches (Kapli et al., 2020). Data alignment is also a challenging task since alignment errors would hamper phylogenetic inferences. It is thus crucial to identify the homologous nucleotides or amino-acid residues at every position of the sequences (Philippe et al., 2017; Kapli et al., 2020). Alignment methods can be classified into several categories, and for each category, a panel of softwares is available (for a review, see Philippe et al., 2017; Kapli et al., 2020). Lastly, the choice of phylogenetic reconstruction methods constitutes one of the most essential criteria to avoid phylogenomic result inconsistency. Character-based methods, and in particular the probabilistic maximum likelihood and Bayesian methods, are based on an explicitly evolutionary model of sequences (Delsuc et al., 2005; Kapli et al., 2020). The use of the most complex models will thus reduce the probability of inconsistency, except when sequences evolve heterogeneously and are not identically distributed (Kolaczkowski & Thornton, 2004; Delsuc et al., 2005). Research on more realistic models of sequence evolution is a work-in-process (Delsuc et al., 2005; Philippe et al., 2017). Detailed guidelines and a phylogenomic pipeline recently proposed by Kapli et al. (2020) could easily be applied to trout phylogenomic studies.

Last but not least, the raw data generated, considered part of the “Big Data science”, and all steps from data assembly to phylogenetic inference need huge storage requirements (Giribet, 2015). Although some public databases allow to store raw data, assemblies or data matrices, satisfactory repositories are still lacking (Giribet, 2015). Moreover, these amounts of generated genomic data and the complexity in evolutionary models come at a high computational cost (Giribet, 2015). For instance, most heuristic searches with probabilistic methods will be nearly impossible with more than 200 species (Philippe et al., 2005). Faster and more efficient algorithms are thus required to overcome the current and upcoming size of phylogenomic datasets (Giribet, 2015).

Nevertheless, genomics, thanks to a much larger number of loci available, has increased our ability to evaluate the degree of genetic isolation of populations and to detect introgression in many species. For this reason, genomic data can play an important role in species delimitation under many species concepts (Rannala & Yang, 2020). In order to propose clear and objective definition of species, powerful methods, such as coalescent-based species delimitation (Fujita et al., 2012), were developed. Because species can show low genetic divergence but high divergence in other traits, the whole process of lineage diversification cannot be explained by these methods alone (Fujita et al., 2012, Cicero et al., 2021). Some authors advocate that studies aimed at delimiting species are best framed in a more integrative context, by taking into account, notably, morphological and ecological characters as well as life-history traits of taxa across their distribution and habitats (Fujita et al., 2012; Cicero et al., 2021). Indeed, when the taxa in question are of conservation concern, it is important to use multiple lines of evidence into taxonomic decision, even if this evidence can now, in theory, be provided by genomic data (for some examples, see Stanton et al., 2019). Trout systematics will thus benefit from a more integrative view of the taxonomy.

Implications and prospective directions

In conservation biology, taxa identification (taxonomy) and the understanding of their evolutionary relationships (systematics) is fundamental to design efficient biodiversity conservation priorities and management strategies (Allendorf, 2012). In taxonomy and systematics, the central and fundamental unit is the species. Therefore, conservation strategies have long been focused on species. In order to protect trout biodiversity, some authors suggested thus to maintain species names for some trout populations despite discrepancies between morphology and genetics. For instance, *S. letnica* (Karaman, 1924) populations of the Lake Ohrid in the Balkans represent a monophyletic group inside the AD lineage, whereas differences in morphology, intralacustrine distribution, ecology and the season of spawning were observed between populations (Sell & Spirkovski, 2004; Sušnik et al., 2007). In order to preserve the Lake Ohrid biodiversity, Sušnik et al. (2007) recommended, however, to keep the taxonomic epithet *S. letnica* for the endemic Ohrid trout. At a larger geographic scale, *S. marmoratus*, characterized by a marbled-colour pattern, is supposed to inhabit northern and southern parts of the Adriatic Sea drainage, and it is often associated to the mitochondrial MA lineage (Sanz, 2018). However, the marble-colour pattern was also found in some trouts from Norway (Skaala & Solberg, 1997), while MA haplotypes were identified in Central Italy as well as in French, Turkish or Greek populations (Fig. 2). These cases are just some examples among some others underlying the urgent need to revise the trout systematics in an integrative view. In this purpose, it will be crucial to consider all types of diversity (morphology, ecology, genetics, life-history traits, and so on), but also to choose appropriate morphological and genetic/genomic proxies, and taxon sampling (including type specimens) to describe a part of these diversities.

On the one hand, because species is a fundamental unit in conservation and, on the other hand, because less attention has been focused on the definition and delimitation of conservation units from intraspecific diversity, numerous known subspecies are elevated to the species rank for conservation purposes (Isaac et al., 2004; Berrebi et al., 2013; Zachos et al., 2013). Subspecies seem, however, also relevant in biodiversity conservation, with many taxa listed under the International Union for the Conservation of Nature Red List criteria (Gippoliti & Amori, 2007; Braby et al., 2012; IUCN, 2022), and recognized as a taxonomic unit by the International Code of Nomenclature (Article 5.2; https://www.iczn.org/the-code/the-code-online/; Braby et al., 2012). The utility of subspecies in taxonomy and conservation biology has been debated for decades, notably, because subspecies are not defined conceptually with objective criteria allowing their delimitation (Braby et al., 2012). Moreover, discrepancies exist, at the subspecies level, between molecular and phenotypic characters (Phillimore & Owens, 2006; Cicero et al., 2021). Other intraspecific conservation units – e.g., the evolutionary significant units (ESU; Ryder, 1986; Waples, 1991; Dizon et al., 1992; Moritz, 1994; Crandall et al., 2000), the operational conservation units (OCU; Doadrio et al., 1996) or the management units (MU; Taylor & Dizon, 1999) - have been proposed. Several definitions exist to define an ESU, but the most popular one is that proposed by Moritz (1994): an ESU can be defined as populations that are monophyletic for mtDNA and showing significant divergence in nucDNA allele frequencies. An OCU is rather a continuous area limited by geographic boundaries, and inhabited by one or more populations sharing the same genetic pattern (Doadrio et al., 1996), while a MU is for populations of conspecific individuals among which the degree of connectivity is sufficiently low so that each population should be monitored and managed separately (Palsbøll et al., 2006). Some of these units were, in fact, already suggested to manage and conserve natural diversity of Iberian trout populations (Machordom et al., 2000; Almodóvar et al., 2006). Even if the definition of most of these conservation units relies on genetic/genomic data, other types of information (e.g. life history traits, habitat type, phenotype, gene flow pattern, genomic clusters) are proposed to define conservation units (Allendorf, 2012; Hashemzadeh Segherloo et al., 2021). In the near future, genomic approaches could be really appropriate to estimate the level of vulnerability of *Salmo* native populations as well as the extant of threats acting on them.

I cannot agree more with Kottelat & Freyhof (2007) when they say that, despite the innumerable studies on trout, this interest has resulted in very little data usable in the taxonomic framework, unfortunately still today. To cite Kottelat & Freyhof (2007), it is thus more than time that the “hordes” of geneticists and taxonomists studying trout start working together to better preserve natural diversity of trout in its native distribution. This will be probably allowed in a near future thanks to the combination of NGS technologies, a more integrative approach of the trout systematics and the appropriate definition of conservation units.

References

Alexandrou, M. A., B. A. Swartz, N. J. Matzke & T. H. Oakley, 2013. Genome duplication and multiple evolutionary origins of complex migratory behaviour in Salmonidae. Molecular Phylogenetics and Evolution 69: 514-523.

Allendorf, F. W., 2012. Units of conservation. In Allendorf, F. W., G. H. Luikart & S. N. Aitken (eds), Conservation and Genetics of Populations. John Wiley and Sons, Chichester: 316-351.

Almodóvar, A., G. G. Nicola, B. Elvira & J. L. García-Marín, 2006. Introgression variability among Iberian brown trout Evolutionary Significant Units: the influence of local management and environmental features. Freshwater Biology 51: 1175-1187.

Anđelković, S. J., 1989. Tertiary fish of Yugoslavia: a stratigraphic–paleonthologic–paleoecological study. Jugoslavenska akademija znanosti i umjetnosti 38: 1–121.

Antunes, A., P. Alexandrino & N. Ferrand, 1999. Genetic characterization of Portuguese brown trout (*Salmo trutta* L.) and comparison with other European populations. Ecology of Freshwater Fish 8: 194-200.

Antunes, A., R. Faria, S. Weiss & P. Alexandrino, 2001. Complex evolutionary history in the brown trout: Insights on the recognition of conservation units. Conservation Genetics 2: 337-347.

Antunes, A., A. R. Templeton, R. Guyomard & P. Alexandrino, 2002. The role of nuclear genes in intraspecific evolutionary inference: genealogy of the *transferrin* gene in brown trout. Molecular Biology and Evolution 19: 1272-1287.

Apostolidis, A., Y. Karakousis & C. Triantaphyllidis, 1996. Genetic divergence and phylogenetic relationships among *Salmo trutta* L. (brown trout) populations from Greece and other European countries. Heredity 76: 551-560.

Apostolidis, A. P., P. K. Apostolou, A. Georgiadis & R. Sandaltzopoulos, 2007. Rapid identification of *Salmo trutta* lineages by multiplex PCR utilizing primers tailored to discriminate single nucleotide polymorphisms (SNPs) of the mitochondrial control region. Conservation Genetics 8: 1025-1028.

Apostolidis, A. P., M.-J. Madeira, M. M. Hansen & A. Machordom, 2008. Genetic structure and demographic history of brown trout (*Salmo trutta*) populations from southern Balkans. Freshwater Biology 53: 1555-1566.

Aurelle, D. & P. Berrebi, 2001. Genetic structure of brown trout (*Salmo trutta* L.) populations from south-western France: data from mitochondrial control region variability. Molecular Ecology 10: 1551-1561.

Bardakci, F., N. Degerli, O. Ozdemir & H. H. Basibuyuk, 2006. Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. Journal of Fish Biology 68: 36-55.

Behnke, R. J., 1986. About trout. Trout Winter 1986: 42-47.

Bernatchez, L., 2001. The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. Evolution 55: 351-379.

Bernatchez, L., R. Guyomard & F. Bonhomme, 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. Molecular Ecology 1: 161-173.

Bernatchez, L. & A. Osinov, 1995. Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. Molecular Ecology 4: 285-298.

Berrebi, P., C. Tougard, S. Dubois, Z. Shao, I. Koutseri, S. Petkovski & A. Crivelli, 2013. Genetic diversity and conservation of the Prespa trout in the Balkans. International Journal of Molecular Sciences 14 : 23454-23470.

Berrebi, P., V. Caputo Barucchi, A. Splendiani, S. Muracciole, A. Sabatini, F. Palmas, C. Tougard, M. Arculeo & S. Marić, 2019. Brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. Hydrobiologia 826: 209-231.

Birnie-Gauvin, K., E. B. Thorstad & K. Aarestrup, 2019. Overlooked aspects of the *Salmo salar* and *Salmo trutta* lifecycles. Reviews in Fish Biology and Fisheries 29: 749-766.

Bouza, C., J. Castro, L. Sánchez & P Martínez, 2001. Allozymic evidence of parapatric differenciation of brown trout (*Salmo trutta* L.) within an Atlantic river basin of the Iberian Peninsula. Molecular Ecology 10: 1455-1469.

Braby, M. F., R. Eastwood & N. Murray, 2012. The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness?. Biological Journal of the Linnean Society 106: 699-716.

Budy, P., G. P. Thiede, J. Lobón-Cervía, G. Gonzalez Fernandez, P. McHugh, A. McIntosh, L. Asbjørn Vøllestad, E. Becares & P. Jellyman, 2013. Limitation and facilitation of one of the world's most invasive fish: an international comparison. Ecology 94: 356-367.

Cagigas, M. E., E. Vázquez, G. Blanco & J. A. Sánchez, 2002. Phylogeographical lineages in brown trout (*Salmo trutta*): investigating microgeographical differentiation between native populations from Northern Spain. Freshwater Biology 47: 1879-1892.

Casanova, A., S. Heras, A. Abras, M. I. Roldán, C. Bouza, M. Vera, J. L. García-Marín & P. Martínez, 2022. Genomic hatchery introgression in brown trout (*Salmo trutta* L.): development of a diagnostic SNP panel for monitoring the impacted Mediterranean Rivers. Genes 13: 255.

Cicero, C., N. A. Mason, R. A. Jiménez, D. R. Wait, C. Y. Wang-Claypool & R. C. Bowie, 2021. Integrative taxonomy and geographic sampling underlie successful species delimitation. The Auk 138: ukab009.

Cloutier, A., T. B. Sackton, P. Grayson, M. Clamp, A. J. Baker & S. Edwards, 2019. Whole-genome analyses resolve the phylogeny of flightless birds (Palaeognathae) in the presence of an empirical anomaly zone. Systematic Biology 68: 937-955.

Cortey, M., C. Pla & J.-L. García-Marín, 2004. Historical biogeography of Mediterranean trout. Molecular Phylogenetics and Evolution 33: 831-844.

Cortey, M., M. Vera, C. Pla & J.-L. García-Marín, 2009. Northern and Southern expansions of Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. Biological Journal of the Linnean Society 97: 904-917.

Crandall, K. A., O. R. P. Beninda-Emonds, G. M. Mace & R. K. Wayne, 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15: 290-295.

Crespi, B. J. & M. J. Fulton, 2004. Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. Molecular Phylogenetics and Evolution 31: 658-679.

Crête-Lafrenière, A., L. K. Weir & L. Bernatchez, 2012. Framing the Salmonidae family phylogenetic portrait: a more complete picture from increased taxon sampling. PLoS One 7: e46662.

Crivelli, A. J., 2006. *Salmo pallaryi*. The IUCN Red List of Threatened Species 2006: e.T61190A12440688.https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T61190A12440688.en. Downloaded on 05 February 2021.

Cronquist, A., 1978. Once again, what is a species? Beltsville Symposia in Agricultural Researches 3: 3-20.

Delsuc, F., H. Brinkmann & H. Philippe, 2005. Phylogenomics and the reconstruction of the tree of life. Nature Reviews Genetics 6: 361-375.

Díaz-Arce, N., H. Arrizabalaga, H. Murua, X. Irigoien & N Rodríguez-Ezpeleta, 2016. RAD-seq derived genome-wide nuclear markers resolve the phylogeny of tunas. Molecular Phylogenetics and Evolution 102: 202-207.

Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster & J. Sisson, 1992. Rethinking the stock concept: a phylogeographic approach. Conservation Biology 6: 24-36.

Doadrio, I., A. Perdices & A. Machordom, 1996. Allozymic variation of the endangered killifish *Aphanius iberus* and its application to conservation. Environmental Biology of Fishes 45: 259-271.

Doadrio, I., S. Perea & A. Yahyaoui, 2015. Two new species of Atlantic trout (Actinopterygii, Salmonidae) from Morocco. Graellsia 71: e031.

Dong, C., L. Chen, J. Feng, J. Xu, S. Mahboob, K. Al-Ghanim, X. Li & P. Xu, 2016. Genome wide identification, phylogeny, and expression of *Aquaporin* genes in common carp (*Cyprinus carpio*). PLos One 11: e0166160.

Dudu, A., O. Popa, S. E. Georgescu, A. Dinischiotu & M. Costache, 2010. Molecular analysis of Romanian salmonid species based on 16S rRNA and 12S rRNA sequences. Archiva Zootechnica 13: 30-40.

Dudu, A., S. E. Georgescu, O. Popa, A. Dinischiotu & M. Costache, 2011. Mitochondrial 16S and 12S rRNA sequence analysis in four salmonid species from Romania. Acta Zoologica Academiae Scientiarum Hungaricae 57: 233-246.

Elliott, J. M., 1994. Wild brown trout *Salmo trutta*: an important national and international resource. Freshwater Biology 21: 1-5.

Elliott, M., 2018. Preface. In Lobón-Cerviá, J. & N. Sanz (eds), Brown trout: biology, ecology and management. John Wiley and Sons, Chichester: XV-XVI.

Ferguson, A. & F. M. Mason, 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout *Salmo trutta* L. in Lough Melvin, Ireland. Journal of Fish Biology 18: 629-642.

Froese, R. & D. Pauly, 2021. FishBase, The Global Database of Fishes. World Wide Web electronic publication. www.fishbase.org, version (06/2021).

Fujita, M. K., A. D. Leaché,, F. T. Burbrink, J. A. McGuire & C. Moritz, 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends in Ecology & Evolution 27: 480-488.

Fumagalli, L., A. Snoj, D. Jesenšek, F. Ballox, T. Jug, O. Duron, F. Brossier, A. J. Crivelli & P. Berrebi, 2002. Extreme genetic differentiation among the remnant populations of marble trout (*Salmo marmoratus*) in Slovenia. Molecular Ecology 11: 2711-2716.

García-Marín, J. L., N. Sanz & C. Pla, 1998. Proportions of native and introduced brown trout in adjacent fished and unfished Spanish rivers. Conservation Biology 12: 313-319.

García-Marín, J.-L., F. M. Utter & C. Pla, 1999. Postglacial colonization of brown trout in Europe based on distribution of allozyme variants. Heredity 82: 46-56.

Gippoliti, S. & G. Amori, 2007. The problem of subspecies and biased taxonomy in conservation lists: the case of mammals. Folia Zoologica 56: 113-117.

Giribet, G., 2015. New animal phylogeny: future challenges for animal phylogeny in the age of phylogenomics. Organisms Diversity and Evolution 16: 419-426.

Giuffra, E., L. Bernatchez & R. Guyomard, 1994. Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. Molecular Ecology 3: 161-171.

Gorjanović-Kramberger, D. 1891. Palaeoichthyoložki prilozi (Collectae palaeoichthyologicae). Rad Jugoslavenske Akademije Znanosti i Umjetnosti. Knjiga CVI. Razred Matematićko-prirodoslovni 12: 59–129.

Gratton, P., G. Allegrucci, A. Gandolfi & V. Sbordoni, 2013. Genetic differentiation and hybridization in two naturally occurring sympatric trout *Salmo* spp. forms from a small karstic lake. Journal of Fish Biology 82: 637-657.

Gratton, P., G. Allegrucci, V. Sbordoni & A. Gandolfi, 2014. The evolutionary jigsaw puzzle of the surviving trout (*Salmo trutta* L. complex) diversity in the Italian region. A multilocus Bayesian approach. Molecular Phylogenetics and Evolution 79: 292-304.

Griffiths, A. M., D. Bright & J. R. Stevens, 2009. Complete mitochondrial control region sequences indicate a distinct variety of brown trout *Salmo trutta* in the Aral Sea. Journal of Fish Biology 74: 1136-1142.

Guinand, B., M. Oral & C. Tougard, 2021. Brown trout phylogenetics: a persistent mirage towards (too) many species. Journal of Fish Biology 99: 298-307.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk & O. Gascuel, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321.

Guyomard, R., & F. Krieg, 1986. Mise en évidence d'un flux génétique entre populations naturelles de truite fario et souche de repeuplement dans deux rivières de Corse. Bulletin Français de la Pêche et de la Pisciculture 303: 134-140.

Hamilton, K. E., A. Ferguson, J. B. Taggart, T. Tómasson, A. Walker & E. Fahy, 1989. Post-glacial colonization of brown trout, *Salmo trutta* L.: *Ldh-5* as a phylogeographic marker locus. Journal of Fish Biology 35: 651-664.

Hansen, M. M. & V. Loeschcke, 1996. Genetic differentiation among Danish brown trout populations, as detected RFLP analysis of PCR amplified mitochondrial DNA segments. Journal of Fish Biology 48: 422-436.

Hansen, T., P. G. Fjelldal, S. Lien, M. Smith, C. Corton, K. Oliver, J. Skelton, E. Betteridge, J. Doulcan, O. Fedrigo, J. Mountcastle, E. Jarvis, S. A. McCarthy, W. Chow, K. Howe, J. Torrance, J. Wood, Y. Sims, L. Haggerty, R. Challis, J. Threlfall, D. Mead, R. Durbin & M. Blaxter, 2021. The genome sequence of the brown trout, *Salmo trutta* Linneaus 1758 (version 1; peer review: 3 approved) Wellcome Open Research 2021, 6:108.

Hashemzadeh Segherloo, I., J. Freyhof, P. Berrebi, A.-L. Ferchaud, M. Geiger, J. Laroche, B. A. Levin, E. Normandeau & L. Bernatchez, 2021. A genomic perspective on an old question: *Salmo* trouts or *Salmo trutta* (Teleostei: Salmonidae)? Molecular Phylogenetics and Evolution 162: 107204.

Horreo, J. L., 2017. Revisiting the mitogenomic phylogeny of Salmoninae: new insights thanks to recent sequencing advances. PeerJ 5: e3828.

Höss, M., 2000. Neanderthal population genetics. Nature 404: 453-454.

Hughes, L. C., G. Órti, Y. Huang, Y. Sun, C. C. Baldwin, A. W. Thompson. D. Arcila, R. Betancur-R., C. Li, L. Becker, N. Bellora, X. Zhao, X. Li, M. Wang, C. Fang, B. Xie, Z. Zhou, H. Huang, S. Chen, B. Venkatesh & Q. Shi, 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transciptomic and genomic data. Proceedings of the National Academy of Sciences of the United States of America 115: 6249-6254.

Hurst, C. D., S. E. Bartlett, W. S. Davidson & I. J. Bruce, 1999. The complete mitochondrial DNA sequence of the Atlantic salmon, *Salmo salar*. Gene 239: 237-242.

Hutching, J. A. 2014. Unintentional selection, unanticipated insights: introductions, stocking and the evolutionary ecology of fishes. Journal of Fish Biology 85: 1907-1926.

Hynes, R. A., E. J. Duke & P. Joyce, 1989. Mitochondrial DNA as a genetic marker for brown trout, *Salmo trutta* L., populations. Journal of Fish Biology 35:687-701.

Isaac, N. J. B., J. Mallet & G. M. Mace, 2004. Taxonomic inflation: its influence on macroecology and conservation. Trends in Ecology and Evolution 19: 464-469.

IUCN (2022) The IUCN Red List of Threatened Species. Version 2021-3. http://www.iucnredlist.org. Downloaded on 9 March 2022.

Kalayci, G., R. C. Ozturk, E. Capkin & I. Altinok, 2018. Genetic and molecular evidence that the brown trout *Salmo trutta* belonging to the Danubian lineage are a single biological species. Journal of Fish Biology 93: 792-804.

Kanjuh, T., A. Marić, M. Piria, I. Špelić, I. Maguire & P. Simonović, 2020. Diversity of brown trout, *Salmo trutta* (Actinopterygii: Salmoniformes: Salmonidae), in the Danube river basin of Croatia revealed by mitochondrial DNA. Acta Ichthyologica et Piscatoria 50: 292-300.

Kapli, P., Z. Yang & M. J. Telford, 2020. Phylogenetic tree building in the genomic age. Nature Reviews Genetics 21: 428-444.

Karakoukis, Y. & C. Triantaphyllidis, 1990. Genetic structure and differentiation among Greek brown trout (*Salmo trutta* L.) populations. Heredity 64: 297-304.

Keller, I., A. Taverna & O. Seehausen, 2010. A pyrosequencing assay for the rapid discrimination of mitochondrial lineages in the *Salmo trutta* complex. Molecular Ecology Resources 11: 196-199.

Kohout, J., A. Šedivá, A. Apostolou, T. Stefanov, S. Marić, M. Gaffaroğlu & V. Šlechta, 2013. Genetic diversity and phylogenetic origin of brown trout *Salmo trutta* populations in eastern Balkans. Biologia 68: 1229-1237.

Kolaczkowski, B. & J. W. Thornton, 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. Nature 431: 980-984.

Kottelat, M. & J. Freyhof, 2007. Hanbook of European freshwater fishes. Publications Kottelat and Freyhof, Cornol and Berlin.

Lahnsteiner, F. & A. Jagsch, 2005. Changes in phenotype and genotype of Austrian *Salmo trutta* populations during the last century. Environmental Biology of Fishes 74: 51-65.

Lecaudey, L. A., U. K. Schliewen, A. G. Osinov, E. B. Taylor, L. Bernatchez & S. J. Weiss, 2018. Inferring phylogenetic structure, hybridization and divergence times within Salmoninae (Teleostei: Salmonidae) using RAD-sequencing. Molecular Phylogenetics and Evolution 124: 82-99.

Leitwein, M., P.-A. Gagnaire, E. Desmarais, S. Guendouz, M. Rohmer, P. Berrebi & B. Guinand, 2016. Genome-wide nucleotide diversity of hatchery-reared Atlantic and Mediterranean strains of brown trout *Salmo trutta* compared to wild Mediterranean populations. Journal of Fish Biology 89: 2717-2734.

Leitwein, M., B. Guinand, J. Pouzadoux, E. Desmarais, P. Berrebi & P.-A. Gagnaire, 2018. A dense brown trout (*Salmo trutta*) linkage map reveals recent chromosomal rearrangements in the *Salmo* genus and the impact of selection on linked neutral diversity. G3: Genes, Genomes, Genetics 7: 1365-1376.

Levin, B., E. Simonov, S. Rastorguev, E. Boulygina, F. Sharko, S. Tsygankova, B. Gabrielyan, H. Roubeyan, R. Mayden & A. Nedoluzhko, 2018. High-throughput sequencing of the mitochondrial genomes from archived fish scales: an example of the endangered putative species flock of Sevan trout *Salmo ischchan*. Hydrobiologia 822: 217-228.

Levin, B., E. Simonov, B. K. Gabrielyan, R. L. Mayden, S. M. Rastorguev, H. R. Roubenyan, F. S. Sharko & A. V. Nedoluzhko, 2022. Caucasian treasure: genomics sheds light on the evolution of half-extinct Sevan trout, *Salmo ischchan*, species flock. Molecular Phylogenetics and Evolution, 167: 107346.

Li, F., Y. Sun, J. Chen, X. Jiang & S. Zou, 2016. The complete mitochondrial genome of *Salmo trutta fario* Linnaeus (Salmoniformes, Salmoninae). Mitochondrial DNA B 1: 491-492.

Linnaeus, C., 1758. Systema naturæ. Salvius, Holmiae.

Lobón-Cerviá, J., 2018. Princess of the streams: the brown trout *Salmo trutta* L. as aquatic royalty. In Lobón-Cerviá, J. & N. Sanz (eds), Brown trout: biology, ecology and management. John Wiley and Sons, Chichester: 1-13.

Lo Brutto, S., N. Hristovski & M. Arculeo, 2010. Genetic divergence between morphological forms of brown trout *Salmo trutta* L. in the Balkan region of Macedonia. Journal of Fish Biology 76: 1220-1227.

Machordom, A., J. Suárez, A. Almodóvar & J. M. Bautista, 2000. Mitochondrial haplotype variation and phylogeography of Iberian brown trout populations. Molecular Ecology 9: 1324-1338.

Macqueen, D. J. & I. A. Johnston, 2014. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proceedings of the Royal Society, Serie B 281: 20132881.

Marić, S., S. Sušnik Bajec, J. Schöffmann, V. Kostov & A. Snoj, 2017. Phylogeography of stream-dwelling trout in the Republic of Macedonia and a molecular genetic basis for revision of the taxonomy proposed by S. Karaman. Hydrobiologia 785: 249-260.

McKeown, N. J., R. A. Hynes, R. A. Duguid, A. Ferguson & P. A. Prodöhl, 2010. Phylogeographic structure of brown trout *Salmo trutta* in Britain and Ireland: glacial refugia, postglacial colonization and origins of sympatric populations. Journal of Fish Biology 76: 319-347.

Meraner, A., P. Gratton, F. Baraldi & A. Gandolfi, 2013. Nothing but a trace left? Autochthony and conservation status of Northern Adriatic *Salmo trutta* inferred from PCR multiplexing, mtDNA control region sequencing and microsatellite analysis. Hydrobiologia 702: 201-213.

Meyer, A., 1993. Evolution of mitochondrial DNA in fishes. In Hochachka, P. W. & T. P. Mommsen (eds.), Biochemistry and molecular biology of fishes. Elsevier, Amsterdam: 1–38.

Moghadam, H. K., M. M. Ferguson & R. G. Danzmann, 2011. Whole genome duplication: challenges and considerations associated with sequence orthology assignment in Salmoninae. Journal of Fish Biology 79: 561-574.

Moritz, C., 1994. Defining ‘Evolutionarily Significant Units’ for conservation. Trends in Ecology and Evolution 9: 373-375.

Muhlfield, C. C., D. C. Dauwalter, R. P. Kovach, J. L. Kershner, J. E. Williams & J. Epifanio, 2018. Trout in hot water: a call for global action. Science 360: 866-867.

Nedoluzhko, A. V., S. M. Rastorguev, E. Simonov, E. S. Boulygina, F. S. Sharko, S. V. Tsygankova, B. K. Gabrielyan, H. R. Roubenyan & B. A. Levin, 2018a. Two complete mitochondrial genomes of extinct form of the Sevan trout *Salmo ischchan danilewskii*. Mitochondrial DNA B 3: 40-41.

Nedoluzhko, A. V., E. Simonov, S. M. Rastorguev, E. S. Boulygina, F. S. Sharko, S. V. Tsygankova, V. Q. Nguyen, B. K. Gabrielyan, H. R. Roubenyan & B. A. Levin, 2018b. Sequencing of two mitochondrial genomes of endangered form of the Sevan trout *Salmo ischchan aestivalis*. Mitochondrial DNA B 3: 469-471.

Ninua, L., D. Tarkhnishvili & E. Gvazava, 2018. Phylogeography and taxonomic status of trout and salmon from the Ponto-Caspian drainages, with inferences on European brown trout evolution and taxonomy. Ecology and Evolution 8: 2645-2658.

Oleinik, A. G., L. A. Skurikhina & A. D. Kukhlevsky, 2017. The Lactate Dehydrogenase gene *LDH-C1*, a new molecular marker for phylogenetic analysis of salmonid fishes (Salmoniformes: Salmonidae). Russian Journal of Marine Biology 43: 499-502.

Osinov, A. G. & L. Bernatchez, 1996. 'Atlantic' and 'Danubian' phylogenetic groupings of brown trout *Salmo trutta* complex: Genetic divergence, evolution, and conservation. Journal of Ichthyology 36: 723-746.

Parhi, J., P. S. Tripathy, H. Priyadarshi, S. C. Mandal & P. K. Pandey, 2019. Diagnosis of mitogenome for robust phylogeny: a case of Cyprinifomes fish group. Gene 713: 143967.

Palsbøll, P. J., M. Bérubé & F. W. Allendorf, 2006. Identification of management units using population genetic data. Trends in Ecology and Evolution 22: 11-16.

Pasquier, J., C. Cabau, T. Nguyen, E. Jouanno, D. Severac, I. Braasch, L. Journot, P. Pontarotti, C. Klopp, J. H. Postlethwait, Y. Guiguen & J. Bobe, 2016. Gene evolution and gene expression after whole genome duplication in fish: the PhyloFish database. BMC Genomics 17: 368.

Patarnello, T., L. Bargelloni, F. Caldara & L. Colombo, 1994. Cytochome *b* and 16S rRNA sequence variation in the *Salmo trutta* (Salmonidae, Teleostei) species complex. Molecular Phylogenetics and Evolution 3: 69-74.

Perea, S., M. Al Amouri, E. G. Gonzalez, L. Alcaraz, A. Yahyaoui & I. Doadrio, 2020. Influence of historical and human factors on genetic structure and diversity patterns in peripheral populations: Implications for the conservation of Moroccan trout. bioRxiv.

Phillimore, A. B. & I. P. F. Owens, 2006. Are subspecies useful in evolutionary and conservation biology? Proceedings of the Royal Society of London, Series B, Biological Sciences 273: 1049-1053.

Philippe, H. & E. Douzery, 1994. The pitfalls of molecular phylogeny based on four species, as illustrated by the Cetacea/Artiodactyla relationships. Journal of Mammalian Evolution 2: 133-152.

Phillipe, H., E. A. Snell, E. Bapteste, P. Lopez, P. W. H. Holland & D. Casane, 2004. Phylogenomics of Eukaryotes: impact of missing data on large alignments. Molecular Biology and Evolution 21: 1740-1752.

Phillipe, H., F. Delsuc, H. Brinkmann & N. Lartillot, 2005. Phylogenomics. Annual Review of Ecology, Evolution, and Systematics 36: 541-562.

Phillipe, H., D. M. de Vienne, V. Ranwez, B. Roure, D. Baurain & F. Delsuc, 2017. Pitfalls in supermatrix phylogenomics. European Journal of Taxonomy 283.

Phillips, R. B., M. P. Matsuoka, I. Konon & K. M. Reed, 2000. Phylogenetic analysis of mitochondrial and nuclear sequences supports inclusion of *Acantholingua ohridana* in the genus *Salmo*. Copeia 2000: 546-550.

Presa, P., B. G. Pardo, P. Martínez & L. Bernatchez, 2002. Phylogeographic congruence between mtDNA and rDNA ITS markers in brown trout. Molecular Biology and Evolution 19: 2161-2175.

Pustovrh, G., S. Sušnik Bajec & A. Snoj, 2011a. Evolutionary relationship between marble trout of the northern and southern Adriatic basin. Molecular Phylogenetics and Evolution 59: 761-766.

Pustovrh, G., A. Snoj & S. Sušnik Bajec, 2011b. A set of SNPs enabling identification of trouts and their hybrids in *Salmo* genus. Conservation Genetics Resources 3: 147-150.

Pustovrh, G., A. Snoj & S .Sušnik Bajec, 2014. Molecular phylogeny of *Salmo* of the western Balkans, based upon multiple nuclear loci. Genetics Selection Evolution 46: 7.

Rannala B. & Z. Yang, 2020. Species Delimitation. In Scornavacca, C., F. Delsuc & N. Galtier (eds), Phylogenetics in the Genomic Era. No commercial publisher | Authors open access book: 5.5:1–5.5:18.

Razpet, A., S. Sušnik, T. Jug & A. Snoj, 2007. Genetic variation among trout in the river Neretva basin, Bosnia and Herzegovina. Journal of Fish Biology 70: 94-110.

Rezaei, A., 2015. Studies on cytochrome *c* oxidase subunit II gene in *Salmo trutta caspius*. Banat's Journal of Biotechnology 6: 5-12.

Rezaei, A. & S. Akhshabi, 2017. Studies of mitochondrial genomics in *Salmo trutta fario*. Mitochondrial DNA B 2: 307-3018.

Rezaei, A., S. Akhshabi & H. R. Jamalzadeh, 2017. Studies on the mitochondrial genomics in *Salmo trutta caspius* population in three Rivers of South Caspian Sea. Research Journal of Biotechnology 12: 49-61.

Riffel, M., V. Storch & A. Schreiber, 1995. Allozyme variability of brown trout (*Salmo trutta* L.) populations across the Rhenanian-Danubian watershed in southwest Germany. Heredity 74: 241-249.

Rossi, A. R., G. Petrosino, V. Milana, M. Martinoli, A. Rakaj & L. Tancioni, 2019. Genetic identification of native populations of Mediterranean brown trout *Salmo trutta* L. complex (Osteichthyes: Salmonidae) in central Italy. The European Zoological Journal 86: 424-431.

Ryder, O. A., 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution 1:9–10.

Ryman, N. & G. Ståhl, 1980. Genetic changes in hatchery stocks of brown trout (*Salmo trutta*). Canadian Journal of Fisheries and Aquatic Sciences 37: 82-87.

Sahoo, P. K., L. Singh, L. Sharma, R. Kumar, V. K. Singh, S. Ali, A. K. Singh & A. Barat, 2016. The complete mitogenome of brown trout (*Salmo trutta fario*) and its phylogeny. Mitochondrial DNA A 27: 4563-4565.

Saint-Pé, K., M. Leitwein, L. Tissot, N. Poulet, B. Guinand, P. Berrebi, G. Marselli, J.-M. Lascaux, P.-A. Gagnaire & S. Blanchet, 2019. Development of a large SNPs resource and a low-density SNP array for brown trout (*Salmo trutta*) population genetics. BMC Genomics 20: 582.

Samy, J. K. A., T. D. Mulugeta, T. Nome, S. R. Sandve, F. Grammes, P. K. Kent, S. Lien & D. I. Våge, 2017. SalmoBase: an integrated molecular data resource for salmonid species. BMC Genomics 18: 482.

Sanz, N., J.-L. García-Marín & C. Pla, 2000. Divergence of brown trout (*Salmo trutta*) within glacial refugia. Canadian Journal of Fisheries and Aquatic Sciences 57: 2201-2210.

Sanz, N., 2018. Phylogeographic history of brown trout: a review. In Lobón-Cerviá, J. & N. Sanz (eds), Brown trout: biology, ecology and management. John Wiley and Sons, Chichester: 17-63.

Sass, C., W. J. D. Iles, C. F. Barrett, S. Y. Smith & C. D. Specht, 2016. Revisiting the Zingiberales: using multiplexed exon capture to resolve ancient and recent phylogenetic splits in a charismatic plant lineage. PeerJ 4:e1584.

Schenekar, T., E. Lerceteau-Köhler & S. Weiss, 2014. Fine-scale phylogeographic contact zone in Austrian brown trout *Salmo trutta* reveals multiple waves of post-glacial colonization and a pre-dominance of natural versus anthropogenic admixture. Conservation Genetics 15: 561-572.

Sell, J. & Z. Spirkovski, 2004. Mitochondrial DNA differentiation between two forms of trout *Salmo letnica*, endemic to the Balkan Lake Ohrid, reflects their reproductive isolation. Molecular Ecology 13: 3633-3644.

Shedko, S. V., I. L. Miroshnichenko & G. A. Nemkova, 2012. Phylogeny of salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of nuclear *RAG1* gene. Russian Journal of Genetics 48: 575-579.

Shedko, S. V., I. L. Miroshnichenko & G. A. Nemkova, 2013. Phylogeny of salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of mtDNA data. Russian Journal of Genetics 49: 623-637.

Skaala, Ø. & G. Nævdal, 1989. Genetic differentiation between freshwater resident and anadromous brown trout, *Salmo trutta*, within watercourses. Journal of Fish Biology 34: 597-605.

Skaala, Ø. & G. Solberg, 1997. Biochemical genetic variability and taxonomy of a marmorated salmonid in River Otra, Norway. Nordic Journal of Freshwater Research 73: 3–12.

Splendiani, A., M. Giovannotti, P. Nisi Cerioni, M. L. Caniglia & V. Caputo, 2006. Phylogeographic inferences on the native brown trout mtDNA variation in central Italy. Italian Journal of Zoology 73: 179-189.

Splendiani, A., T. Fioravanti, M. Giovannotti, A. Negri, P. Ruggeri, L. Olivieri, P. Nisi Cerioni, M. Lorenzoni & V. Caputo Barucchi, 2016. The effects of paleoclimatic events on Mediterranean trout: preliminary evidences from ancient DNA. PLoS One 11: e0157975.

Splendiani, A., T. Fioravanti, M. Giovannotti, L. Olivieri, P. Ruggeri, P. Nisi Cerioni, S. Vanni, F. Enrichetti & V. Caputo Barucchi, 2017. Museum samples could help to reconstruct the original distribution of *Salmo trutta* complex in Italy. Journal of Fish Biology 90: 2443-2451.

Splendiani, A., F. Palmas, A. Sabatini & V. Caputo Barucchi, 2019. The name of the trout: considerations on the taxonomic status of the *Salmo trutta* L., 1758 complex (Osteichthyes: Salmonidae) in Italy. The European Zoological Journal 86: 432-442.

Snoj, A., B. Marčeta, S. Sušnik, E. Melkič, V. Meglič & P. Dovč, 2002. The taxonomic status of the "sea trout" from the north Adriatic Sea, as revealed by mitochondrial and nuclear DNA analysis. Journal of Biogeography 29: 1179-1185.

Snoj, A., B. Glamuzina, A. Razpet, J. Zablocki, I. Bogut, E. Lerceteau-Köhler, N. Pojskić & S. Sušnik, 2010. Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. Hydrobiologia 651: 199-212.

Snoj, A., S. Marić, S. Sušnik Bajec, P. Berrebi, S. Janjani & J. Schöffmann, 2011. Phylogeographic structure and demographic patterns of brown trout in North-West Africa. Molecular Phylogenetics and Evolution 61: 203-211.

Snoj, A., J. Bravničar, S. Marić, S. Sušnik Bajec, H. Benaissa & J. Schöffmann, 2021. Nuclear DNA reveals multiple waves of colonisation, reticulate evolution and a large impact of stocking on trout in north-west Africa. Hydrobiologia 848: 3389-3405.

Stanton, D. W., P. Frandsen,, R. K. Waples, R. Heller, I.-R. M. Russo, P. A. Orozco-terWengel, C.-E. Tingskov Pedersen, H. R. Siegismund & .M. W. Bruford, 2019. More grist for the mill? Species delimitation in the genomic era and its implications for conservation. Conservation Genetics 20: 101-113.

Suárez, J., J. M. Bautista, A. Almodóvar & A. Machordom, 2001. Evolution of the mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the biogeographical role of the Iberian Peninsula. Heredity 87: 198-206.

Sušnik, S., J. Schöffmann & A. Snoj, 2004. Phylogenetic position of *Salmo* (*Platysalmo*) *platycephalus* Behnke 1968 from south-central Turkey, evidenced by genetic data. Journal of Fish Biology 64: 947-960.

Sušnik, S., J. Schöffmann & S. Weiss, 2005. Genetic verification of native brown trout from the Persian Gulf (Catak Cay River, Tigris basin). Journal of Fish Biology 67: 879-884.

Sušnik, S., I. Knizhin, A. Snoj & S. Weiss, 2006. Genetic and morphological characterization of a Lake Ohrid endemic, *Salmo* (*Acantholingua*) *ohridanus* with the comparison to sympatric *Salmo trutta*. Journal of Fish Biology 68: 2-23.

Sušnik, S., S. Weiss, T. Odak, B. Delling, T. Treer & A. Snoj, 2007. Reticulate evolution: ancient introgression of the Adriatic brown trout mtDNA in softmouth trout *Salmo obtusirostris* (Teleostei: Salmonidae). Biological Journal of the Linnean Society 90: 139-152.

Taylor, B. L. & A. E. Dizon, 1999. First policy the science: why management unit based solely on genetic criteria cannot work. Molecular Ecology 8: S11-S16.

Tošić, A., D. Škraba, V. Nikolić, D. Mrdak & P. Simonović, 2014. New mitochondrial DNA haplotype of brown trout *Salmo trutta* L. from Crni Timok drainage area in Serbia. Turkish Journal of Fisheries and Aquatic Sciences 14: 37-42.

Tougard, C., F. Justy, B. Guinand, E. J. P. Douzery & P. Berrebi, 2018. *Salmo macrostigma* (Teleostei, Salmonidae): nothing more than a brown trout (*S. trutta*) lineage? Journal of Fish Biology 93: 302-310.

Turan, D., M. Kottelat & S. Engin, 2009. Two new species of trouts, resident and migratory, sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae). Ichthyological Exploration of Freshwaters 20: 333-364.

Turan, D., G. Kalaycı, Y. Bektaş, C. Kaya & E. Bayçelebi, 2020. A new species of trout from the northern drainages of Euphrates River, Turkey (Salmoniformes: Salmonidae). Journal of Fish Biology 96: 1454-1462.

Vera, M., M. Cortey, N. Sanz & J.-L. García-Marín, 2010. Maintenance of an endemic lineage of brown trout (*Salmo trutta*) within the Duero river basin. Journal of Zoological Systematics and Evolutionary Research 48: 181-187.

Vera, M., I. Sourinejad, C. Bouza, R. Vilas, A. Pino-Querido, M. R. Kalbassi & P. Martínez, 2011. Phylogeography, genetic structure, and conservation of the endangered Caspian brown trout, *Salmo trutta caspius* (Kessler, 1877), from Iran. Hydrobiologia 664: 51-67.

Vera, M., J.-L. García-Marín, P. Martinez & C. Bouza, 2015. Phylogenetic diversity within the endemic brown trout Duero lineage: implications for conservation and management. Marine and Freshwater Research 66: 1066-1071.

Vera, M., C. Bouza, A. Casanova, S. Heras, P. Martínez & J. L. García-Marín, 2019. Identification of an endemic Mediterranean brown trout mtDNA group within a highly perturbed aquatic system, the Llobregat River (NE Spain) Hydrobiologia 827: 277-291.

Vladimirov, V. I., 1946. The trout from the diatomite deposits of the Pliocene. Doklady Akademiâ Nauk Armânskoj SSR 4: 123–128.

Vladimirov, V. I. 1948. Armenian brook trout and its relations to other representatives of the genus *Salmo*. Trudy of the Sevan Hydrobiological Station 10: 87–178.

Vuorinen, J. & J. Piironen, 1984. Electrophoretic identification of Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 41: 1834-1837.

Wandeler, P., S. Smith, P. A. Morin, R. A. Pettifor & S. M. Funk, 2003. Patterns of nuclear DNA degeneration over time - a case study in historic teeth samples. Molecular Ecology 12: 1087-1093.

Waples, R. S., 1991. Pacific salmon, *Onchorhynchus* spp., and the definition of “species” under endangered species act. Marine Fisheries Review 53: 11-22.

Weiss, S., A. Antunes, C. Schlötterer & P. Alexandrino, 2000. Mitochondrial haplotype diversity among Portuguese brown trout *Salmo trutta* L. populations: relevance to the post-Pleistocene recolonization of northern Europe. Molecular Ecology 9: 691-698.

Wheeler, Q. D., 2001. Systematics, overview. Encyclopedia of Biodiversity 5: 569-588.

Wiens, J. J., 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Systematic Biology 52: 528-538.

Zachos, F. E., M. Apollonio, E. V. Barmann, M. Festa-Bianchet, U. Gohlich, J. C. Habel, E. Haring, L. Kruckenhauser, S. Lovari, A. D. McDevitt, C. Pertoldi, G. E. Rossner, M. R. Sanchez-Villagra, M. Scandura & F. Suchentrunk, 2013. Species inflation and taxonomic artefacts-A critical comment on recent trends in mammalian classification. Mammalian Biology 78: 1-6.

Acknowledgements

The present review is a follow-up to a presentation done as invited speaker at the "Associazione Italiana Ittilogi Acque Dolci" thematic congress that took place in Cagliari (Sardinia, Italy) in September 2019. For this reason, I would like to thanks the organizers, Andrea Sabatini and Francesco Palmas (Università degli Studi di Cagliari, Sardinia, Italy) for their kind invitation and their warm welcome. I am also grateful to Bruno Guinand (Institut des Sciences de l'Evolution de Montpellier, France) who encouraged me to write this review. Thanks are also expressed to Andrea Splendiani and Vicenzo Caputo Barucchi (Università Politecnica delle Marche, Ancona, Italy), Aleš Snoj (University of Ljubljana, Slovenia) and Jose-Luis García-Marín (Universitat de Girona, Spain) who provided some trout tissue samples. I also warmly thank Andrea Splendiani and two anonymous reviewers for their helpful comments on the manuscript.

Data availability

Mitogenome sequences that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/ under the accession numbers MW632104 to MW632113.

Funding

The French Government (CNRS) supported this study. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics declarations

Conflict of interest

There is no conflict of interest concerning the manuscript.

Ethical approval

New DNA sequences were obtained from DNA stored at – 20 °C or fin clips stored in ethanol 96% and collected in the frame of previous studies. Therefore, no specific permissions were required for locations and activities involved in this study.

Figure legend

Fig. 1 Number of publications dealing with trout systematics found in public reference databases (Web of Sciences, Google Scholar, PubMed) in December 2021 using the words "*Salmo*" and "phylogeny", "systematics" or "diversity". A reference was considered when, at least, two *Salmo* species or *S. trutta* evolutionary lineages were included in the studied dataset, and phylogenetic relationships were represented by a tree or a network. The list of references considered is in Table S1. MtDNA, mitochondrial DNA; nucDNA, nuclear DNA; NGS, next-generation sequencing technologies.

Fig. 2 Approximate native distribution of the nine *Salmo trutta* mitochondrial DNA evolutionary lineages based on references listed in Table S1.

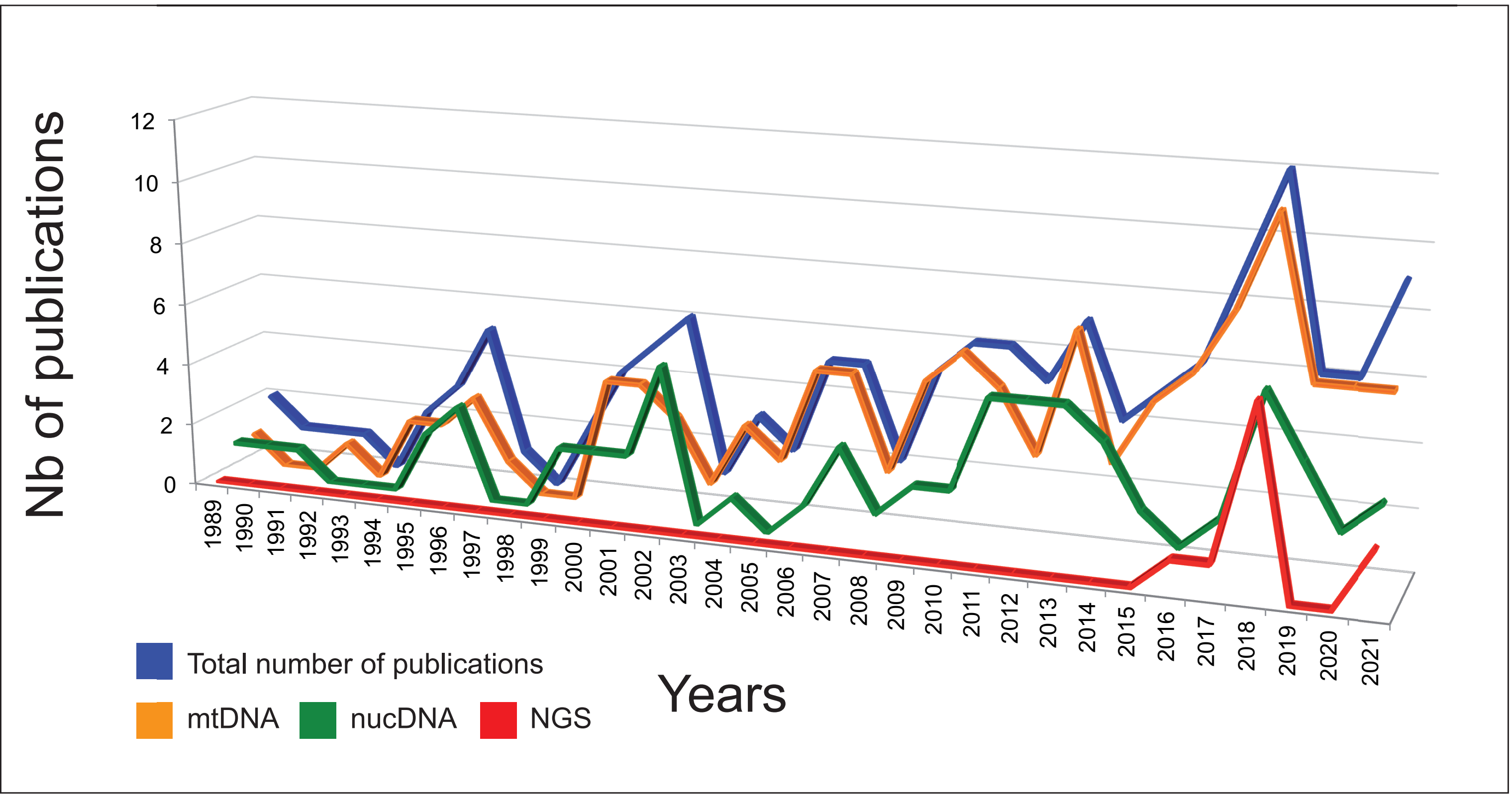
Fig. 3 Maximum likelihood phylogenetic trees reconstructed from the mitochondrial control region (A), the mitochondrial cytochrome *b* gene (B), the concatenation of these two mitochondrial regions (C) and the complete mitogenome (D) using PhyML v3 (Guindon et al., 2010). Informative sites (IS) are indicated in brackets. Numbers at nodes are for bootstrap percentages (BP≥50%). Black circles indicate nodes with BP = 100%. White circles are for node with BP < 50%. Acquisition of new molecular data was done according to Tougard et al. (2018). Sample list and information related to phylogenetic analyses are in Table S3. AD, Adriatic; AT, Atlantic. DA, Danubian; DU, Duero; MA, marble; ME, Mediterranean; NA, North African.

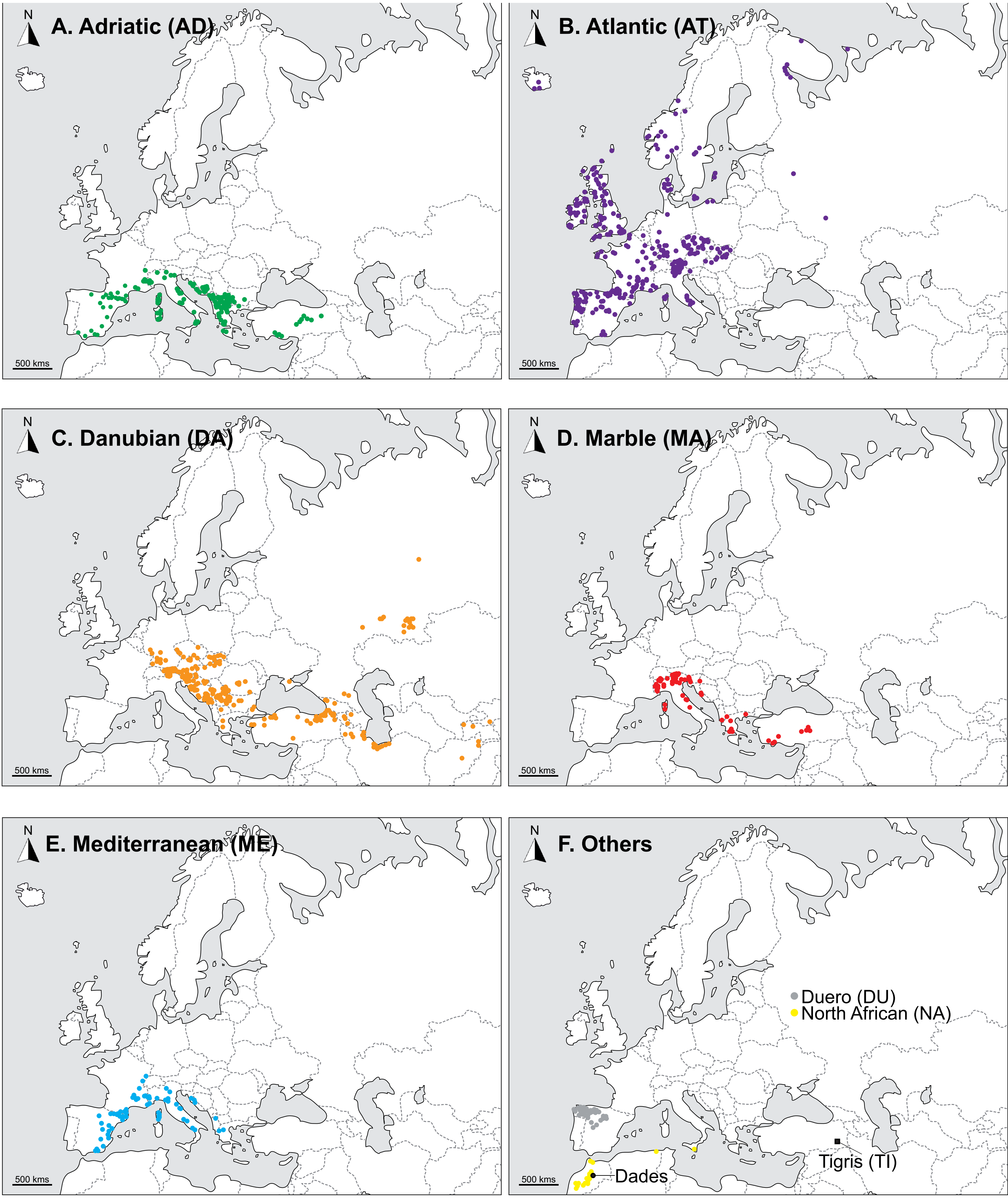
Supplementary information

Table S1 Detailed list of references taken into account for Figures 1 and 2.

Table S2 Relationships between evolutionary lineages and morphospecies based on genetic studies.

Table S3 Details on models of sequence evolution and sampling (accession number, locality and reference) used for phylogenetic reconstruction with PhyML v3 (Guindon et al., 2010).


Nb of publications
12
10
8
6
4
2
0
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
Years
Total number of publications
mtDNA
nucDNA
NGS


A. Adriatic (AD)

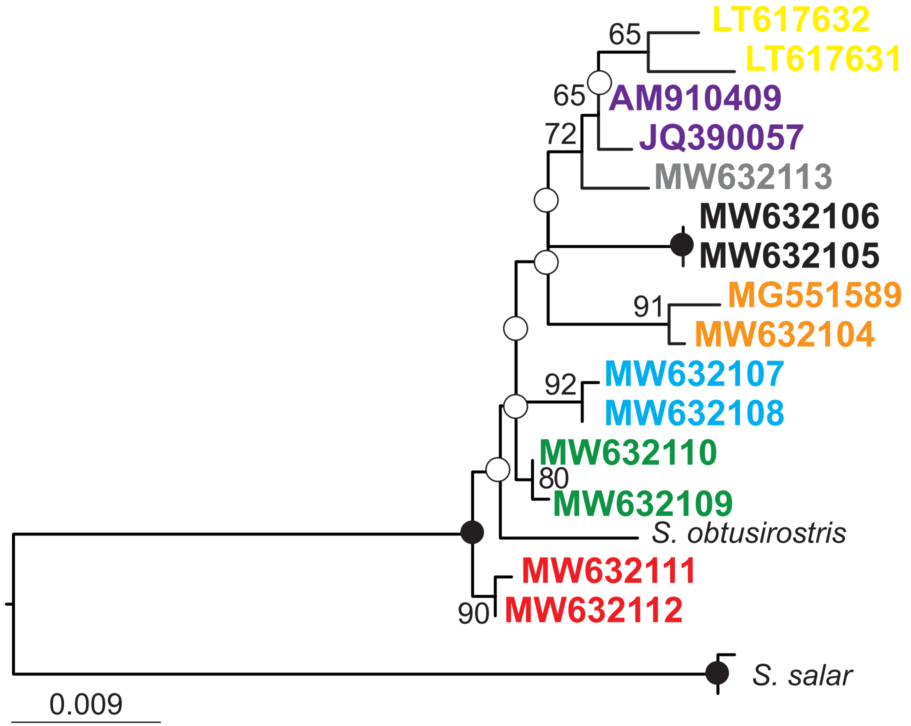
B. Atlantic (AT)

C. Danubian (DA)

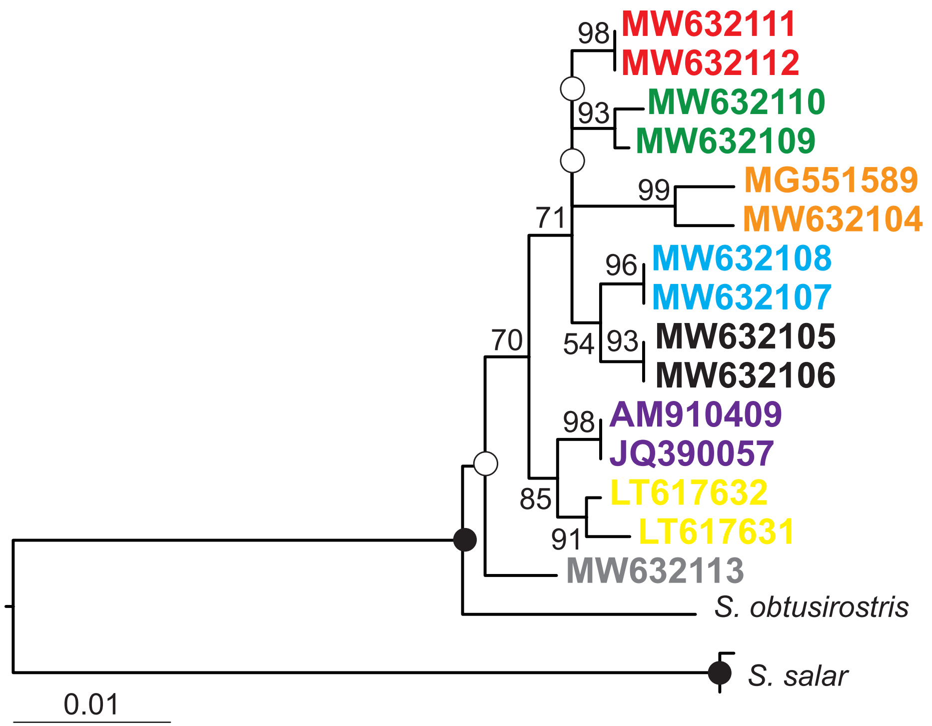
D. Marble (MA)

E. Mediterranean (ME)

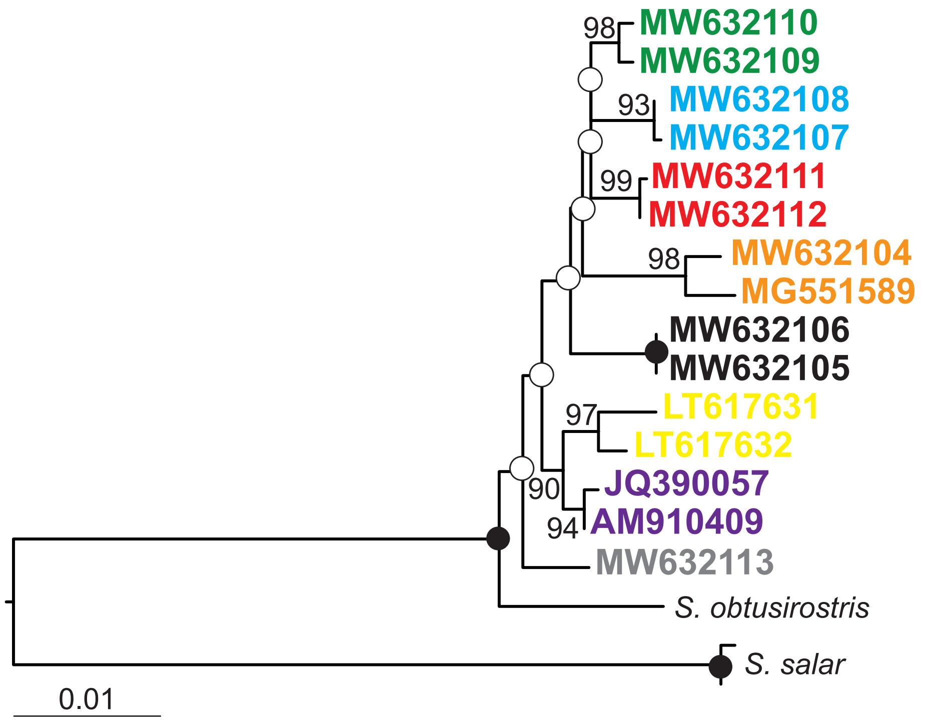
F. Others



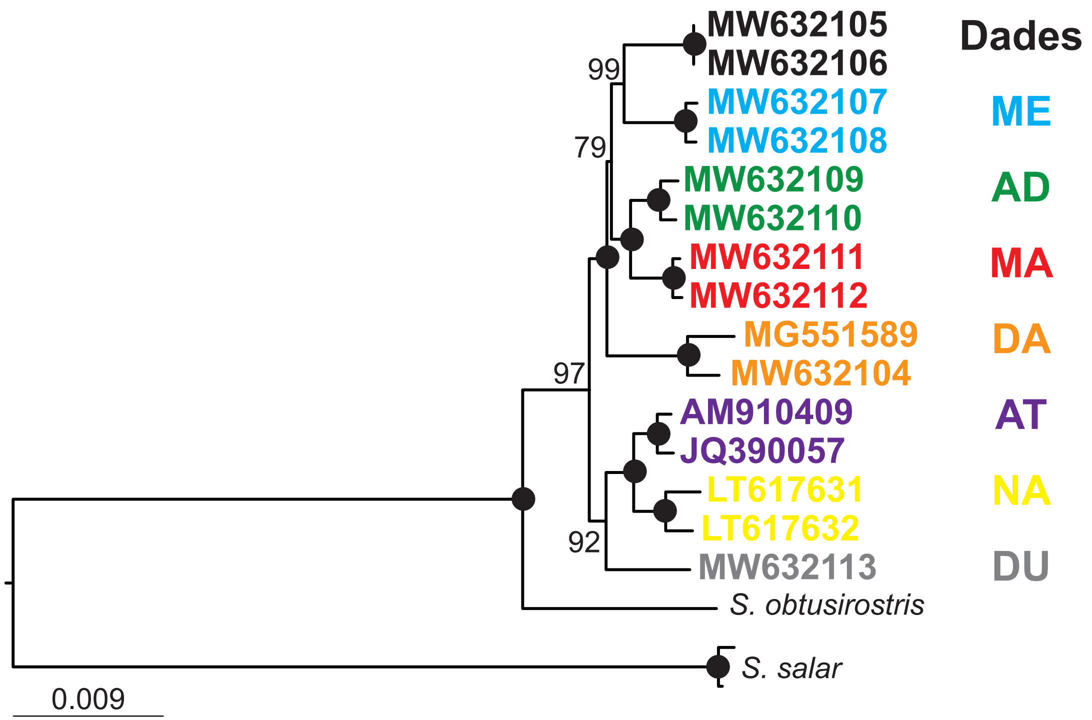
A. Control Region (28 IS)



B. Cytochrome *b* (30 IS)



C. Control Region + Cytochrome *b* (58 IS)



D. Complete Mitogenome (387 IS)