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# Brown trout phylogenetics: a persistent mirage towards (too) many species

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“... such abundant literature and insightful studies may be little more than a mirage”

J. Lobón-Cerviá (2018)

*The mirage surrounding one extensively studied species*

Because of its socio-economic importance in fisheries or as highly-prized sport species, the brown trout, *Salmo trutta* (Linnaeus, 1758) has acquired one iconic status. A statement commonly done about this umbrella species affected by habitat degradation is that trout is a highly polytypic Pan-European species and/or species complex naturally distributed over >1 million km<sup>2</sup>, actually ranging from northern continental Europe and Iceland to North Africa, and extending eastward perhaps to northern foothills of Himalaya in Kyrgyzstan and Tajikistan (e.g. Lobón-Cerviá, 2018). The diversity of phenotypes and life histories has motivated the description of numerous taxonomic units, often ranked as species and subspecies. The number of trout within the *S. trutta* complex varies considerably among authors. It spans from 23 (International Union for Conservation of Nature [IUCN], 2020) to generally 30-35 (Kottelat & Freyhof, 2007; Sanz, 2018), and to more than 50 or 60 (Behnke, 1986; Froese & Pauly, 2019; Jonsson & Jonsson, 2011). The delimitation of trout taxa by ichthyologists was certainly first impacted by poor awareness of phenotypic plasticity and speciation as a process, then based on species concepts based on diagnosability, while these taxa are not reproductively isolated and contradict Mayr's (1942) biological species concept. Reliance in “non-adaptive taxonomic types” (Savvaitova, 1995) and in supremacy of one or other species concept could have participated to the belief that one or a huge number of taxa are existing, while obscuring the very information that provide insight in the diversification process (e.g., Willis, 2017). Trout may also have suffered taxonomic inflation (i.e., the recognition of unnecessary taxa; Isaac *et al.*, 2004) in order to support management and conservation decisions.

Authors interested in trout however agree on a point: the complex is complex and the rise of molecular phylogeny during the 80's tried to address this issue to gain

understanding in both the phylogeny of the *Salmo* genus and the phylogeny of the brown trout, thus in their origin, diversification and other aspects of their evolutionary history. The brown trout complex received an interest not found in any other (Pan-)European fish 'species' so far. Indeed, outside population genetics issues that target one single 'species' or – generally speaking – a single operational taxonomic unit (OTU), a literature review estimated the number of original phylogeographic and phylogenetic studies considering at least two OTUs – clades, lineages or (sub)species – to approx. 130 (Fig. 1). Such interest should have led to significant improvements of knowledge, including taxonomic progress.

While a review of knowledge based also on over 100 studies has been already provided by Sanz (2018), we however believe we are facing a mirage of extensive knowledge in trout. If we do not oppose to contents of her work, a difference between Sanz's (2018) review and this opinion piece could be framed in the well-known 'splitter-lumper' dichotomy. Hereby, our goal is to be 'splitters' in order to stress that the genealogical relationships resulting from speciation within the brown trout complex remains unfortunately neglected. Otherwise, works as summarized by Sanz (2018) *rather* depict 'lumping' in which most molecular data produced to help for the delimitation of trout species or another taxonomic entity are aggregated to one existing reference, *S. trutta* sensu stricto (hereafter, StSS) whose origin is offered below. This opinion piece hence suggests to reconsider the outcomes and to come back on some issues that initially motivated trout molecular studies, i.e. providing a sound phylogenetic frame and the evolutionary history of described taxa, before to establish, redefine or improve – or not - operational criteria necessary to species delimitation.

#### *Thirty years of research in few lines: what is really known?*

If former allozyme studies anticipated the rise of (mt)DNA studies in trout and identified phylogenetic/phylogeographic signals (reviews in Ferguson, 1989; Guyomard, 1989; García-Marín *et al.*, 1999), works by Bernatchez *et al.* (1992), Bernatchez and Osinov (1995), and Bernatchez (2001) represent the main first attempts to investigate trout diversity

using sequencing molecular tools in a Pan-European perspective. Since these works, main observations might be briefly summarized:

- (1)** The five original mtDNA StSS lineages reported in Bernatchez *et al.* (1992) and Bernatchez (2001) – namely Atlantic, Danubian, Mediterranean, Adriatic and marmoratus (Fig. 2) – still anchored most studies in the field, but, their respective distribution area became more complex, resulting in the co-occurrence of mtDNA clades in some areas (e.g., Italy, Corsica, Balkans) rather than their allopatric distribution, as initially described (Sanz, 2018 for review);
- (2)** Few other StSS lineages have been added to the original ones in Spain (Duero: Suárez *et al.*, 2001), Turkey (Tigris: Bardakci *et al.*, 2006; Sušnik *et al.*, 2005), Morocco (Dades; Snoj *et al.*, 2011) and Northern Africa (Tougard *et al.*, 2018) (Fig. 2). Sub-lineages related to the Atlantic lineage have been reported in Central Europe (e.g. Cortey *et al.*, 2009);
- (3)** Some species within the complex have lost their initial status and now related to one of the original StSS clade (Kalayci *et al.*, 2018; McKeown *et al.*, 2010; Sanz, 2018; Snoj *et al.*, 2010; Sušnik *et al.*, 2007a; Tougard *et al.*, 2018). High rate of invalid taxa is not surprising in trout (Jonsson & Jonsson, 2011), and in salmonids in general (e.g. Adams & Maitland, 2007). However, the problem goes beyond taxon names and affects the understanding of evolutionary history. For example, Lobón-Cerviá (2018) reports a possible synonymy between *S. macrostigma* and *S. cettii*, when Tougard *et al.* (2018) questioned the reality of *S. macrostigma*. Authors proposed *S. cettii* as belonging to the Adriatic StSS (e.g., Gratton *et al.*, 2014), while Tougard *et al.* (2018) linked *S. macrostigma* to the North African StSS (Fig. 2). One or two species? One or two evolutionary histories? What is/are the realised distribution(s)? Confusion reigns. Furthermore, some OTUs should be hybrids (Razpet *et al.*, 2007; McKeown *et al.*, 2010; Gratton *et al.*, 2014). Otherwise, mtDNA data confirmed the classification of some species outside StSS: *S. obtusirostris* restricted to the western Balkans (Snoj *et al.*, 2002) and *S. ohridanus* endemic to Lake Ohrid (Phillips *et al.*, 2000; Sušnik *et al.*,

2006), with additional support coming from nuclear data (e.g. Pustovrh *et al.*, 2014; Lecaudey *et al.*, 2018). A proposal to erect the marmoratus lineage as a species outside StSS has been made by Pustovrh *et al.* (2014) based on nuclear data, but not thoroughly validated so far.

**(4)** One origin in the Middle East or/and Mesopotamia is postulated with a probable colonization of northern Europe by the Caspian and the Black Sea, and a colonization of Southern Europe following a Mediterranean route, southern of the Anatolian plateau (Bardakci *et al.*, 2006 and references therein). This point is probable and evidence also comes from comparisons with other species groups (e.g. *Squalius* and *Chondrostoma*; Durand *et al.*, 2002, 2003). The phylogeny presented in Fig. 2 based on StSS mtDNA haplotypes captured little of such information, placing in this case all the western European lineages (Duero, Atlantic and North African) at the root of the mtDNA brown trout phylogeny. It thus emphasizes one Atlantic refuge/Western European for European fish (e.g. Bryja *et al.*, 2010; Culling *et al.*, 2006; Durand *et al.*, 1999) that has been discussed in trout (e.g. Sanz 2018) and Atlantic salmon (*S. salar*; e.g., Finnegan *et al.*, 2013). A refuge is however not an origin. MtDNA taxonomic frames provide interesting but incomplete versions of evolutionary histories (Dellicour & Flot, 2018; Toews & Brelsford, 2012), and no Pan-European nuclear-DNA study is available in trout so far. Sanz (2018) reviewed and proposed mtDNA estimates for divergence time among *S. ohridanus*-*S. obtusirostris* and *S. trutta* that rarely exceed 2 Myr (i.e. Early Pleistocene, concordant with the oldest fossil attributed to *S. trutta*; Vladimirov, 1946). However, recent fossil-calibrated, nuclear estimates provided divergence times around 4-6 Myr between brown trout and *S. ohridanus*-*S. obtusirostris*, and divergence with Atlantic salmon dating back from the Middle Miocene (15-10 Myr) (Crête-Lafrenière *et al.*, 2012; Lecaudey *et al.*, 2018). Unfortunately, for both mtDNA and nuclear estimates, this did not extend to other OTUs than those reported in Fig. 2.

**(5)** Finally, if we emphasize the lack of updated molecular phylogeny encompassing most proposed trout taxa in this piece, no complete morphology-based phylogeny has

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been proposed either. Only partial attempts including few taxa have been produced (e.g., Salmanov & Dorofeyeva, 2001). Correlations between molecular and morphological observations supporting taxonomy resulted in mixed results (see Sanz, 2018; Delling *et al.*, 2020). Further investigations are requested to understand the molecular underpinnings of observed phenotypic and life-history differences that led to trout biocomplexity. To date, only Jacobs *et al.* (2018) really addressed this issue at a genomic scale for the *S. trutta*/*S. ferox* 'species' pair.

The initial frame that focused on StSS is thus valid, now better evaluated, and it has been enriched by additional lineages and better delineation of relationships to few other taxa. It however remains mostly dependent on uniparentally inherited mtDNA data (Fig. 1). Overall, the number of supported taxa and more importantly their relationships, their rate and patterns of diversification, their colonization histories, and their links to phenotypic diversification that support taxonomic descriptions remain obscure. Deep knowledge is a mirage and we may question if scientists built a complex to not face their icon.

#### *Two more frames*

If a coherent phylogenetic frame is still missing, two other frames strongly cohabit in trout molecular studies: one concerns species definition, taxonomy and their associated revisions (*supra* and Fig. 2), while a second is sounded in conservation issues – often crucial in trout - and should be 'taxonomy independent' (Splendiani *et al.*, 2019; see also Stanton *et al.*, 2019). These two frames are generally considered fundamental to design efficient biodiversity conservation priorities and management strategies (Allendorf, 2012; Eizaguirre & Baltazar-Soares, 2014), but their uncoupling has been promoted by some agencies (e.g. Haig *et al.*, 2006). Briefly said, operational criteria to species delimitation should not be confused with operational criteria for conservation (e.g. Raposo *et al.*, 2020). In trout, the inappropriateness of considering the "old" zoogeographic frame for conservation and the preservation of its evolutionary diversity has been already discussed (Antunes *et al.*, 2001; Apostolidis *et al.*, 2011; McKeown *et al.*, 2010; Schenekar *et al.*, 2014). Its use remains

unfortunately pregnant while focus on local (meta)populations could be advertised to prioritize issues that are prompted to more efficient management and deal with biological characteristics, ecological and/or evolutionary potentials (Antunes *et al.*, 2001; Splendiani *et al.*, 2019). However, because species is the only recognized unit to frame conservation policies and because rare and endemic species rather than widely distributed ones are favoured, the two frames remain unfortunately lumped in trout by putting the species status or the lineage at the conservation forefront. Numerous trout studies thus focused on events occurring at temporal and/or spatial scales that are poorly relevant to conservation management and quite descriptive at the level of OTUs (e.g. updated distribution of haplotypes pointing new records or levels of diversity that are not related to life history or ecological data).

Other approaches that consider “the best of all worlds” might be substituted (e.g., Mee *et al.*, 2015). Pillars of sands should be avoided, and, by abusively mixing the two frames, the goal to address the big phylogenetic picture, to reduce taxonomic uncertainty and to better understand the processes that shaped the brown trout complex has been progressively lost. This big picture has perhaps become still more difficult to address as new putative trout species have been described over the last fifteen years, notably in Morocco (Delling & Doadrio, 2005; Doadrio *et al.*, 2015) and the Anatolian and Taurus regions, including Tigris and Euphrates drainages (e.g., Turan *et al.*, 2011, 2014, 2017, 2020). These are areas where novel StSS lineages were described (Dades, Tigris, see above), and perhaps close to the putative centre of origin of brown trout for Anatolian trout. We thus do not currently know how these taxa are related, if they clustered within or outside StSS (could be considered as valid taxa or not, *sensu* Galtier, 2019), and which portion of trout evolutionary history they may allow to better understand.

#### *The future*

The next step in trout study is to handle the full complexity of phylogenetic relationships to learn how trout diversity arises and changes. Picking up only previously described ‘species’



one by one for further molecular comparison with StSS has become a dead-end. After decades of studies using maternally inherited mtDNA markers or - more generally - reduced marker sets, “going large” with next generation sequencing (NGS) technologies that provide access to thousands of nuclear loci (e.g. single nucleotide polymorphisms [SNPs]) and genomic features (e.g., Wellenreuther *et al.*, 2019) to delineate the underlying genealogy is undoubtedly the key in trout. Panels of genome-wide distributed SNP markers have proved suitable for phylogenomic analysis (Leaché & Oaks, 2017) and updated the understanding about the patterns and the mechanisms of lineage diversification, together with species delimitation (e.g., Chan *et al.*, 2020; Díaz-Arce *et al.*, 2016; Hughes *et al.*, 2020; Leaché *et al.*, 2014; Song *et al.*, 2017). This includes fish species complex in sticklebacks (Guo *et al.*, 2019), roaches (Baumsteiger *et al.*, 2017), cichlids (e.g. Willis, 2017); suckers (Bangs *et al.*, 2020), topminnows (Duvernell *et al.*, 2019), and coregonids (*Coregonus ardeti* species flock; Ackiss *et al.*, 2020). Based on several thousands of polymorphisms, Copus *et al.* (2018) proposed to reduce the *Gila robusta* complex to a single species. NGS data also provided with better assessment of origin, colonization routes, demographic histories including admixture and introgression, and/or identification of stage of divergence during the speciation process in diverse fish species (e.g., Jeffries *et al.*, 2016; Fang *et al.*, 2018; Lucek *et al.*, 2018; Ravinet *et al.*, 2018; Rougemont & Bernatchez, 2018). For example, the pattern of reticulate evolution between *S. obtusirostris* and the Adriatic clade of *S. trutta* described by Sušnik *et al.* (2007b) should receive in depth evaluation. Trout, however, covers perhaps more putative OTUs than any other examples, and OTU-rich taxa appeared yet especially challenging to investigate these issues (e.g. Dincă *et al.*, 2019; Chambers & Hillis, 2020). Actually, it is recognized that a limited number of sampled genomes per OTU may provide access to substantial information, including “evolutionary process connectivity” (i.e. how contemporary observed connectivity and diversity reflect long term selective/adaptive and demographic history, including patterns of genetic admixture and speciation; Gagnaire, 2020). The ever-decreasing cost to access genome data would probably no longer constrains this issue.

The release of important genomic resources such as the Atlantic salmon (Lien *et al.*, 2016; GenBank: GCA\_000233375.4) and brown trout (GenBank: GCA\_901001165.1) genomes provide substrates to progress in trout phylogeny. SNPs are accessible in *S. trutta*, but mostly used however in a population genomics framework (e.g., Andersson *et al.*, 2017; Drywa *et al.*, 2013; Leitwein *et al.*, 2016; Saint-Pé *et al.*, 2019). Jacobs *et al.* (2018) used SNPs to document the divergence in the *S. trutta/S. ferax* 'species' pair occurring in Loch Maree catchment (Scotland), but this did not represent a phylogenetic study. Only Lecaudey *et al.* (2018) used SNPs in a phylogenetic context, but they were interested in higher phylogenetic relationships than the ones sustaining the brown trout complex.

The production of a more complete phylogeny including many taxa seem possible, but challenges have to be overcome. This includes the relatively young history of divergence for some OTUs, taxon sampling, choosing marker types, or identification of orthologous/paralogous genes (single-copy vs gene duplicates) in trout. As paralogs impede investigations in salmonids (Lecaudey *et al.*, 2018), databases (Pasquier *et al.*, 2016; Samy *et al.*, 2017) and tools providing guidelines for *de novo* or graph-based inference methods for orthologous gene identification have been developed (Kapli *et al.*, 2020; Smith & Hahn, 2020). Many of them seem however already identified owing to transcriptome assembly in the brown trout (Carruthers *et al.*, 2018) and consolidate resources to address this challenging key task. While the number of markers cannot be considered sufficient to accurate phylogeny estimation (Pyron, 2015) and their nature may lead to conflicting results (e.g. Reddy *et al.*, 2017), results by Collins and Hrbek (2018) suggested good performance of SNP data coming from restriction-site-associated DNA (RAD) analysis to address relatively recent and shallow phylogenies. However, relevance of models behind RAD-based and other SNP analysis remains to be considered further (Bravo *et al.*, 2019). Among methods, the multispecies coalescent (MSC) emerged to tackle complex phylogenies in the age of increasing access to genomic data (e.g., Rannala & Yang, 2017). MSC is a statistical framework based on coalescent theory that explicitly

accounts for gene tree discordance due to incomplete lineage sorting and was initially devoted to identify ‘species’ as entities between which genetic exchanges have been negligible. It may now address more complex demographic modelling during speciation (e.g., Hey *et al.*, 2018; Long & Kubatko, 2018), have been extended to phylogenetic network as the multispecies network coalescent (e.g., Wen *et al.*, 2016; Zhang *et al.*, 2018), and recognized as “broadly” efficient (Hillis, 2019; Kapli *et al.*, 2020; Rannala & Yang, 2017). Guidelines and tools regarding coalescent-based species delimitations and further testing procedures are regularly enriched (e.g., Hey and Pinho, 2012; Carstens *et al.*, 2013; Flouri *et al.*, 2020; Jones, 2017; Kapli *et al.*, 2020; Liu *et al.*, 2019; Luo *et al.*, 2018). In fish, ancestral geographic distribution or estimation of divergence times among closely related lineages benefited of such new statistical modelling approaches in sticklebacks (Fang *et al.*, 2020). In cichlids, Olave and Meyer (2020) illustrated capacities to deal with recent radiation events that might also be present in some lacustrine trout (e.g., *S. ischchan* in Lake Sevan, Armenia, in which four sympatric morphs were recorded; Dadikyan, 1986).

Despite access to statistical procedures and genomic resources, species are hypotheses and not inherently subjectives, because of artificial cut-offs in the process of divergence (e.g. Hey, 2001; De Queiroz, 2007; Pante *et al.*, 2015). MSC – or any other method (e.g. approximate Bayesian computations) - is then not a magic bullet and improving trout phylogeny that describe a speciation process by analyzing genome-wide data sets is not sufficient to establish criteria for taxon delimitation (e.g. Chambers & Hillis, 2020; Stanton *et al.*, 2019; Sukumaran & Knowles, 2017; Willis, 2017). Result accuracy and criteria for taxon delimitation have to be considered in light of complementary knowledge coming from different perspectives, including phenotypic and ecological information (Barley *et al.*, 2018; Campillo *et al.*, 2020; Hillis, 2019; Solís-Lemus *et al.*, 2015; Sukumaran & Knowles, 2017), as well as geographical/distributional consideration (e.g., De Queiroz, 2007; Hillis, 2019, 2020). In the roundtail chub (*G. robusta*) complex, Copus *et al.* (2018) showed that morphological variation reflected phenotypic plasticity and that only molecular variation could offer consistent material for taxa delimitation. Willis (2017) also discussed

the real necessity of species delimitation in peacock cichlids (*Cichla pinima* species complex), because their evolutionary history could not be unambiguously rendered as discrete units. Concurrently, Kautt *et al.* (2018) showed that Midas cichlid species complex from Nicaraguan crater's lakes can be described as independent evolutionary lineages that established differences in morphology, ecological settings and distribution, suggesting the likely existence of criteria for taxa/species delimitation in less than 2000 generations.

'Going large' offers to explore many facets of trout speciation, strengthen the interplay between systematics, phylogeography and speciation genomics (Galtier, 2019), and operate to renew links with conservation issues (Coates *et al.*, 2018; Stanton *et al.*, 2019; Raposo *et al.*, 2020). It may allow for the definition of shared operational criteria for trout (sub-)species delimitations and to set aside a current situation where many trout taxa – while reproductively non-isolated - have been described as tips of one unknown phylogeny. Carstens *et al.* (2013) and others proposed to be conservative when delimiting species and the current situation militates for recognizing only few trout species. Actually only *S. ohridanus* and *S. obtusirostris* seem to have received (relatively) sufficient molecular support as independent taxa. Much work has still to be done and trout from the Anatolian and Taurus regions are certainly the most interesting taxa that might contribute to elucidate the trout phylogenetic puzzle.

Finally, because of the availability of OTUs present only as museum specimens (e.g. *S. pallaryi*), the study of ancient or degraded DNA has also to be more deeply considered in trout. Few studies considered museum specimens, only using mtDNA (Splendiani *et al.* 2016, 2017; Levin *et al.*, 2018; Tougard *et al.*, 2018). Study of nuclear ancient DNA is challenging, but may improve knowledge (Chassaing *et al.*, 2016; Lopez *et al.*, 2020).

#### *In the meantime*

If the bar is too high before to gather extensive genome-wide data, a pragmatic approach remains the study of complete mitogenomes. Trout mitogenomes are recent and now retrieved from both contemporary and archived samples (e.g., Nedoluzhko *et al.* 2018;

Sahoo *et al.*, 2016; Tougard *et al.*, 2018; first *S. salar* mitogenome by Hurst *et al.*, 1999). While mtDNA is uniparentally inherited, produces over-pronounced geographic structuring and is a notable source of mito-nuclear discordance (i.e. produces very partial pictures of connectivity and gene flow among OTUs; Petit & Excoffier, 2009; Toews & Brelsford, 2012), phylogenies using complete mitogenome remain largely indicative of the speciation process and paleogeographic events (Ben Chehida *et al.*, 2020; Jacobsen *et al.*, 2012; Wang *et al.*, 2019; see also Collins & Hrbek, 2018)). Mitogenomes enabled support to complex situations in which specific status was not previously assessed and/or ambiguous (e.g. Beheregaray *et al.*, 2017; Buckley *et al.*, 2018), providing also support for a more integrative and corroborative taxonomy in which morphology and molecular species delineation have been shown to match (Pedraza-Marrón *et al.*, 2019) or not (Vernygora *et al.*, 2018).

In the meantime, we may also ask ourselves if progress in molecular trout studies do not also depend on a large cooperative research network that may gather samples from many OTUs, from many selected locations (and museums) and further ecological information to push trout research to new areas. If local research groups exist (e.g., Balkan Trout Restoration Group: <http://www.balkan-trout.com/research.htm>), transnational studies remain few, mostly binational, and large-scale international programs regarding trout date back to two decades ago (TroutConcert; Laikre, 1999). New collaborations are certainly necessary to reach novel achievements and make the mirage of trout molecular studies disappear to both splitters and lumpers.

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## References

- Ackiss, A. S., Larson, W. A., & Stott, W. (2020). Genotyping-by-sequencing illuminates high levels of divergence among sympatric forms of coregonines in the Laurentian Great Lakes. *Evolutionary Applications*, **13**, 1037-1054. <https://doi.org/10.1111/eva.12919>
- Adams, C. E., & Maitland, P. S. (2007). Arctic charr in Britain and Ireland – 15 species or one?. *Ecology of Freshwater Fish*, **16**, 20-28. <https://doi.org/10.1111/j.1600-0633.2006.00179.x>
- Allendorf, F. W. (2012). Units of conservation. In: F.W. Allendorf, G.H. Luikart & S.N. Aitken (Eds.), *Conservation and Genetics of Populations, 2<sup>nd</sup> Edition* (pp.316-351). Hoboken, NJ: Wiley
- Andersson, A., Jansson, E., Wennerström, L., Chiriboga, F., Arnyast, M., Kent, M. P. *et al.* (2017). Complex genetic diversity patterns of cryptic, sympatric brown trout (*Salmo trutta*) populations in tiny mountain lakes. *Conservation Genetics*, **18**, 1213-1227. <https://doi.org/10.1007/s10592-017-0972-4>
- Antunes, A., Faria, R., Weiss, S., & Alexandrino, P. (2001). Complex evolutionary history in the brown trout: insights on the recognition of conservation units. *Conservation Genetics*, **2**, 337-347. <https://doi.org/10.1023/A:1012518112891>
- Apostolidis, A. P., Stoumboudi, M. T., Kalogianni, E., Cote, G., & Bernatchez, L. (2011). Genetic divergence among native trout *Salmo trutta* populations from southern Balkans based on mitochondrial DNA and microsatellite variation. *Journal of Fish Biology*, **79**, 1950-1960. <https://doi.org/10.1111/j.1095-8649.2011.03136.x>
- Bangs, M. R., Douglas, M. R., Chafin, T. K., & Douglas, M. E. (2020). Gene flow and species delimitation in fishes of Western North America: Flannelmouth (*Catostomus latipinnis*) and bluehead sucker (*C. pantosteus discobolus*). *Ecology and Evolution*, **10**, 6477-6493. <https://doi.org/10.1002/ece3.6384>
- Bardakci, F., Degerli, F., Ozdemir, O., & Basibuyuk, H. H. (2006). Phylogeography of the Turkish brown trout, *Salmo trutta* L.: mitochondrial DNA PCR RFLP variation. *Journal of Fish Biology*, **68**, 36-55. <https://doi.org/10.1111/j.0022-1112.2006.00948.x>
- Barley, A. J., Brown, J. M., & Thomson, R. C. (2018). Impact of model violations on the inference of species boundaries under the multispecies coalescent. *Systematic Biology*, **67**, 269-284. <https://doi.org/10.1093/sysbio/syx073>

- Baumsteiger, J., Moyle, P. B., Aguilar, A., O'Rourke, S. M., & Miller, M. R. (2017). Genomics clarifies taxonomic boundaries in a difficult species complex. *PLoS ONE*, **12**, e0189417. <https://doi.org/10.1371/journal.pone.0189417>
- Behnke, R. J. (1986). Brown trout. *Trout*, **27**, 42-47.
- Beheregaray, L. B., Pfeiffer, L. V., Attard, C. R. M., Sandoval-Castillo, J., Domingos, F. M. C. B., Faulks, L. K., Gilligan D. M., & Umack, P. J. (2017). Genome-wide data delimits multiple climate-determined species ranges in a widespread Australian fish, the golden perch (*Macquaria ambigua*). *Molecular Phylogenetics and Evolution*, **111**, 65-75. <https://doi.org/10.1016/j.ympev.2017.03.021>
- Ben Chehida, Y., Thumloup, J., Schumacher, C., Harkins, T., Aguilar, A., Borrell, A., Ferreira, M., *et al.* (2020). Mitochondrial genomics reveals the evolutionary history of the porpoises (Phocoenidae) across the speciation continuum. *Scientific Reports*, **10**, 15190. <https://doi.org/10.1038/s41598-020-71603-9>
- 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. <https://doi.org/10.1111/j.0014-3820.2001.tb01300.x>
- Bernatchez, L., Guyomard, R., & Bonhomme, F. (1992). DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Molecular Ecology*, **1**, 161-173. <https://doi.org/10.1111/j.1365-294x.1992.tb00172.x>
- Bernatchez, L., & Osinov, A. (1995) Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology*, **4**, 285-297. <https://doi.org/10.1111/j.1365-294x.1995.tb00222.x>
- Bravo, G. A., Antonelli, A., Bacon, C. D., Bartoszek, K., Blom, M. P. K., Huynh, S., Edwards, S. V. *et al.* (2019). Embracing heterogeneity: coalescing the Tree of Life and the future of phylogenomics. *PeerJ*, **14**, e6399. <https://doi.org/10.7717/peerj.6399>
- Bryja, J., Smith, C., Konečný, A., & Reichard, M. (2010). Range-wide population genetic structure of the European bitterling (*Rhodeus amarus*) based on microsatellite and mitochondrial DNA analysis. *Molecular Ecology*, **19**, 4708-4722. <https://doi.org/10.1111/j.1365-294X.2010.04844.x>
- Buckley, S. J., Domingos, F. M. C. B., Attard, C. R. M., Brauer, C. J., Sandoval-Castillo, J., Lodge, R., Umack, P. J., *et al.* (2018). Phylogenomic history of enigmatic pygmy perches: implications for biogeography, taxonomy and conservation. *Royal Society Open Science*, **5**, 172125. <https://doi.org/10.1098/rsos.172125>
- Campillo, L. C., Barley, A. J. & Thomson, R. C. (2020). Model-based species delimitation: are coalescent species reproductively isolated? *Systematic Biology*, **69**, 708-721. <https://doi.org/10.1093/sysbio/syz072>
- Carruthers, M., Yurchenko, A. A., Augley, J. J., Adams, C. E., Herzyk, P., & Elmer, K. R. (2018). *De novo* transcriptome assembly, annotation and comparison of four ecological and evolutionary model salmonid fish

species. *BMC Genomics*, **19**, 32. <https://doi.org/10.1186/s12864-017-4379-x> (erratum available at: <https://doi.org/10.1186/s12864-018-4840-5>)

- Carstens, B. C., Pelletier, T. A., Reid, N. M., & Sattler, J. D. (2013). How to fail at species delimitation. *Molecular Ecology*, **22**, 4369-4383. <https://doi.org/10.1111/mec.12413>
- Chambers, E. A., & Hillis, D. M. (2020). The multispecies coalescent over-splits species in the case of geographically widespread taxa. *Systematic Biology*, **69**, 184-193. <https://doi.org/10.1093/sysbio/syz042>
- Chan, K. O., Hutter, C. R., Wood, P. L. Jr, Grismer, L. L., Das, I., & Brown, R. M. (2020). Gene flow creates a mirage of cryptic species in a Southeast Asian spotted stream frog complex. *Molecular Ecology*, **29**, 3970-3987. <https://doi.org/10.1111/mec.15603>
- Chassaing, O., Desse-Berset, N., Hänni, C., Hughes, S., & Berrebi, P. (2016). Phylogeography of the European sturgeon (*Acipenser sturio*): A critically endangered species. *Molecular Phylogenetics and Evolution*, **94**, 346-357. <https://doi.org/10.1016/j.ympev.2015.09.020>
- Coates, D. J., Byrne, M., & Moritz, C. (2018). Genetic diversity and conservation units: dealing with the species-population continuum in the age of genomics. *Frontiers in Ecology and Evolution*, **6**, 165. <https://doi.org/10.3389/fevo.2018.00165>
- Collins, R. A., & Hrbek, T. (2018). An *in silico* comparison of protocols for dated phylogenomics. *Systematic Biology*, **67**, 633-650. <https://doi.org/10.1093/sysbio/syx089>
- Copus, J.M., Montgomery, W.L., Forsman, Z.H., Bowen, B.W., & Toonen, R.J. (2018). Geopolitical species revisited: genomic and morphological data indicate that the roundtail chub *Gila robusta* species complex (Teleostei, Cyprinidae) is a single species. *PeerJ.*, **6**, e5605. <https://doi.org/10.7717/peerj.5605>
- Cortey, M., Vera, M., Pla, C., & García-Marín, J. L. (2009). Northern and Southern expansions of Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. *Biological Journal of the Linnean Society*, **97**, 904–917. <https://doi.org/10.1111/j.1095-8312.2009.01220.x>
- Crête-Lafrenière, A., Weir, L. K., & Bernatchez, L. (2012). Framing the Salmonidae family phylogenetic portrait: A more complete picture from increased taxon sampling. *PLoS One*, **7**, e46662. <https://doi.org/10.1371/journal.pone.0046662>
- Culling, M. A., Janko, K., Boron, A., Vasil'ev, V. P., Côté, I. M., & Hewitt, G. M. (2006). European colonization by the spined loach (*Cobitis taenia*) from Ponto-Caspian refugia based on mitochondrial DNA variation. *Molecular Ecology*, **15**, 173-190. <https://doi.org/10.1111/j.1365-294X.2005.02790.x>
- Dadikyan, M. G. (1986). *Fishes of Armenia*. Yerevan, Armenia: AN Arm SSR (in Russian)
- Dellicour, S., & Flot, J.-F. (2018). The hitchhiker's guide to single-locus species delimitation. *Molecular Ecology Resources*, **18**, 1234–1246. <https://doi.org/10.1111/1755-0998.12908>



- Delling, B., & Doadrio, I. (2005). Systematics of the trouts endemic to Moroccan lakes, with description of a new species (Teleostei: Salmonidae). *Ichthyological Exploration of Freshwaters*, **16**, 49–64. <https://doi.org/10.1111/j.1095-8312.2009.01220.x>
- Delling, B., Sabatini, A., Muracciole, S., Tougard, C., Berrebi, P. (2020). Morphologic and genetic characterisation of Corsican and Sardinian trout with comments on *Salmo* taxonomy. *Knowledge & Management of Aquatic Ecosystems*, **421**, 21. <https://doi.org/10.1051/kmae/2020013>
- De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, **56**(6), 879-86. <https://doi.org/10.1080/10635150701701083>
- Díaz-Arce, N., Arrizabalaga, H., Murua, H., Irigoien, X., & Rodríguez-Ezpeleta, N. (2016). RAD-seq derived genome-wide nuclear markers resolve the phylogeny of tunas. *Molecular Phylogenetics and Evolution*, **102**, 202-207. <https://doi.org/10.1016/j.ympev.2016.06.002>
- Dincă, V., Lee, K. M., Vila, R., & Mutanen, M. (2019). The conundrum of species delimitation: a genomic perspective on a mitogenetically super-variable butterfly. *Proceedings Biological Sciences*, **286**, 20191311. <https://doi.org/10.1098/rspb.2019.1311>
- Doadrio, I., Perea, S., & Yahyaoui, A. (2015). Two new species of Atlantic trout (Actinopterygii, Salmonidae) from Morocco. *Graellsia*, **71**, e031. <https://doi.org/10.3989/graellsia.2015.v71.142>
- Drywa, A., Poćwierz-Kotus, A., Wąs, A., Dobosz, S., Kent, M. P., Lien, S., Bernaś, R., & Wenne, R. (2013). Genotyping of two populations of Southern Baltic Sea trout *Salmo trutta* m. *trutta* using an Atlantic salmon derived SNP-array. *Marine Genomics*, **9**, 25-32. <https://doi.org/10.1016/j.margen.2012.08.001>
- Durand, J.-D., Persat, H., & Bouvet, Y. (1999). Phylogeography and postglacial dispersion of the chub (*Leuciscus cephalus*) in Europe. *Molecular Ecology*, **8**, 989-97. <https://doi.org/10.1046/j.1365-294x.1999.00654.x>
- Durand, J.-D., Tsigenopoulos, C. S., Unlü, E., & Berrebi, P. (2002). Phylogeny and biogeography of the family Cyprinidae in the Middle East inferred from cytochrome b DNA - evolutionary significance of this region. *Molecular Phylogenetics and Evolution*, **22**, 91-100. <https://doi.org/10.1006/mpev.2001.1040>
- Durand, J.-D., Bianco, P. G., Laroche, J., & Gilles, A. (2003). Insight into the origin of endemic Mediterranean ichthyofauna: phylogeography of *Chondrostoma* genus (Teleostei, Cyprinidae). *Journal of Heredity*, **94**, 315-28. <https://doi.org/10.1093/jhered/esg074>
- Duvernell, D. D., Westhafer, E., & Schaefer, J. F. (2019). Late Pleistocene range expansion of North American top minnows accompanied by admixture and introgression. *Journal of Biogeography*, **46**, 2126-2140. <https://doi.org/10.1111/jbi.13597>
- Eizaguirre, C., & Baltazar-Soares, M. (2014) Evolutionary conservation - evaluating the adaptive potential of species. *Evolutionary Applications*, **7**, 963–967. <https://doi.org/10.1111/eva.12227>

- Fang, B., Merilä, J., Matschiner, M., & Momigliano, P. (2020). Estimating uncertainty in divergence times among three-spined stickleback clades using the multispecies coalescent. *Molecular Phylogenetics and Evolution*, **142**, 106646. <https://doi.org/10.1016/j.ympev.2019.106646>
- Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M., & Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. *Molecular Phylogenetics and Evolution*, **127**, 613-625. <https://doi.org/10.1016/j.ympev.2018.06.008>
- Ferguson, A. (1989). Genetic differences among brown trout, *Salmo trutta*, stocks and their importance for the conservation and management of the species. *Freshwater Biology*, **21**, 35–46. <https://doi.org/10.1111/j.1365-2427.1989.tb01346.x>
- Finnegan, A. K., Griffiths, A. M., King, R. A., Machado-Schiaffino, G., Porcher, J. P., Garcia-Vazquez, E., Bright, D., & Stevens, J. R. (2013). Use of multiple markers demonstrates a cryptic western refugium and postglacial colonisation routes of Atlantic salmon (*Salmo salar* L.) in Northwest Europe. *Heredity*, **111**, 34-43. doi: 10.1038/hdy.2013.17
- Flouri, T., Jiao, X., Rannala, B., & Yang, Z. (2020). A Bayesian implementation of the multispecies coalescent model with introgression for phylogenomic analysis. *Molecular Biology and Evolution*, **37(4)**, 1211-1223. <https://doi.org/10.1093/molbev/msz296>
- Fraser, D. J., & Bernatchez, L. (2001). Adaptive evolutionary conservation: Towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752. <https://doi.org/10.1046/j.1365-294X.2001.t01-1-01411.x>
- Froese, R., & Pauly, D. (2019). FishBase. World Wide Web electronic publication. Available at: [www.fishbase.org](http://www.fishbase.org) (last accessed 18/12/2019).
- Gagnaire, P-A. (2020). Comparative genomics approach to evolutionary process connectivity. *Evolutionary Applications*, **13**, 1320–1334. <https://doi.org/10.1111/eva.12978>
- Galtier, N. (2019). Delineating species in the speciation continuum: A proposal. *Evolutionary Applications*, **12**, 657-663. <https://doi.org/10.1111/eva.12748>
- García-Marín, J. L., Utter, F. M., & Pla, C. (1999). Post-glacial colonization of brown trout in Europe based on distribution of allozyme variants. *Heredity*, **82**, 79-86. <https://doi.org/10.1038/sj.hdy.6884470>
- Gratton, P., Allegrucci, G., Sbordoni, V., & Gandolfi, A. (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. <https://doi.org/10.1016/j.ympev.2014.06.022>
- Guo, B., Fang, B., Shikano, T., Momigliano, P., Wang, C., Kravchenko, A., & Merilä, J. (2019). A phylogenomic perspective on diversity, hybridization and evolutionary affinities in the stickleback genus *Pungitius*. *Molecular Ecology*, **28**, 4046-4064. <https://doi.org/10.1111/mec.1520>

- Guyomard, R. (1989). Diversité génétique de la truite commune. *Bulletin Français de la Pêche et de la Pisciculture*, **314**, 118-135. <https://doi.org/10.1051/kmae:1989008>
- Haig, S. M., Beever, E. A., Chambers, S. M., Draheim, H. M., Dugger, B. D., Dunham, S., Elliott-Smith, E., et al. (2006). Taxonomic considerations in listing subspecies under the U.S. Endangered Species Act. *Conservation Biology*, **20**, 1584-1594. <https://doi.org/10.1111/j.1523-1739.2006.00530.x>
- Hey, J. (2001). The mind of the species problem. *Trends in Ecology & Evolution*, **16**, 326-329. [https://doi.org/10.1016/s0169-5347\(01\)02145-0](https://doi.org/10.1016/s0169-5347(01)02145-0)
- Hey, J., & Pinho, C. (2012). Population genetics and objectivity in species diagnosis. *Evolution*, **66**, 1413-1429. <https://doi.org/10.1111/j.1558-5646.2011.01542.x>
- Hey, J., Chung, Y., Sethuraman, A., Lachance, J., Tishkoff, S., Sousa, V. C., & Wang, Y. (2018). Phylogeny estimation by integration over isolation with migration models. *Molecular Biology and Evolution*, **35**, 2805-2818. <https://doi.org/10.1093/molbev/msy162>
- Hillis, D. M. (2019). Species delimitation in herpetology. *Journal of Herpetology*, **53**, 3–12. <https://doi.org/10.1670/18-123>
- Hillis, D. M. (2020). The detection and naming of geographic variation within species. *Herpetological Review*, **51**, 52–56.
- Hughes, L. C., Cardoso, Y. P., Sommer, J. A., Cifuentes, R., Cuello, M., Somoza, G. M., Gonzales-Castro, M., Malabarba, L. R., Cussac, V., Habit, E. M., Betancur-R, R., & Orti, G. (2020). Biogeography, habitat transitions and hybridization in a radiation of South American silverside fishes revealed by mitochondrial and genomic RAD data. *Molecular Ecology*, **29**, 738-751. <https://doi.org/10.1111/mec.15350>
- Hurst, C. D., Bartlett, S. E., Davidson, W. S., & Bruce, I. J. (1999). The complete mitochondrial DNA sequence of the Atlantic salmon, *Salmo salar*. *Gene*, **239**, 237-242. [https://doi.org/10.1016/s0378-1119\(99\)00425-4](https://doi.org/10.1016/s0378-1119(99)00425-4)
- Isaac, N. J. B., Mallet, J., & Mace, G. M. (2004). Taxonomic inflation: Its influence on macroecology and conservation. *Trends in Ecology and Evolution*, **19**, 464–469. <https://doi.org/10.1016/j.tree.2004.06.004>
- IUCN (2020). The IUCN Red List of Threatened Species. Version 2020-2. Available at <http://www.iucnredlist.org> (last accessed 10 July 2020)
- Jacobs, A., Hughes, M. R., Robinson, P. C., Adams, C. E., & Elmer, K. R. (2018). The genetic architecture underlying the evolution of a rare piscivorous life history form in brown trout after secondary contact and strong introgression. *Genes (Basel)*, **9**, 280. <https://doi.org/10.3390/genes9060280>
- Jacobsen, M. W., Hansen, M. M., Orlando, L., Bekkevold, D., Bernatchez, L., Willerslev, E., & Gilbert, M. T. (2012). Mitogenome sequencing reveals shallow evolutionary histories and recent divergence time between morphologically and ecologically distinct European whitefish (*Coregonus* spp.). *Molecular Ecology*, **21**, 2727-2742. <https://doi.org/10.1111/j.1365-294X.2012.05561.x>

- Accepted Article
- Jeffries, D. L., Copp, G. H., Lawson Handley, L., Olsén, K. H., Sayer, C. D., & Hänfling, B. (2016). Comparing RADseq and microsatellites to infer complex phylogeographic patterns, an empirical perspective in the Crucian carp, *Carassius carassius*, L. *Molecular Ecology*, **25**, 2997-3018. <https://doi.org/10.1111/mec.13613>
- Jones, G. (2017). Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology*, **74**, 447-467. <https://doi.org/10.1007/s00285-016-1034-0>
- Jonsson, B., & Jonsson, N. (2011). *Ecology of Atlantic Salmon and Brown trout: Habitat as a Template for Life Histories*. Heidelberg: Springer-Verlag
- Kalayci, G., Ozturk, R. C., Capkin, E., & Altinok, I. (2018). Genetic and molecular evidence that brown trout *Salmo trutta* belonging to the Danubian lineage are a single biological species. *Journal of Fish Biology*, **93**, 793-804. <https://doi.org/10.1111/jfb.13777>
- Kapli, P., Yang, Z., & Telford, M. J. (2020). Phylogenetic tree building in the genomic age. *Nature Review Genetics*, **21**, 428-444. <https://doi.org/10.1038/s41576-020-0233-0>
- Kautt, A. F., Machado-Schiaffino, G., & Meyer A. (2018). Lessons from a natural experiment: Allopatric morphological divergence and sympatric diversification in the Midas cichlid species complex are largely influenced by ecology in a deterministic way. *Evolution Letters*, **2**, 323-340. <https://doi.org/10.1002/evl3.64>
- Kottelat, M., & Freyhof, J. (2007). *Handbook of European Freshwater Fishes*. Cornol, Switzerland: Publications Kottelat and Berlin, Germany: Freyhof.
- Laikre, L. (ed.) (1999) Conservation genetics management of brown trout (*Salmo trutta*) in Europe. Available at: [http://www.popgen.su.se/pdf/Troutconcert\\_consreport.pdf](http://www.popgen.su.se/pdf/Troutconcert_consreport.pdf) (last accessed: 15 October 2020).
- Leaché, A. D., & Oaks, J.R. (2017). The utility of single nucleotide polymorphism (SNP) data in phylogenetics ; *Annual Reviews of Ecology, Evolution & Systematics*, **46**, 69-84. <https://doi.org/10.1146/annurev-ecolsys-110316-022645>
- Leaché, A. D., Fujita, M. K., Minin, V. N., & Bouckaert, R. R. (2014). Species delimitation using genome-wide SNP data. *Systematic Biology*, **63**, 534–542. <https://doi.org/10.1093/sysbio/syu018>
- Lecaudey, L. A., Schliwen, U. K., Osinov, A. G., Taylor, E. B., Bernatchez, L., & Weiss, S. J. (2018). Inferring phylogenetic structure, hybridization and divergence times within Salmoninae (Teleostei: Salmonidae) using RAD-sequencing. *Molecular Phylogenetics and Evolution*, **124**, 82-99. <https://doi.org/10.1016/j.ympev.2018.02.022>
- Leitwein, M., Gagnaire, P.-A., Desmarais, E., Guendouz, S., Rohmer, M., Berrebi, P., & Guinand, B. (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. <https://doi.org/10.1111/jfb.13131>

- Levin, B., Simonov, E., Rastorguev, S., Boulygina, E., Sharko, F., Tsygankova, S., Gabrielyan, B., *et al.* (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. <https://doi.org/10.1007/s10750-018-3688-7>
- Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., Hvidsten, T. R., *et al.* (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature*, **533**, 200-205. <https://doi.org/10.1038/nature17164>
- Liu, L., Anderson, C., Pearl, D., & Edwards, S. V. (2019). Modern phylogenomics: building phylogenetic trees using the multispecies coalescent model. *Methods in Molecular Biology*, **1910**, 211-239. [https://doi.org/10.1007/978-1-4939-9074-0\\_7](https://doi.org/10.1007/978-1-4939-9074-0_7)
- Lobón-Cerviá, J. (2018). Princess of the streams: the brown trout *Salmo trutta* L. as aquatic royalty. In J. Lobón-Cerviá & N. Sanz (Eds.), *Brown trout – Biology, ecology and management* (pp. 1–13). Hoboken, NJ: Wiley. <https://doi.org/10.1002/9781119268352.ch1>
- Long, C., & Kubatko, L. (2018). The effect of gene flow on coalescent-based species-tree inference. *Systematic Biology*, **67**, 770-785. <https://doi.org/10.1093/sysbio/syy020>
- Lopez, L., Turner, K. G., Bellis, E. S., & Lasky, J. R. (2020). Genomics of natural history collections for understanding evolution in the wild. *Molecular Ecology Resources*, **20**, 1153-1160. <https://doi.org/10.1111/1755-0998.13245>
- Lucek, K., Keller, I., Nolte, A. W., & Seehausen, O. (2018). Distinct colonization waves underlie the diversification of the freshwater sculpin (*Cottus gobio*) in the Central European Alpine region. *Journal of Evolutionary Biology*, **31**, 1254-1267. <https://doi.org/10.1111/jeb.13339>
- Luo, A., Ling, C., Ho, S. Y. W., & Zhu, C. D. (2018). Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Systematic Biology*, **67**, 830-846. <https://doi.org/10.1093/sysbio/syy011>
- Mayr, E. (1942). *Systematics and the origin of species*. New York : Columbia University Press.
- McKeown, N. J., Hynes, R. A., Duguid, R. A., Ferguson, A., & Prodöhl, P. A. (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. <https://doi.org/10.1111/j.1095-8649.2009.02490.x>
- Mee, J. A., Bernatchez, L., Reist, J. D., Rogers, S. M., & Taylor, E. B. (2015). Identifying designatable units for intraspecific conservation prioritization: a hierarchical approach applied to the lake whitefish species complex (*Coregonus* spp.). *Evolutionary Applications*, **8**, 423-441. <https://doi.org/10.1111/eva.12247>

- Nedoluzhko, A., Rastorguev, S., Simonov, E., Boulygina, E., Sharko, F., Tsygankova, S., Gabrielyan, B. K., *et al.* (2018). Two complete mitochondrial genomes of extinct form of the Sevan trout *Salmo ischchan danilewskii*. *Mitochondrial DNA Part B*, **3**, 40-41. <https://doi.org/10.1080/23802359.2017.1419096>
- Olave, M., & Meyer, A. (2020). Implementing large genomic single nucleotide polymorphism data sets in phylogenetic network reconstructions: a case study of particularly rapid radiations of cichlid fish. *Systematic Biology*, **69**, 848-862. <https://doi.org/10.1093/sysbio/syaa005>
- Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., *et al.* (2015). Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Molecular Ecology*, **24**, 525-544. <https://doi.org/10.1111/mec.13048>
- Pasquier, J., Cabau, C., Nguyen, T., Jouanno, E., Severac, D., Braasch, I., Journot, L., *et al.* (2016). Gene evolution and gene expression after whole genome duplication in fish: the PhyloFish database. *BMC Genomics*, **17**, 368. <https://doi.org/10.1186/s12864-016-2709-z>
- Pedraza-Marrón, C. D. R., Silva, R., Deeds, J., Van Belleghem, S. M., Mastretta-Yanes, A., Domínguez-Domínguez, O., Rivera-Vega, R. A. *et al.* (2019). Genomics overrules mitochondrial DNA, siding with morphology on a controversial case of species delimitation. *Proceedings Biological Sciences*, **286**, 20182924. <https://doi.org/10.1098/rspb.2018.2924>
- Petit, R. J., & Excoffier, L. (2009). Gene flow and species delimitation. *Trends in Ecology & Evolution*, **24**, 386–393. doi: 10.1016/j.tree.2009.02.011
- Phillips, R. B., Mastuoka, M. P., Konon, I., & Reed, K. M. (2000). Phylogenetic analysis of mitochondrial and nuclear sequences supports inclusion of *Acantholingua ohridana* in the genus *Salmo*. *Copeia*, **2000(2)**, 546-550. [https://doi.org/10.1643/0045-8511\(2000\)000\[0546:PAOMAN\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2000)000[0546:PAOMAN]2.0.CO;2)
- Pustovrh, G., Snoj, A., & Bajec Sušnik S. (2014). Molecular phylogeny of *Salmo* of the western Balkans, based upon multiple nuclear loci. *Genetics Selection Evolution*, **46**, 7. <https://doi.org/10.1186/1297-9686-46-7>
- Pyron, R. A. (2015). Post-molecular systematics and the future of phylogenetics. *Trends in Ecology & Evolution*, **30**, 384-389. <https://doi.org/10.1016/j.tree.2015.04.016>
- Rannala, B., & Yang, Z. (2017). Efficient Bayesian species tree inference under the multispecies coalescent. *Systematic Biology*, **66**, 823-842. <https://doi.org/10.1093/sysbio/syw119>
- Raposo, M. A., Kirwan, G. M., Lourenço, A. C. C., Sobral, G., Brockmann, F. A., & Stopiglia, R. (2020). On the notions of taxonomic ‘impediment’, ‘gap’, ‘inflation’ and ‘anarchy’, and their effects on the field of conservation. *Systematics and Biodiversity*. <https://doi.org/10.1080/14772000.2020.1829157>
- Ravinet, M., Yoshida, K., Shigenobu, S., Toyoda, A., Fujiyama, A., & Kitano, J. (2018). The genomic landscape at a late stage of stickleback speciation: High genomic divergence interspersed by small localized regions of introgression. *PLoS Genetics*, **14**, e1007358. <https://doi.org/10.1371/journal.pgen.1007358>

- Razpet, A., Sušnik, S., Jug, T., & Snoj, A. (2007). Genetic variation among trout in the River Neretva basin, Bosnia and Herzegovina. *Journal of Fish Biology*, **70**, 94-110. <https://doi.org/10.1111/j.1095-8649.2007.01392.x>
- Reddy, S., Kimball, R. T., Pandey, A., Hosner, P. A., Braun, M. J., Hackett, S. J., Han, K. L., *et al.* (2017). Why do phylogenomic data sets yield conflicting trees? Data type influences the avian tree of life more than taxon sampling. *Systematic Biology*, **66**, 857-879. <https://doi.org/10.1093/sysbio/syx041>
- Rougemont, Q., & Bernatchez, L. (2018). The demographic history of Atlantic salmon (*Salmo salar*) across its distribution range reconstructed from approximate Bayesian computations. *Evolution*, **72**, 1261-1277. <https://doi.org/10.1111/evo.13486>
- Sahoo, P. K., Singh, L., Sharma, L., Kumar, R., Singh, V. K., Ali, S., Singh, A. K., *et al.* (2016). The complete mitogenome of brown trout (*Salmo trutta fario*) and its phylogeny. *Mitochondrial DNA Part A*, **27**, 4563-4565. <https://doi.org/10.3109/19401736.2015.1101565>
- Saint-Pé, K., Leitwein, M., Tissot, L., Poulet, N., Guinand, B., Berrebi, P., Marselli, G., *et al.* (2019). Development of a large SNPs resource and a low-density SNP array for brown trout (*Salmo trutta*) population genetics. *BMC Genomics*, **20**, 582. <https://doi.org/10.1186/s12864-019-5958-9>
- Salmanov, A. V., & E. A. Dorofeyeva. (2001). Comparative osteology of river and lake trouts (genus *Salmo*). *Proceedings of Zoological Institute of the Russian Academy of Sciences*, **287**, 149-195.
- Samy, J. K. A., Mulugeta, T. D., Nome, T., Sandve, S. R., Grammes, F., Kent, M.P., Lien, S., *et al.* (2017). SalmoBase: an integrated molecular data resource for Salmonid species. *BMC Genomics*, **18**, 482. <https://doi.org/10.1186/s12864-017-3877-1>
- Sanz, N. (2018). Phylogeography history of brown trout: A review. In J. Lobón-Cerviá & N. Sanz (Eds.), *Brown trout – Biology, ecology and management* (pp. 17–63). Hoboken, NJ: Wiley. <https://doi.org/10.1002/9781119268352.ch2>
- Savvaitova, K. (1995). Patterns of diversity and process of speciation in Arctic char. *Nordic Journal of Freshwater Research*, **71**, 81–91.
- Schenekar, T., Lerceteau-Köhler, E., & Weiss, S. (2014). Fine-scale phylogeographic contact zone in Austrian Brown trout *Salmo trutta* reveals multiple waves of post-glacial colonization and predominance of natural vs anthropogenic admixture. *Conservation Genetics*, **15**, 561-572. <https://doi.org/10.1007/s10592-013-0561-0>
- Smith, M. L., & Hahn, M. W. (2020). New approaches for inferring phylogenies in the presence of paralogs. *Trends in Genetics*. Advance online publication. <https://doi.org/10.1016/j.tig.2020.08.012>.
- Snoj, A., Glamuzina, B., Razpet, A., Zablocki, J., Bogut, I., Lercetau-Köhler, E., Pojskic, N., *et al.* (2010). Resolving taxonomic uncertainties using molecular systematics: *Salmo dentex* and the Balkan trout community. *Hydrobiologia*, **651**, 199–212. <https://doi.org/10.1007/s10750-010-0297-5>

- Snoj, A., Marić, S., Bajec, S. S., Berrebi, P., Janjani, S., & Schöffmann J. (2011). Phylogeographic structure and demographic patterns of brown trout in North-West Africa. *Molecular Phylogenetics and Evolution*, **61**, 203–211. <https://doi.org/10.1016/j.ympev.2011.05.011>
- Snoj, A., Melkiè, E., Sušnik, S., Muhamedagić, S., & Dovè, P. (2002). DNA phylogeny supports revised classification of *Salmothymus obtusirostris*. *Biological Journal of the Linnean Society*, **77**, 399–411. <https://doi.org/10.1046/j.1095-8312.2002.00130.x>
- Solís-Lemus, C., Knowles, L.L., & Ané C. (2015). Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution*, **69**, 492–507. <https://doi.org/10.1111/evo.12582>
- Song, S, Zhao, J., & Li, C. (2017). Species delimitation and phylogenetic reconstruction of the siniperacids (Perciformes: Siniperacidae) based on target enrichment of thousands of nuclear coding sequences. *Molecular Phylogenetics and Evolution*, **11**, 44–55. <https://doi.org/10.1016/j.ympev.2017.03.014>
- Splendiani, A., Fioravanti, T., Giovannotti, M., Negri, A., Ruggeri, P., Olivieri, L, Cerioni, P. N., *et al.* (2016). The effects of paleoclimatic events on Mediterranean trout: Evidences from ancient DNA. *PLoS ONE*, **11**, e0157975. <https://doi.org/10.1371/journal.pone.0157975.g005>
- Splendiani, A., Fioravanti, T., Giovannotti, M., Olivieri, L., Ruggeri, P., Nisi Cerioni, P., Vanni, S., *et al.* (2017). Museum samples could help to reconstruct the original distribution of *Salmo trutta* complex in Italy. *Journal of Fish Biology*, **90**, 2443–2451. <https://doi.org/10.1111/jfb.13307>
- Splendiani, A., Palmas, F., Sabatini, A. & Caputo, V. (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. <https://doi.org/10.1080/24750263.2019.1686544>
- Stanton, D. W. G., Frandsen, P., Waples, R. K., Heller, R., Russo, I.-R.-M., Orozco-terWengel, P. A., Tongskov, C.-E., *et al.* (2019). More grist for the mill? Species delimitation in the genomic era and its implications for conservation. *Conservation Genetics*, **20**, 101–113. <https://doi.org/10.1007/s10592-019-01149-5>
- Suárez, J., Bautista, J. M., Almodóvar, A., & Machordom, A. (2001). Evolution of the mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the biogeographical role of the Iberian Peninsula. *Heredity*, **87**, 198–206. <https://doi.org/10.1046/j.1365-2540.2001.00905.x>
- Sukumaran, J., & Knowles, L. L. (2017). Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences of the USA*, **114**, 1607–1612. <https://doi.org/10.1073/pnas.1607921114>
- Sušnik, S., Schöffmann, J., & Weiss, S. (2005). Genetic verification of native brown trout from the Persian Gulf (Catak Cay River, Tigris basin). *Journal of Fish Biology*, **67**, 879–884. <https://doi.org/10.1111/j.0022-1112.2005.00780.x>
- Sušnik, S., Snoj, A., Wilson, I. F., Mrdak, D., & Weiss, S. (2007). Historical demography of brown trout (*Salmo trutta*) in the Adriatic drainage including the putative *S. letnica* endemic to Lake Ohrid. *Molecular Phylogenetics and Evolution*, **44**, 63–76. <https://doi.org/10.1016/j.ympev.2006.08.021>



- Sušnik, S., Weiss, S., Tomljanovic, T., Delling, B., Treer, T., Snoj, A. (2007b). 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.
- Toews, D. P. L., & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, **21**, 3907–3930. <https://doi.org/10.1111/j.1365-294X.2012.05664.x>
- Tougaard, C., Justy, F., Guinand, B., Douzery, E. J. P., & Berrebi, P. (2018). *Salmo macrostigma* (Teleostei, Salmonidae): Nothing more than a brown trout (*S. trutta*) lineage? *Journal of Fish Biology*, **93**, 302-310. <https://doi.org/10.1111/jfb.13751>
- Turan, D., Doğan, E., Kaya, C., & Kanyılmaz, M. (2014). *Salmo kottelati*, a new species of trout from Alakır Stream, draining to the Mediterranean in southern Anatolia, Turkey (Teleostei, Salmonidae). *ZooKeys*, **462**, 135–151. <https://doi.org/10.3897/zookeys.462.8177>
- Turan, D., Kalaycı, G., Bektaş, Y., Kaya, C., & Bayçelebi, E. (2020). A new species of trout from the northern drainages of Euphrates River, Turkey (Salmoniformes: Salmonidae). *Journal of Fish Biology*, **96**, 1454-1462. <https://doi.org/10.1111/jfb.14321>
- Turan, D., Kottelat, M., & Bektaş, Y. (2011). *Salmo tigridis*, a new species of trout from Tigris River, Turkey (Teleostei: Salmonidae). *Zootaxa*, **2**, 993: 23–33. <https://doi.org/10.11646/zootaxa.2993.1.2>
- Turan, D., Kottelat, M., & Kaya, C. (2017). *Salmo munzuricus*, a new species of trout from the Euphrates River drainage, Turkey (Teleostei: Salmonidae). *Ichthyological Explorations of Freshwaters*, **28**, 55-63
- Vernygora, O. V., Davis, C. S., Murray, A. M., & Sperling, F. A. H. (2018). Delimitation of *Alosa* species (Teleostei: Clupeiformes) from the Sea of Azov: integrating morphological and molecular approaches. *Journal of Fish Biology*, **93**, 1216-1228. <https://doi.org/10.1111/jfb.13847>
- Vladimirov, V. I. (1946). The trout from the Diatomite deposits of the Pliocene. *Doklady Akademii Nauk Armânskoj SSR*, **4**, 123–128 (in Russian)
- Wang, X, Zhang, Y., Zhang, H, Qin, G., & Lin, Q. (2019). Complete mitochondrial genomes of eight seahorses and pipefishes (Syngnathiformes: Syngnathidae): insight into the adaptive radiation of syngnathid fishes. *BMC Evolutionary Biology*, **19**, 119. <https://doi.org/10.1186/s12862-019-1430-3>
- Wellenreuther, M., Mérot, C., Berdan, E., & Bernatchez, L. (2019). Going beyond SNPs: The role of structural genomic variants in adaptive evolution and species diversification. *Molecular Ecology*, **28**, 1203-1209. <https://doi.org/10.1111/mec>
- Wen, D., Yu, Y., & Nakhleh L. (2016). Bayesian inference of reticulate phylogenies under the multispecies network coalescent. *PLoS Genetics*, **12(5)**, e1006006. <https://doi.org/10.1371/journal.pgen.1006006>. (Erratum in: *PLoS Genetics*, 2017, 13(2), e1006598)

Willis, S. C. (2017). One species or four? Yes!...and, no. Or, arbitrary assignment of lineages to species obscures the diversification processes of Neotropical fishes. *PLoS One*, **12**, e0172349. <https://doi.org/10.1371/journal.pone.0172349>

Zhang, C., Ogilvie, H.A., Drummond, A. J., & Stadler, T. (2018). Bayesian inference of species networks from multilocus sequence data. *Molecular Biology and Evolution*, **35(2)**, 504-517. <https://doi.org/10.1093/molbev/msx307>

## Captions of the Figures

**Fig. 1:** Evolution of the number of publications over the 1989-2020 period dealing with trout systematics found in public reference databases (Web of Sciences, Google Scholar, PubMed) in June 2020 using “Salmo”, “phylogeny”, or “systematics” as queries, then curated (e.g. reviews reporting data from former studies were discarded to avoid to count a study twice). A reference was considered relevant when, at least, two *Salmo* taxonomic species or *S. trutta* evolutionary lineages were included in the studied dataset, and phylogenetic relationships were represented by a tree or a network. The full list of references ( $N = 128$ ) used in this study is available upon request to the contact author. MtDNA: PCR-based sequence of mitochondrial DNA genes; nucDNA: PCR-based sequence of nuclear genes and microsatellites; NGS: data obtained through next-generation sequencing technologies, including nuclear SNPs and mitogenomes. Studies strictly based on allozymic data were not considered.

**Fig. 2:** Simplified Bayesian phylogenetic tree reconstructed from a concatenated dataset including the complete mitochondrial control region (1019 base pairs) and cytochrome *b* (1140 base pairs) genes. Numbers at nodes are for posterior probabilities ( $\geq 0.80$ ) and bootstrap percentages ( $\geq 50\%$ ), while “-” are for nodes weakly supported in either the Bayesian or maximum likelihood analyses. Lineage and species names are indicated on the right. Outside the Atlantic salmon taken as outgroup, note that only four taxa are considered in this tree (*S. trutta*, *S. ohridanus*, *S. obtisrostris* and *S. marmoratus* as the marble trout lineage is often considered a separate OTU [e.g., Pustovrh *et al.*, 2014]), meaning this phylogeny includes only 17.4% (4 out of 23) species recognized by IUCN. The horizontal bar under the phylogenetic tree indicates the number of nucleotide substitutions per site. For details about sampling and phylogenetic analyses, see Tougard *et al.* (2018).



