Rare and fleeting: an example of interspecific recombination in animal mitochondrial DNA

Kate L. Ciborowski[1], Sofía Consuegra[1,2],
Carlos García de Leániz[2], Mark A. Beaumont[3],
Jinliang Wang[1] and William C. Jordan[1,*]

[1]*Institute of Zoology, Regent's Park, London NW1 4RY, UK*
[2]*School of Biological Sciences, University of Wales,
Swansea SA2 8PP, UK*
[3]*School of Animal and Microbial Sciences, University of Reading,
Whiteknights, Reading RG6 6AJ, UK*
*Author for correspondence (bill.jordan@ioz.ac.uk).

Recombination is thought to occur only rarely in animal mitochondrial DNA (mtDNA). However, detection of mtDNA recombination requires that cells become heteroplasmic through mutation, intramolecular recombination or 'leakage' of paternal mtDNA. Interspecific hybridization increases the probability of detecting mtDNA recombinants due to higher levels of sequence divergence and potentially higher levels of paternal leakage. During a study of historical variation in Atlantic salmon (*Salmo salar*) mtDNA, an individual with a recombinant haplotype containing sequence from both Atlantic salmon and brown trout (*Salmo trutta*) was detected. The individual was not an F1 hybrid but it did have an unusual nuclear genotype which suggested that it was a later-generation backcross. No other similar recombinant haplotype was found from the same population or three neighbouring Atlantic salmon populations in 717 individuals collected during 1948–2002. Interspecific recombination may increase mtDNA variability within species and can have implications for phylogenetic studies.

1. INTRODUCTION

Recombination of mitochondrial DNA (mtDNA) occurs commonly in plants and fungi but was traditionally thought to be rare or absent in animal mtDNA (Birky 2001). However, there is evidence that at least some of the molecular machinery required for recombination does exist in animal mitochondria (Thyagarajan *et al.* 1996). In recent years, there have been a number of examples from natural populations of mtDNA recombinants in species including nematodes (Lunt & Hyman 1997), butterflies (Andolfatto *et al.* 2003), fish (Hoarau *et al.* 2002), humans (Kraytsberg *et al.* 2004), scorpions (Gantenbein *et al.* 2005) and lizards (Ujarvi *et al.* 2007). Moreover, surveys of mtDNA sequence databases have revealed previously undetected recombination events (Ladoukakis & Zouros 2001; Piganeau *et al.* 2004; Tsaousis *et al.* 2005).

In order for recombinant mtDNA molecules to be detected, a state of cellular heteroplasmy is required, i.e. more than one mitochondrial lineage needs to be present in a cell (through mutation, intramolecular recombination or 'leakage' of paternal mtDNA), otherwise recombinant molecules will be identical to parental molecules. Animal mtDNA has conventionally been assumed to have strict maternal inheritance and consequently cells are generally homoplasmic (Birky 2001). However, paternal leakage of mtDNA from sperm, demonstrated in several species (e.g. Gyllensten *et al.* 1991; Magoulas & Zouros 1993; Schwartz & Vissing 2002; Kvist *et al.* 2003), can cause heteroplasmy and increase the probability of detection of recombination events. Sperm contain mitochondria, and it is thought that up to 100 functional mitochondria (and their genomes) enter the oocyte cytoplasm at fertilization in mammals (Sutovsky *et al.* 2000). In several mammalian species (including mice, cattle and humans), it has been shown that the mitochondria are tagged with ubiquitin which allows recognition by oocyte proteolytic enzymes and commits sperm-derived mitochondria to destruction, before or during the third proteolytic cleavage (Sutovsky *et al.* 2000).

Rokas *et al.* (2003) theorized that survival of paternal mitochondria may be higher in interspecific crosses than within-species matings because molecular recognition systems may be relaxed in such crosses. Studies have demonstrated that in mice there is a higher probability of paternal leakage in interspecific when compared with intraspecific crosses (Shitara *et al.* 1998). Sutovsky *et al.* (2000) suggested this may be because the ubiquitin-activating and ubiquitin-conjugating enzymes may not be directly compatible across species. High levels of divergence between haplotypes, for example at the interspecific level, would aid in detection of recombination events. Here, we report an example of interspecific mtDNA recombination identified as part of a study of long-term temporal genetic changes in four Spanish Atlantic salmon (*Salmo salar*) populations.

2. MATERIAL AND METHODS

Scale samples were collected from angled adult Atlantic salmon in the rivers Asón, Nansa, Pas and Deva in northern Spain during 1948–2002. Variation in mtDNA was assayed through a combination of restriction fragment length polymorphism (RFLP) analysis and direct DNA sequencing (Ciborowski *et al.* 2007) and individuals were genotyped at five polymorphic microsatellite loci (EST47, *Ssa*A124, *Ssa*D486, *Ssosl*85 and *Sasa-UBA-3UTR*; K.L. Ciborowski *et al.* 2007, unpublished data). To test whether the individual carrying the putative recombinant mtDNA haplotype was an F1 hybrid, the 5S rDNA locus was amplified (Pendas *et al.* 1995). Small amounts of DNA from the scale sample for this individual precluded any longer range PCRs of mtDNA.

Sequences were searched for similarity with existing sequences in GenBank using BLAST (Altschul *et al.* 1990). Possible recombination events were detected using a bootscanning method implemented in SIMPLOT (Lole *et al.* 1999). The recombinant sequence was queried with a sliding window size of 200 bp and step size of 20 bp against the most common *S. salar* sequence in the sample (GenBank Accession DQ237848), a *Salmo trutta* sequence from the River Nansa, northern Spain (EF513754) and an *Oncorhynchus mykiss* (rainbow trout) sequence (L29771). The integrity of the open reading frame in the recombinant sequence was checked using the vertebrate mitochondrial genetic code and the program TRANSLATE (http://bioinformatics.org/sms2/translate.html).
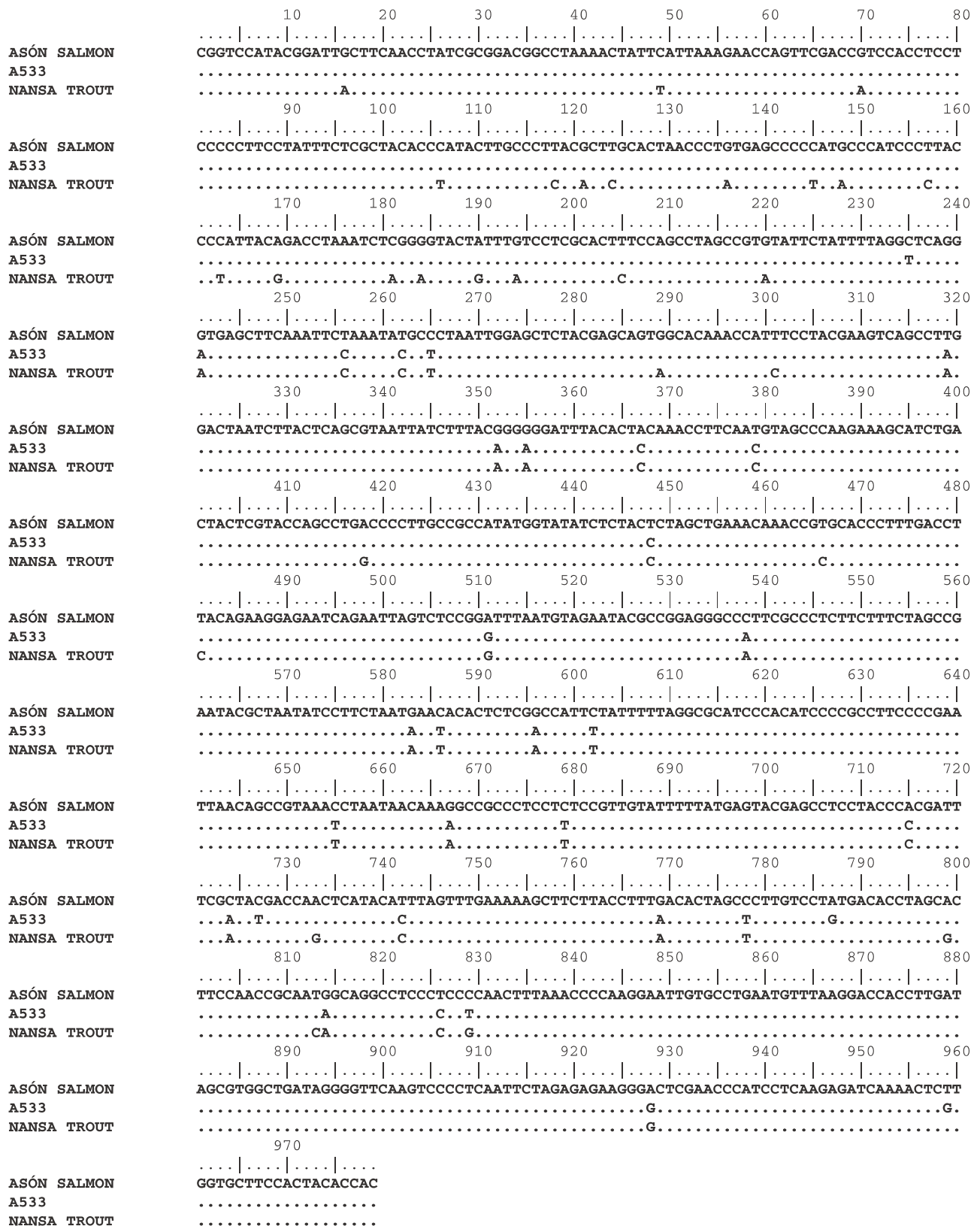
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                              10        20        30        40        50        60        70        80
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          CGGTCCATACGGATTGCTTCAACCTATCGCGGACGGCCTAAAACTATTCATTAAAGAACCAGTTCGACCGTCCACCTCCT
A533                 ................................................................................
NANSA TROUT          ..............A...........................................T.................A.......
                              90       100       110       120       130       140       150       160
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          CCCCCTTCCTATTTCTCGCTACACCCATACTTGCCCTTACGCTTGCACTAACCCTGTGAGCCCCATGCCCATCCCTTAC
A533                 ................................................................................
NANSA TROUT          .......................T..........C..A..C..........A........T..A......C...
                             170       180       190       200       210       220       230       240
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          CCCATTACAGACCTAAATCTCGGGGTACTATTTGTCCTCGCACTTTCCAGCCTAGCCGTGTATTCTATTTTAGGCTCAGG
A533                 .......................................................................T.....
NANSA TROUT          ..T.....G...........A..A.....G...A.........C.............A.................
                             250       260       270       280       290       300       310       320
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          GTGAGCTTCAAATTCTAAATATGCCCTAATTGGAGCTCTACGAGCAGTGGCACAAACCATTTCCTACGAAGTCAGCCTTG
A533                 A..............C.....C..T.....................................................A.
NANSA TROUT          A..............C.....C..T.................A...........C...............A.
                             330       340       350       360       370       380       390       400
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          GACTAATCTTACTCAGCGTAATTATCTTTACGGGGGGATTTACACTACAAACCTTCAATGTAGCCCAAGAAAGCATCTGA
A533                 ........................A..A.............C..........C.......................
NANSA TROUT          ........................A..A.............C..........C.......................
                             410       420       430       440       450       460       470       480
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          CTACTCGTACCAGCCTGACCCCTTGCCGCCATATGGTATATCTCTACTCTAGCTGAAACAAACCGTGCACCCTTTGACCT
A533                 ..............................................C...............................
NANSA TROUT          ...............G...................C.............C...................
                             490       500       510       520       530       540       550       560
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          TACAGAAGGAGAATCAGAATTAGTCTCCGGATTTAATGTAGAATACGCCGGAGGGCCCTTCGCCCTCTTCTTTCTAGCCG
A533                 ...............................G.....................A........................
NANSA TROUT          C..............................G.....................A....................
                             570       580       590       600       610       620       630       640
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          AATACGCTAATATCCTTCTAATGAACACACTCTCGGCCATTCTATTTTTAGGCGCATCCCACATCCCCGCCTTCCCCGAA
A533                 .......................A..T.........A.....T.................................
NANSA TROUT          .......................A..T.........A.....T.................................
                             650       660       670       680       690       700       710       720
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          TTAACAGCCGTAAACCTAATAACAAAGGCCGCCCTCCTCTCCGTTGTATTTTTATGAGTACGAGCCTCCTACCCACGATT
A533                 ..............T.........A.........T.................................C.....
NANSA TROUT          ..............T.........A.........T.................................C.....
                             730       740       750       760       770       780       790       800
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          TCGCTACGACCAACTCATACATTTAGTTTGAAAAAGCTTCTTACCTTTGACACTAGCCCTTGTCCTATGACACCTAGCAC
A533                 ...A..T.........C.......................A........T.......G............
NANSA TROUT          ...A.........G.......C.......................A........T...............G.
                             810       820       830       840       850       860       870       880
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          TTCCAACCGCAATGGCAGGCCTCCCTCCCCAACTTTAAACCCCAAGGAATTGTGCCTGAATGTTTAAGGACCACCTTGAT
A533                 .............A...........C..T.................................
NANSA TROUT          ...........CA...........C..G.................................
                             890       900       910       920       930       940       950       960
                     ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ASÓN SALMON          AGCGTGGCTGATAGGGGTTCAAGTCCCCTCAATTCTAGAGAGAAGGGACTCGAACCCATCCTCAAGAGATCAAAACTCTT
A533                 ..........................................G............................G.
NANSA TROUT          ..........................................G............................
                             970
                     ....|....|....|....
ASÓN SALMON          GGTGCTTCCACTACACCAC
A533                 ...................
NANSA TROUT          ...................
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Figure 1. Alignment of the mtDNA *ND1* sequence from A533 with that of an Atlantic salmon and brown trout, both also from northern Spanish populations.

3. RESULTS AND DISCUSSION

We found a unique and anomalous mtDNA *ND1* gene RFLP pattern in an individual (A533) caught in the River Asón in 1953. Direct sequencing of the mtDNA for this individual (EF513755) and a subsequent BLAST search returned homologies with both Atlantic salmon and brown trout (*S. trutta*) mtDNA *ND1*

sequences. A visual inspection of an alignment of the sequence from A533, a representative Spanish Atlantic salmon mtDNA *ND1* sequence (from Ciborowski *et al.* 2007) and a Spanish brown trout mtDNA *ND1* sequence, suggested two separate regions (figure 1). The 5′ region of the A533 sequence appeared to have high similarity with the Atlantic salmon sequence, and
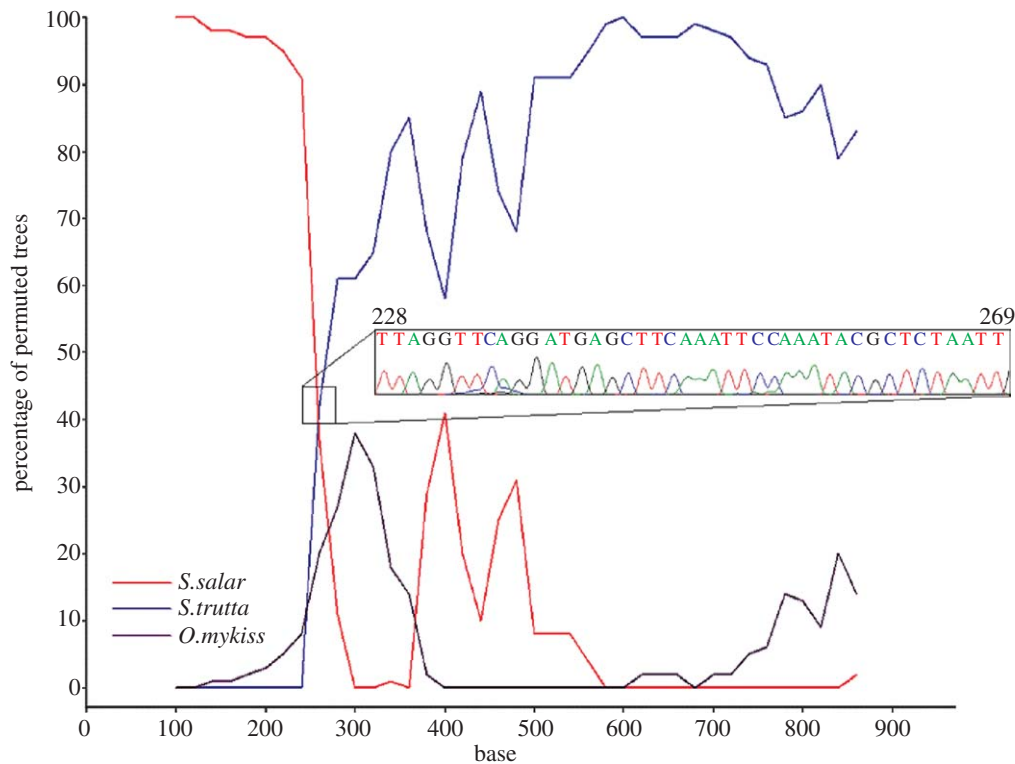
Figure 2. Bootscan using the mtDNA ND1 sequence from A533 as a query with those from Atlantic salmon (*S. salar*), brown trout (*S. trutta*) and rainbow trout (*O. mykiss*) as references. The 'percentage of permuted trees' is a bootstrap value for trees which have query and reference sequences as sister taxa. Inset: experimental chromatogram for the 228–269 bp region.

then abruptly to have similarity with the brown trout sequence in the 3′ region. Using a bootscanning method for detecting recombination events (Lole *et al.* 1999), recombination between Atlantic salmon and brown trout in the mtDNA *ND1* gene was detected around position 240 (figure 2). No evidence of brown trout sequence was detected in any of the other Atlantic salmon samples (*n*=717) and the absence of ambiguous peaks in the sequencing chromatogram (figure 2 inset) argues against contamination of the sample by DNA from both species. The recombinant sequence is also unlikely to be derived from a nuclear-encoded mitochondrial pseudogene (i.e. a Numt) as we found no evidence for amplification of more than one sequence from any other individual, either from RFLP patterns (*n*=717) or direct sequencing (*n*=55; Ciborowski *et al.* 2007), and Numts are unknown from fish species in general (Bensasson *et al.* 2001; Venkatesh *et al.* 2006).

Amplification of the 5S rDNA locus—which has fixed variants of differing size in Atlantic salmon and brown trout (Pendas *et al.* 1995)—from A533 yielded only a PCR product of the expected size for Atlantic salmon. However, the nuclear genotype of A533 was anomalous, i.e. at five microsatellite loci typed, eight of the alleles found in this individual were not found in a sample of Atlantic salmon from the same river in the same decade (*n*=31–36 individuals per locus). Collectively, the evidence suggests that A533 was not an F1 hybrid, but the result of a later-generation backcross. Without similar data from the sympatric trout population, it is not possible to definitively assign the relative contributions of Atlantic salmon and brown trout to the nuclear genome of A533.

Atlantic salmon and brown trout are sympatric in the River Asón, and hybridization between the species has been reported at relatively high frequencies in Spanish rivers (García de Leániz & Verspoor 1989). While the recombinant mtDNA haplotype had an intact open reading frame (in the sequenced section, at least) and could have produced a functional protein, in general, Atlantic salmon×brown trout hybrids display reduced survival and fecundity. While in some situations, hybrids can produce viable backcross offspring, they are generally triploid and therefore infertile (Galbreath & Thorgaard 1995), limiting the possibility of introgression of genes from one species to the other (Verspoor & Hammar 1991). As A533 was angled as an adult salmon, it appears that its viability was not overly compromised. However, if A533 was infertile (its microsatellite genotype did not suggest that it was triploid) or male, the recombinant mtDNA haplotype would not have been inherited by future generations via this individual. Even if A533 was fertile and female the low-frequency recombinant haplotype would have been liable to loss through genetic drift. Indeed, no other such recombinant mtDNA *ND1* haplotype was observed in similar samples collected from 1950 to 2002 in the River Asón or three neighbouring Atlantic salmon populations over the same time span (Ciborowski *et al.* 2007).

To our knowledge, this is one of only a few clear examples of interspecific mtDNA recombination in the literature (Piganeau *et al.* 2004; Tsaousis *et al.* 2005). Given the rare and fleeting nature of the occurrence of the recombinant haplotype identified here, it would have gone undetected in typical surveys

of mtDNA variability within species, which are often less intensive and limited to contemporary samples. Interspecific recombination may increase intraspecific mtDNA diversity but, given reduced viability and fecundity in Atlantic salmon×brown trout hybrids and backcrosses, it is an unlikely mechanism for doing so in these species. Although our detection of the interspecific recombination event was aided by relatively high sequence divergence (5.62%) between Atlantic salmon and brown trout at this region of the mtDNA *ND1* gene and the relatively long tracts of sequence involved, interspecific recombination between shorter sections of mtDNA genomes showing lower levels of divergence may go undetected and have an insidious effect on phylogenetic studies involving mtDNA. Importantly, interspecific mtDNA recombination may be another consideration in evaluating the usefulness of mtDNA barcoding for species identification (Rokas *et al.* 2003; Rubinoff *et al.* 2006).

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