The complete mitochondrial genome of a new invader fish in France, the pink salmon Oncorhynchus gorbuscha (Walbaum, 1792) (Teleostei, Salmonidae)

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The complete mitochondrial genome of a new invader fish in France, the pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792) (Teleostei, Salmonidae)

by

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Abstract. – The pink salmon *Oncorhynchus gorbuscha* (Teleostei, Salmonidae) is a North Pacific species. It arrived in Northern Europe through freshwater migration each odd year since 2017, creating management issues. In this study, we sequenced a complete mitochondrial genome for a pink salmon caught in the Bresle River (France) with a museum voucher. The sequence is 16,695 bp in length and is similar to previous mitogenomes published within this genus. Length heteroplasmy on the control region for this species was detected, as described in previous molecular studies. We tested several primers commonly used for metabarcoding studies on four markers (12S, 16S, COI and Cytb), and all allow the discrimination of this species. This mitogenome is a reference for molecular identification, for instance in like environmental DNA studies.

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Résumé. – Le génome mitochondrial complet d'un nouveau poisson téléostéen invasif en France, le saumon rose *Oncorhynchus gorbuscha* (Walbaum, 1792) (Teleostei, Salmonidae).

Le saumon rose *Oncorhynchus gorbuscha* (Teleostei, Salmonidae) est une espèce du Pacifique Nord arrivée en Europe du Nord avec une migration en eau douce chaque année impaire depuis 2017, générant des problèmes de gestion. Dans cette étude, nous avons séquencé le génome mitochondrial complet d'un saumon rose d'un spécimen enregistré en collection et capturé dans la rivière Bresle (France). La séquence a une longueur de 16 695 pb et est similaire à d'autres mitogénomes publiés chez ce genre. Cependant, nous avons mis en évidence une hétéroplasmie de longueur sur la région de contrôle pour cette espèce confirmant les études moléculaires précédentes. Nous avons également testé plusieurs amorces couramment utilisées pour les études de métabarcoding sur quatre gènes (12S, 16S, COI et Cytb). Tous les marqueurs permettent la discrimination de cette espèce. Notre mitogénome peut être utilisé comme référence pour l'expertise moléculaire, par exemple pour des approches d'ADN environnemental.

INTRODUCTION

The anadromous Pacific salmons *Oncorhynchus* spp. are native to the Pacific coasts of North-western America and North-eastern Asia and have a high interest for aquaculture, recreative fishing and commercial fisheries (Froese and Pauly, 2022). Many species were therefore introduced all over the world since the end of the 19th century with varying success (Scott and Crossman, 1973). In France, three species were introduced (the rainbow trout *O. mykiss* (Walbaum, 1792), the Coho salmon *O. kisutch* (Walbaum, 1792) and the Chinook salmon *O. tshawytscha* (Walbaum, 1792)) but only *O. mykiss* now remains (Persat *et al*., 2020). Since 2017, another Pacific salmon, the pink salmon *Oncorhynchus gorbuscha* (Walbaum, 1792), has invaded northern Europe every odd year (see Baglinière *et al*., 2020; Beaulaton *et al*., 2021). This species has a very strict and simple life cycle: individuals live two years, spending 18 months in marine waters before breeding in freshwater. Adults measuring about 50 cm migrate in rivers from June to August for Asiatic populations or from July to September for American ones, and die after spawning (Heard, 1991). This species might have an impact on the predation, the competition, the disease transmission on native fish species but also on human health through tapeworm infections with *Diphyllobothrium nihonkaiense* Yamane, Kamo, Bylund & Wikgren, 1986 (Arizono *et al*., 2009; Van der Veer and Nentwig, 2015). Currently, its impact on native European salmonids

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seems to be limited (Mo *et al*., 2018; Beaulaton *et al*., 2021), except maybe on sea lamprey *Petromyzon marinus* Linnaeus, 1758 (Armstrong *et al*., 2018; Mo *et al*., 2018; Bonnyaud and Denys, 2021). However, it was demonstrated that the presence of fish carcasses after spawning enriches the rivers with marine nutrients that potentially impact the ecosystem (Armstrong *et al*., 2018; Mo *et al*., 2018). It can also provide new high-income feeding opportunities through egg or fry consumption by native species (Rasputina *et al*., 2016; Dunlop *et al*., 2021). France recorded the southernmost pink salmon occurrences in Europe with a first observation in the Canche River (Pas-de-Calais department) and two observations in Britanny (Côtes-d'Armor and Finistère departments) in 2017, followed by six additional observations: two in the Bresle River (Seine-Maritime department, Normandy), one in the Somme River (Somme department) and in the Léguer River (Finistère department) and two others in the Manche department in 2021 (Beaulaton *et al*., 2021; Bonnyaud and Denys, 2021).

These observations were done mainly by anglers or video counting-stations, so the number of occurrences is certainly underestimated. Molecular tools like the environmental DNA approach would be necessary to complete the range of methods used for the survey of invasive species (*e.g*., Sepulveda *et al*., 2020). Several studies already used this method in the native and newly invaded areas using as molecular markers sequence fragments of the mitochondrial NADH2, the cytochrome oxidase subunit 1 (COI) and the cytochrome b markers (Mizumoto *et al*., 2018; Duda *et al*., 2021; Gargan *et al*., 2022). However, the popular MiFish and French environmental DNA (eDNA) studies on fishes use the 12S marker (*e.g*., Miya *et al*., 2015; Valentini *et al*., 2016). In the GenBank sequence repository, two mitogenomes are already available but they all lack association to a voucher or an origin other than their hatchery, and therefore cannot be validated as reference sequences (Strohm *et al*., 2016).

In this paper, we describe the mitogenome of *O. gorbuscha* from a voucher specimen caught in the North of France, obtained by a double-multiplexing approach. This mitogenome will be helpful to link the different molecular studies made on this taxon, especially those about the genetic variability within even and odd years populations using different molecular markers (control region, Cytb and NADH2) that are therefore not directly comparable (*e.g*., Sato and Urawa, 2017; Podlesnykh *et al*., 2020) and, as molecular reference, for molecular identification using any marker from DNA barcoding *sensu* Hebert *et al*. (2003) to eDNA analyses (Schroeter *et al*., 2020). We provide the diagnostic sites of this species on metabarcode markers already published for eDNA studies.

MATERIAL AND METHODS

Voucher

The voucher specimen is stored in the National Ichthyological Collections of the Muséum national d'Histoire naturelle (MNHN), catalogued MNHN-IC-2022-0168 (Fig. 1), and originates from the COLISA archive (Marchand *et al*., 2018). The specimen (443 mm of standard length (SL) for 1178 g), a male, was caught on July 23rd, 2021 in the Bresle River at Eu (Normandy; Lat: 50.050628 Long: 1.418690), where the French Biodiversity Agency (OFB) operates a scientific trap since 1981 (Josset *et al*., 2021). Identification was made according to Baglinière *et al*. (2020): presence of a hump on the back, black tongue and elongated black spots on the caudal fin.

Brief material and method

DNA extraction was carried on a fin-clip stored in 95% ethanol on an EpMotion Robot using a MN Biomedical extraction kit and protocol. Three long overlapping PCRs of 6-7 kbp were done following Denys *et al*. (2020). Sequencing was performed using a double-multiplexing approach (Hinsinger *et al*. 2015; Denys *et al*., 2020). Sequencing was performed on Illumina MiSeq at the ICM (Institut du Cerveau et de la Moelle épinière, Paris V) using 250 paired-end standard V2. The mitogenome was assembled with the Geneious 11.2.2 software (Kearse *et al*., 2012) mapping

Figure 1. – Voucher of the sequenced mitogenome, MNHN-IC-2022-0168, 443 mm SL, Bresle river at Eu (Seine-Maritime Dept.), 24th July 2021, OFB coll; credit photo: Q. Josset/OFB.

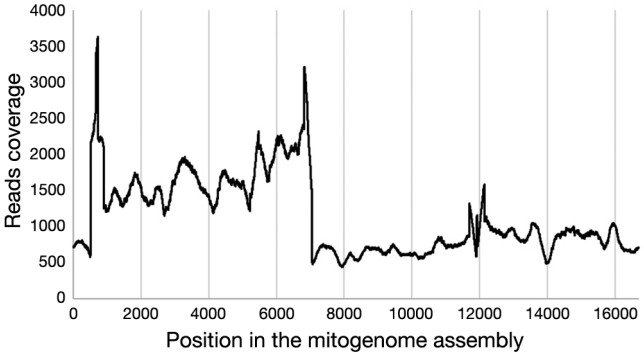

Figure 2. – Coverage depending on position in the mitogenome assembly.

Table I. – Statistics about the assembly of the mitogenome *Oncorhynchus gorbuscha* (GenBank Accession Number PP272106) for the three long PCRs.

	Mt1	Mt2	Mt3
Length (bp)	6748	5385	5382
N reads	44685	14709	20150
Coverage min-max (average; SD)	913-3561 (1702.1; 369.4)	438-1489 (695.4; 220.8)	455-1902 (871.0; 226.4)
Reads lengths min-max (average; SD)	221-251 (249.2; 6.4)	224-251 (248.9; 8.1)	224-251 (248.1; 8.1)

trimmed paired reads on a reference sequence (GenBank accession number EF455489) using the Geneious 11 sensitivity parameter set "low sensitivty/fastest" for the Geneious mapper, and carefully quality controlled by eye along their whole length in addition to the standard controls. The assembly of the control region (CR) had a mismatch with the reference sequence (Appendix 1), so a second assembly by elongation from the assembled sequence without the CR was performed to reconstruct the CR without constraining it through the reference. The consensus sequence was annotated using MitoAnnotator (Iwasaki *et al*., 2013).

Sequence quality

76,484 reads (average length: 249.6) were assembled from the sequencing of the 3 long range PCRs. The read coverage throughout the entire sequence ranges from 438 to 3561, with a mean read coverage of 1147.1 fold (Fig. 2). Assembly statistics for each long fragment are given in Table I.

Phylogenetic reconstruction

Mitogenomes from two *Oncorhynchus gorbuscha* CM029873 and EF455489 (= NC_010959) as well as ten other *Oncorhynchus* species: apache trout *O. apache* (Miller, 1972) MW300342, cutthroat trout *O. clarkii* (Richarson, 1836) KP013107, gila trout *O. gilae* (Miller, 1950) MW300335, chum salmon *O. keta* (Walbaum, 1792) AP010773, coho salmon *O. kisutch* MF621749, masu salmon *O. masou* (Brevoort, 1856) KU523579, rainbow trout *O. mykiss* KP013084, sockeye salmon *O. nerka* (Walbaum, 1792) MH003639 and chinook salmon *O. tshawytscha* AF392054 plus a sequence of brown trout *Salmo trutta* Linnaeus, 1758 MF621761, arctic char *Salvelinus alpinus* Linnaeus, 1758 MN530962 and European grayling *Thymallus thymallus* (Linnaeus, 1758) MN852233 were retrieved from GenBank.

Sequences alignment was performed using the Clustal W plugin in Geneious R11.2.2, as well as nucleotide diversity and pairwise distances. The best evolutionary model was inferred in jModelTest (Darriba *et al*., 2012) and was GTR+I+G for both Akaike and Bayesian information criterion. Phylogenetic analysis was inferred by Maximum Likelihood (ML) using RAxML-HPC2 Workflow on XSEDE (version 8.2.10) (Stamatakis, 2014) with the inferred substitution model and 1,000 bootstrap iterations on the CIPRES Science Gateway (Miller *et al*., 2010) online platform.

Comparison of metabarcode markers

Nine metabarcoding markers from four loci (12S, 16S, COI and Cytb) were tested (Table II). For each locus, we

Table II. – Metabarcoding primers commonly used in the literature and tested in this study to discriminate *Oncorhynchus gorbuscha* from other introduced *Oncorhynchus* species.

Locus	Primers	Sequence 5'-> 3'	Amplicon length (bp)	Reference
12S	MiFish	Forward: GTCGGTAAAACTCGTGCCAGC Reverse: CATAGTGGGGTATCTAATCCCAGTTTG	170	Miya *et al*. (2015)
	Teleo	Forward: ACACCGCCCGTCACTCT Reverse: CTTCCGGTACACTTACCATG	63	Valentini *et al*. (2016)
	Teleo2	Forward: AAACTCGTGCCAGCCACC Reverse: GGGTATCTAATCCCAGTTTG	166	Taberlet *et al*. (2018)
	12S-V5	Forward: TTAGATACCCCACTATGC Reverse: TAGAACAGGCTCCTCTAG	99	Riaz *et al*. (2011)
16S	Fish16sFD/16s2R	Forward: GACCCTATGGAGCTTTAGAC Reverse: CGCTGTTATCCCTADRGTAACT	205	Berry *et al*. (2017)
	16Sar/16Sbr	Forward: CGCCTGTTATCAAAAACAT Reverse: CCGGTCTGAACTCAGATCACGT	581	Duke and Burton (2020)
COI	FishF1/FishR1	Forward: TTCTCAACCAACCACAAAGACATTGG Reverse: TAGACTTCTGGGTGGCCAAAGAATCA	658	Ward *et al*. (2005)
	mlCOIintF/jgHCO2198	Forward: GGWACWGGWTGAACWGTWTAYCCYCC Reverse: TAIACYTCIGGRTGICCRAARAAYCA	313	Leray *et al*. (2013)
CytB	L14912-CYB/H15149-CYB	Forward: TTCCTAGCCATACAYTAYAC Reverse: GGTGGCKCCTCAGAAGGACATTTGKCCYCA	234	Minamoto *et al*. (2012)

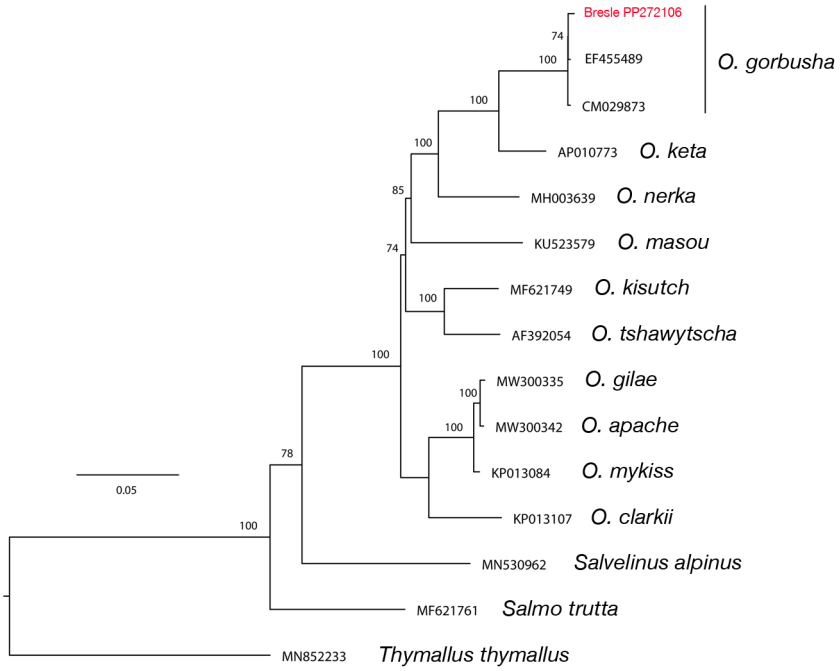

Figure 3. – Maximum Likelihood phylogenetic tree of *Oncorhynchus* mitogenomes; bootstrap values beside the nodes.

compared our sequence with those of the four *Oncorhynchus* species introduced in France available on GenBank and associated with a voucher (Appendix 2). Diagnostic sites characterizing species were then identified with the QUIDDICH package (Kühn and Hasse, 2019) for R (R Core Team, 2022).

RESULTS AND DISCUSSION

Sequence description and phylogenetic analysis

The newly obtained mitogenome has a total length of 16,695 bp and follows the standard vertebrate order, similar to already published mitogenome of *O. gorbuscha*: 13 protein-coding genes including six coding with an incomplete codon stop (COII, ATP6, COIII, NADH3, NADH4 and Cytb), 22 transfer RNA genes including two tRNA-Leu and 2 tRNA-Ser, two ribosomal RNA genes and a control region (Fig. 2; Appendix 3). Intergenic spaces and overlapping sequences were found. The base composition of the entire genome was 27.9% for A, 26.4% for T, 28.7% for C and 17.0% for G. As for the two sequences of *O. gorbuscha* available on GenBank, the mitogenome CM029873 is reversed in the database and had to be reverse complemented to align with the dataset in standard order. The control region of the mitogenome EF455489 does not have the same length (1131 bp *vs*. 1041 bp). Previous genetic studies already highlighted length heteroplasmy among pink salmon populations with this marker with insertions and deletions (Brykov *et al*., 1999; Churikov and Gharrett, 2002; Sato and Urawa, 2017). Alignment of our sequence with all CR sequences from GenBank provided a 100% match with the haplotype OGDL-8 (sequence LC191999), one of the haplotypes widespread in Japan (Sato and Urawa, 2017).

The ML phylogeny (Fig. 3) is consistent with Shedko *et al*. (2013) on mitochondrial data showing generally high support values for nodes as well as with the nuclear RAG1 marker topology (Shedko *et al*., 2012). It differs from phylogenetic trees of Horreo (2017) and Gong *et al*. (2017) by the position of *O. masou*, which is not at a basal position within the *Oncorhynchus* spp. but with a weak robustness. Crespi and Fulton (2004) already demonstrated that the position of *O. masou* depends to the markers analysed and the phylogenetic reconstruction method. Our *O. gorbuscha* mitogenome groups well with the two other sequences available on GenBank, corroborating its identification.

Finally, we tested metabarcoding markers of four genes (12S, 16S, COI, Cytb) in order to know if *O. gorbuscha* can easily be distinguished from the four other *Oncorhynchus* species used for restocking in non-American areas. *O. gorbuscha* is distinguished over these four genes by 10 diagnostic sites on the 12S (Table III), 34 on the 16S (Table IV), 82 on the COI (Table V) and 61 on the Cytb (Table VI). MiFish (Miya *et al*., 2015) and Teleo2 primers (Taberlet *et al*., 2018) between the positions 246/250 and 416 differentiate all 4 species (Table III). However, the fragments corresponding to the 12V-5 primers (Riaz *et al*., 2011) between the positions 444 and 543 and the Teleo primers (Valentini *et al*., 2016) between the positions 849 and 912, while they discriminate well *O. gorbuscha* from other *Oncorhynchus* species, have

Table III. – Diagnostic sites determined on the 12S marker for *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* and *O. tshawytscha*. Diagnostic sites are in bold. The MiFish and Teleo2 fragments (Miya *et al*., 2015; Taberlet *et al*., 2018) between the positions 246/250 and 416, the 12V-5 fragment (Riaz *et al*., 2011) between the positions 444 and 543 as well as the Teleo fragment (Valentini *et al*., 2016) between the positions 849 and 912 are highlighted in grey.

								MiFish / Teleo2					12V-5													Teleo			
	32	38	63	118	122	128	197	307	349	355	370	390	463	490	561	592	643	662	699	730	736	738	747	788	795	864	882	936	947
O. gorbuscha	T	**A**	T	G	**T**	**A**	A	A	**T**	A	T	T	A	G	G	T	C	G	G	T	C	**T**	**G**	**T**	C	**A**	**C**	**A**	**T**
O. kisutch	.	G	**C**	.	C	G	**G**	**G**	C	.	**C**	C	G	A	.	.	.	A	.	.	T	C	A	C	T	G	T	G	C
O. mykiss	**C**	G	.	**A**	C	G	.	.	C	.	.	.	G	A	.	**C**	.	.	**A**	.	.	C	A	C	.	G	T	G	C
O. tshawytscha	.	G	.	.	C	G	.	.	C	**G**	.	C	.	T	**T**	.	**T**	A	.	**C**	T	C	A	C	T	G	T	G	C

Table IV. – Diagnostic sites determined on the 16S marker for *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* and *O. tshawytscha*. Diagnostic sites are in bold. The Fish16sFD/16s2R fragments (Berry *et al*., 2017) between the positions 1170 and 1357 and the 16Sar/16Sbr fragment (Duke and Burton, 2020) between the positions 931 and 1512 are highlighted respectively in dark and light grey.

	18	19	20	29	38	40	44	52	57	59	70	98	114	115	117	118	121	132	149	162	163	164	171	176	180	189	193
O. gorbuscha	C	**T**	C	T	A	C	**T**	**C**	**G**	**T**	**A**	C	**G**	**A**	T	–	T	–	G	–	–	G	A	T	–	**C**	A
O. kisutch	**T**	A	.	.	.	**T**	C	A	A	C	G	.	A	G	.	–	**C**	–	.	–	–	.	.	.	–	T	.
O. mykiss	.	A	**T**	**C**	.	.	C	A	A	C	G	**G**	A	G	.	–	.	–	.	–	–	.	.	**C**	–	T	**G**
O. tshawytscha	.	A	.	.	**–**	.	C	A	A	C	G	.	A	G	**C**	**A**	.	**A**	**A**	**A**	**G**	**A**	**C**	.	**T**	T	.

	241	245	265	266	267	300	309	315	425	427	444	465	470	545	549	550	617	619	648	669	718	721	751	757	759	773	795
O. gorbuscha	A	**C**	**C**	C	**G**	A	**C**	T	G	**A**	**A**	**G**	**G**	A	**A**	T	**C**	T	**–**	G	G	A	T	**T**	A	**G**	**A**
O. kisutch	.	T	T	.	A	.	T	.	.	C	G	A	A	.	C	.	T	.	C	**A**	**A**	.	.	C	.	A	C
O. mykiss	**C**	T	T	**T**	A	.	T	**C**	.	C	G	A	A	**G**	C	**C**	T	**C**	C	.	**T**	.	**C**	C	**C**	A	C
O. tshawytscha	.	T	T	.	A	**G**	T	.	**A**	C	G	A	A	.	C	.	T	.	C	.	.	**G**	.	C	.	A	C

									16Sar/16Sbr		16Sar/16Sbr + Fish16sFD/16s2R														
	808	816	819	823	831	844	864	881	949	1087	1131	1169	1182	1186	1194	1263	1292	1294	1308	1312	1340	1351	1352	1562	1665
O. gorbuscha	T	A	**G**	**G**	C	A	C	G	**G**	C	**T**	**C**	**A/–**	**T**	A	**A**	**T**	C	T	G	A	A	**G**	A	**A**
O. kisutch	.	**G**	A	A	.	.	.	.	A	.	C	A	C	C	**G**	T	C	**T**	.	**A**	.	.	A	**T**	G
O. mykiss	**C**	.	A	A	.	**C**	**A**	.	A	.	C	A	C	C	.	T	C	.	.	.	**T**	**G**	A	.	G
O. tshawytscha	.	.	A	A	**T**	.	.	**A**	A	**T**	C	A	C	C	.	T	C	.	**C**	.	.	.	A	.	G

Table V. – Diagnostic sites determined on the COI marker for *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* and *O. tshawytscha*. Diagnostic sites are in bold. The FishF1/FishR1 fragments (Ward *et al*., 2005) between the positions 48 and 706 and the mlCOIintF/jgCO2198 fragment (Leray *et al*., 2013) between the positions 393 and 706 are highlighted respectively in light and dark grey.

	FishF1/FishR1																								
	21	99	102	108	114	117	123	132	144	148	153	156	159	162	174	210	213	228	258	267	270	276	282	285	294	315
O. gorbuscha	**C**	A	**C**	**A**	G	A	A	**A**	A	**A**	C	G	C	T	**T**	C	**A**	A	G	**A**	**A**	**A**	**T**	**A**	**C**	**G**
O. kisutch	T	.	T	G	**A**	.	**G**	G	.	G	**T**	.	**T**	**C**	C	.	G	.	.	C	T	G	C	T	T	T
O. mykiss	T	**G**	T	G	.	**G**	.	G	**G**	G	.	**A**	.	.	C	.	G	**G**	**A**	C	T	G	C	T	T	T
O. tshawytscha	T	**T**	T	G	.	.	.	G	.	G	.	.	.	.	C	**T**	G	.	.	C	T	G	C	T	T	T

	FishF1/FishR1										FishF1/FishR1 + mlCOIintF/jgCO2198														
	318	327	339	348	354	363	366	372	378	384	402	408	423	426	429	432	438	468	480	498	507	516	519	525	546	552
O. gorbuscha	**C**	**C**	**T**	C/T	**C**	C	T	C	**G**	T	**G**	**T**	**A**	**C**	**C**	**C**	T	**A**	**G**	G	**C**	A	**A**	**A**	A	T
O. kisutch	**G**	T	A	T	T	**G**	**.**	**.**	A	.	C	C	C	A	T	T	.	C	A	.	T	**G**	C	T	.	.
O. mykiss	**T**	T	**G**	**A**	T	.	**G**	**T**	A	**A**	C	C	C	**T**	T	T	.	C	A	.	T	.	**T**	**C**	**C**	**C**
O. tshawytscha	**A**	T	A	C	T	**A**	.	.	A	**C**	C	**A**	C	A	T	T	**G**	C	A	**T**	T	.	C	T	.	.

	FishF1/FishR1 + mlCOIintF/jgCO2198																								
	561	564	568	579	586	591	594	603	615	618	627	643	651	666	669	672	675	729	738	744	747	756	783	789	792	804
O. gorbuscha	T	G	**A**	**A**	C	**A**	C	T	**T**	**C**	**G**	**T**	**T**	**A**	**G**	**G**	**C**	**A/G**	T	**T**	A	**C**	C	T	**G**	C
O. kisutch	.	.	G	**T**	.	C	**T**	.	C	T	A	C	C	G	A	C	G	C	**G**	C	**C**	T	**T**	.	A	.
O. mykiss	**C**	.	G	C	**T**	**T**	.	**C**	C	T	A	C	C	G	A	C	G	C	**C**	C	.	T	.	**C**	A	.
O. tshawytscha	.	**A**	G	C	.	C	.	.	C	T	A	C	C	G	A	C	G	C	.	C	.	T	.	.	A	**T**

	813	819	822	831	834	843	846	861	873	885	888	891	909	915	930	957	993	1005	1017	1024	1029	1035	1044	1053	1062	1065
O. gorbuscha	G	A	**A**	A	**G**	**C**	G	T	**C**	C	G	**G**	C	**C**	**A**	A	**G**	**G**	**C**	C	**A**	**C**	T	**C**	**G**	**C**
O. kisutch	.	.	C	.	A	A	**A**	**C**	T	**T**	.	A	.	T	G	**G**	A	A	T	.	G	T	.	**A**	A	T
O. mykiss	**A**	**G**	C	.	A	**G**	.	.	T	**A**	.	A	**A/T**	T	G	.	A	A	T	**T**	G	T	**C**	T	A	T
O. tshawytscha	.	.	C	**G**	A	A	.	**A**	T	.	**A**	A	.	T	G	.	A	A	T	.	G	T	.	T	A	T

	1068	1083	1098	1099	1122	1125	1146	1152	1188	1194	1208	1209	1212	1215	1224	1227	1275	1278	1287	1293	1299	1302	1305	1326	1332	1344
O. gorbuscha	**C**	**T**	T	**T**	**C**	**C**	**G**	A	A	**C**	**T**	C	C	T	**G**	A	**C**	C	**C**	**A**	C	A	G	C	**G**	**G**
O. kisutch	T	C	**C**	C	T	T	C	**G**	.	G	A	.	.	.	A	**G**	T	**T**	T	.	**T**	**T**	.	.	A	A
O. mykiss	T	C	.	C	T	T	**T**	.	**G**	G	A	.	.	**C**	A	.	T	.	T	.	.	.	.	**T**	A	A
O. tshawytscha	T	C	.	C	T	T	C	.	.	G	A	**T**	**A**	.	A	.	T	.	T	**G**	.	.	**A**	.	A	A

	1347	1359	1368	1380	1384	1443	1459	1470	1491	1500	1506	1515	1518	1524	1527	1536	1541	1546
O. gorbuscha	**A**	G	C	C	T	**A**	**A**	**T**	A	**G**	C	C	G	A	**G**	A	**G**	**T**
O. kisutch	G	.	.	.	.	G	T	A	**G**	A	**T**	.	.	.	A	.	A	A
O. mykiss	G	**A**	.	**A**	**C**	G	**C**	A	.	A	.	**T**	**A**	.	A	.	A	A
O. tshawytscha	G	.	**T**	.	.	G	T	A	.	A	.	.	.	**G**	A	**G**	A	A

Table VI. – Diagnostic sites determined on the Cytb marker for *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* and *O. tshawytscha*. Diagnostic sites are in bold. The L14912-CYB/H15149-CYB fragments (Minamoto *et al.*, 2012) between the positions 48 and 706 is highlighted in grey.

												L14912-CYB/H15149-CYB															
	24	72	78	81	108	115	120	121	126	147	165	168	189	198	204	207	219	222	234	240	243	252	258	276	288	300	309	315
O. gorbuscha	T	**A**	C	C	C	**T**	**C**	**C**	C	**A**	**C**	**T**	T	C	C	C	**C**	**C**	**A**	**T**	C	**T**	A	T	**G**	**T/G**	**T**	**G/A**
O. kisutch	.	C	.	.	.	C	T	T	.	C	T	C	.	.	**T**	.	T	T	C	A	.	A	.	.	A	A	.	C
O. mykiss	**C**	**T**	**T**	.	**A**	C	T	T	**T**	C	T	C	**C**	**T**	.	.	T	T	C	A	.	A	.	**C**	A	A	**C**	C
O. tshawytscha	.	C	.	**T**	.	C	T	T	.	C	T	C	.	.	.	**T**	T	T	C	A	**T**	A	**G**	.	A	A	.	C

	L14912-CYB/H15149-CYB																											
	318	324	341	345	378	381	384	387	390	414	417	447	450	453	462	465	468	471	489	492	498	501	513	522	549	558	567	573
O. gorbuscha	A	**A**	**G**	C	T	**A**	**C**	A	C	**G**	**C**	**T**	**T**	**C**	C	**T**	**G**	**C**	**G**	T	**C**	**A**	**T**	**C**	**T**	**T**	**C**	T
O. kisutch	.	C	A	.	.	C	T	**G**	**A**	A	.	C	C	.	A	C	A	A	A	.	.	G	C	T	C	C	T	.
O. mykiss	**G**	C	A	.	.	C	T	.	.	A	**A**	C	C	**A**	C/A	C	A	A	A	.	**G**	**C**	C	T	C	C	T	**C**
O. tshawytscha	**T**	C	A	**T**	**C**	C	T	.	.	A	.	C	C	.	**G**	C	A	A	A	**C**	.	G	C	T	C	C	T	T

	579	591	624	627	630	639	645	657	660	663	666	684	690	693	708	711	714	717	723	729	732	744	748	750	774	777	783	786
O. gorbuscha	A	**C**	**G**	A	G	C	C	**G**	**T**	C	T	**C**	**C**	**G**	T	**T**	T	A	**A**	**C**	**A**	C	**T**	A	C	**T**	**A**	**G**
O. kisutch	.	T	A	**G**	.	.	.	C	C	.	.	T	A	A	.	C	.	**G**	C	T	T	.	C	.	.	C	C	.
O. mykiss	**G**	T	**T**	.	.	**T**	**T**	**A**	C	.	.	T	A	A	**C**	**A**	**C**	.	C	T	T	**T**	C	.	**T**	C	C	**A**
O. tshawytscha	.	T	A	.	**C**	.	.	C	C	**T**	**C**	T	A	A	.	C	.	.	C	T	T	.	C	**G**	.	C	C	.

	789	795	804	816	819	825	831	840	849	852	876	891	903	906	912	915	918	927	943	945	960	970	975	978	981	982	984	987
O. gorbuscha	C	**G**	C	A	**T**	A	T	**T**	T	C	C	**C**	T	G	C	C	**T**	**A**	**T**	A	**A**	T	T	**A**	**T**	T	A	A
O. kisutch	.	A	.	**G**	C	**G**	**C**	C	.	.	.	G	.	.	**A**	**A**	C	**C**	C	**G**	.	**C**	**C**	G	C	.	**G**	**G**
O. mykiss	**G**	A	**T**	.	C	.	.	C	**C**	.	**T**	G	**C**	.	.	.	C	T	C	T/A	**C**	.	.	G	C	.	.	.
O. tshawytscha	.	A	.	.	C	.	.	C	.	**T**	.	G	.	**A**	**T**	**T**	C	T	C	.	**G**	.	.	G	C	**C**	.	.

	1003	1017	1026	1029	1035	1038	1044	1047	1053	1057	1059	1065	1068	1071	1083	1090	1098	1116	1119
O. gorbuscha	C	A	C	A	**T**	**G**	C	T	C	G	T	**A**	A	C	**T**	G	**T**	**A**	A
O. kisutch	**T**	**G**	.	**G**	C	A	.	.	.	.	.	T	**G**	.	C	.	**A**	C	.
O. mykiss	.	.	**T**	.	C	**C**	**T**	.	**T/A**	.	**C**	T	.	**T**	C	.	**C**	C	.
O. tshawytscha	.	.	.	.	C	A	.	**C**	.	**A**	.	T	.	.	C	**A**	**G**	C	**G**

the exact same sequence for two (12V-5) or all three (Teleo) other species making their distinction impossible. For the other markers used for 16S, COI and Cytb genes, all species are distinguishable (Tables IV-VI).

Thus, this mitogenome can be used as a sequence of reference for different molecular expertise using multiple markers such as eDNA or control of mislabelling food (Wang *et al.*, 2021).

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REFERENCES

Arizono N., Yamada M., Nakamura-Uchiyama F. & Ohnishi K., 2009. *Diphyllobothriasis* associated with eating raw Pacific salmon. *Emerg. Infect. Dis.*, 15(6): 866-870. https://doi.org/10.3201/eid1506.090132

Armstrong J. D., Bean C. W. & Wells A., 2018. The Scottish invasion of pink salmon in 2017. *J. Fish Biol.*, 93: 8-11. https://doi.org/10.1111/jfb.13680

Baglinière J.-L., Josset Q. & Beaulaton L., 2020. Le saumon à bosse ou saumon rose. *In*: Keith P., Poulet N., Denys G., Changeux T., Feunteun E. & Persat H. (Eds), Les Poissons d'eau douce de France. Mèze, Biotope éditions ; Paris, Publications scientifiques du Muséum national d'Histoire naturelle: 512-513.

Beaulaton L., Josset Q. & Baglinière J.-L., 2021. Le Saumon rose (*Oncorhynchus gorbuscha*, Walbaum, 1792) – Conduite à tenir et éléments d'écologie. Report OFB, INRAE, AGROCAMPUS OUEST, Université de Pau et des pays de l'Adour/E2S UPPA: 25 p.

Berry T. E., Osterrieder S. K., Murray D. C., Coghlan M. L., Richardsron A. J., Grealy A. K., Stat M., Beider L. & Bunce M., 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecol. Evol.*, 7: 5435-5453. https://doi.org/10.1002/ece3.3123

Bonnyaud M. & Denys G., 2021. Première occurrence de deux espèces invasives de poissons dans le fleuve Somme : le Saumon rose du Pacifique et le Gobie demi-lune. *L'Avocette*, 45(2): 27-35.

Brykov V. A., Polyakova N., Skurikhina L. A., Kukhlevsky A. D., Kirillova O. N., Churikov D., Pudovkin A. I. & Gharrett A. J., 1999. Analysis of mtDNA indicates weak temporal genetic heterogeneity in pink salmon spawning runs in two rivers on Sakhalin Island. *J. Fish Biol.*, 55: 617-635. https://doi.org/10.1111/j.1095-8649.1999.tb00703.x

Churikov D. & Gharrett A. J., 2002. Comparative phylogeography of the two pink salmon broodlines: an analysis based on a mitochondrial DNA genealogy. *Mol. Ecol.*, 11: 1077-1101. https://doi.org/10.1046/j.1365-294X.2002.01506.x

Crespi B. J. & Fulton M. J., 2004. Molecular systematics of Salmonidae: combined nuclear data yields a robust phylogeny. *Mol. Phylogenet. Evol.*, 31: 658-679. https://doi.org/10.1016/j.ympev.2003.08.012

Darriba D., Taboada G. L., Doallo R. & Posada D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*, 9: 772. https://doi.org/10.1038/nmeth.2109

Denys G. P. J., Secci-Petretto G. & Gomes Dos Santos A., 2020. The complete mitochondrial genome of *Thymallus thymallus* (Linnaeus, 1758) (Actinopterygii, Salmonidae) obtained by long range PCRs and double multiplexing. *Cybium*, 44(2): 91-94. https://doi.org/10.26028/cybium/2020-442-001

Duda J. J., Hoy M. S., Chase D. M., Pess G.R., Brenkman S. J., Mchenry M. M. & Ostberg C. O., 2021. Environmental DNA is an effective tool to track recolonizing migratory fish following large-scale dam removal. *Environ. DNA*, 3: 121-141. https://doi.org/10.1002/edn3.134

Duke E. M. & Burton R. S., 2020. Efficacy of metabarcoding for identification of fish eggs evaluated with mock communities. *Ecol. Evol*., 10: 3463-3476. https://doi.org/10.1002/ece3.6144

Dunlop K., Eloranta A. P., Schoen E., Wipfli M., Jensen J. L. A., Muladal R. & Christense G. N., 2021. Evidence of energy and nutrient transfer from invasive pink salmon (*Oncorhynchus gorbuscha*) spawners to juvenile Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) in northern Norway. *Ecol. Freshw. Fish*, 30: 270-283. https://doi.org/10.1111/eff.12582

Froese R. & Pauly D., 2022. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2022).

Gargan L. M., Mo T. A., Carlsson J. E. L., Ball B., Fossøy F. & Carlsson J., 2022. Development of an environmental DNA assay and field validation for the detection of invasive pink salmon *Oncorhynchus gorbuscha*. *Environ. DNA*, 4: 284-290. https://doi.org/10.1002/edn3.250

Gong L., Liu L.-Q., Guo B.-Y., Ye Y.-Y. & Lü Z.-M., 2017. The complete mitochondrial genome of *Oncorhynchus masou formosanus* (Salmoniformes: Salmonidae) and phylogenetic studies of Salmoninae. *Conserv. Genet. Resour.*, 9: 281-284. https://doi.org/10.1007/s12686-016-0673-1

Heard W. R., 1991. Life history of Pink salmon *Oncorhynchus gorbuscha*. *In*: Groot C. & Marcolis (eds), Pacific Salmon Life Histories. Vancouver, UBC Press: 119-230.

Hebert P. D. N., Ratnasingham S. & De Waard J. R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B.*, 270: S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Hinsinger D. D., Debruyne R., Thomas M., Denys G. P. J., Mennesson M., Utge M. & Dettai A., 2015. Fishing for barcodes in the Torrent: from COI to complete mitogenomes on NGS platforms. *DNA Barcodes*, 3: 170-186. https://doi.org/10.1515/dna-2015-0019

Horreo J. L., 2017. Revisiting the mitogenomic phylogeny of Salmoninae: new insights thanks to recent sequencing advances. *PeerJ*, 5: e3828. https://doi.org/10.7717/peerj.3828

Iwasaki W., Fukunaga T., Isagozawa R., Yamada K., Maeda Y., Satoh T. P., Sado T., Mabuchi K., Takeshima H., Miya M. & Nishida M., 2013. MitoFish and MitoAnnotator: A mitochondrial genome database of fish with an accurate and automatic annotation pipeline. *Mol. Biol. Evol*., 30: 2531-2540. https://doi.org/10.1093/molbev/mst141

Josset Q., Flesselle A., Bernardin A., Macquet T. & Petit L., 2021. Rapport d'activité de l'Observatoire Long Terme de la Bresle – Année 2020. Technical report of the Pôle Gestion des Migrateurs Amphihalins dans leur Environnement: 49 p.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheunung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P. & Drummond A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28: 1647-1649. https://doi.org/10.1093/bioinformatics/bts199

Kühn A.L. & Haase M., 2019. QUIDDICH: QUick IDentification of DIagnostic CHaracters. *J. Zool. Syst. Evol. Res*., 58(1): 22-26. https://doi.org/10.1111/jzs.12347

Leray M., Yang J. Y., Meyer C. P., Mills S. C., Agudelo N., Ranwez V., Boehm J. & Machida R. J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front. Zool.,* 10: 34. https://doi.org/10.1186/1742-9994-10-34

Marchand F., Aymes J.-C., Gueraud F., Domaizon I., Goulon C., Hamelet V., Lange F., Prevost E., Baglinière J.-L., Beaulaton L., Penil C., Josset Q., Flesselle A., Starck A., Jeannot N., Herrard N., Martignon M. & Azam D., 2018 Colisa, the collection of ichthyological samples. https://colisa.fr/. https://doi.org/10.15454/D3ODJM

Miller M. A., Pfeiffer W. & Schwartz T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: 2010 Gateway Computing Environments Workshop (GCE). Ieee: p. 1-8. https://doi.org/10.1109/GCE.2010.5676129

Minamoto T., Yamanaka H., Takahara T., Honio M. N. & Kawabata Z., 2012. Surveillance of fish species composition using environmental DNA. *Limnology*, 13: 193-197. https://doi.org/10.1007/s10201-011-0362-4

Miya M., Sato Y., Fukunaga T., Sado T., Poulsen J. Y., Sato K., Minamoto T., Yamamoto S., Yamanaka H., Araki H., Kondoh M. & Iwasaki W., 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. Open Sci*., 2: 150088. https://doi.org/10.1098/rsos.150088

Mizumoto H., Urabe H., Kanbe T., Fukushima M. & Araki H., 2018. Establishing an environmental DNA method to detect and estimate the biomass of Sakhalin taimen, a critically endangered Asian salmonid. *Limnology*, 19: 219-2277. https://doi.org/10.1007/s10201-017-0535-x

Mo T. A., Thorstad E. B., Sandlund O. T., Berntsen H. H., Fiske P. & Uglem I., 2018. The pink salmon invasion: a Norwegian perspective. *J. Fish Biol*., 93: 5-7. https://doi.org/10.1111/jfb.13682

Persat H., Keith P. & Denys G., 2020. Biogéographie et historique de la mise en place des peuplements ichtyologiques de France métropolitaine. *In*: Keith P., Poulet N., Denys G., Changeux T., Feunteun E. & Persat H., (Eds), Les Poissons d'Eau douce de France. Mèze, Biotope éditions ; Paris, Publications scientifiques du Muséum national d'Histoire naturelle: 43-97.

Podlesnykh A. V., Kukhlevsky A. D. & Brykov V. A., 2020. A comparative analysis of mitochondrial DNA genetic variation and demographic history in populations of even- and odd-year broodline pink salmon, *Oncorhynchus gorbuscha* (Walbaum, 1792), from Sakhalin Island. *Environ. Biol. Fish*, 103: 1553-1564. https://doi.org/10.1007/s10641-020-01040-0

R Core Team, 2022. R: A language and environment for statistical computing. Vienna: R Development Core Team. Available at http://www.R-project.org/

Rasputina E. N., Shustov Y. A. & Tyrkin I. A., 2016. Eggs of pink salmon *Oncorhynchus gorbuscha* as additional non-traditional food of juvenile Atlantic salmon *Salmo salar* in rivers of the Kola Peninsula. *Russ. J. Biol. Invasions*, 7: 294-296. https://doi.org/10.1134/S2075111716030115

Riaz T., Shehzad W., Viari A., Pompanon F., Taberlet P., Coissac E., 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Res*., 39(21): e145. https://doi.org/10.1093/nar/gkr732

Sato S. & Urawa S., 2017. Genetic variation of Japanese pink salmon populations inferred from nucleotide sequence analysis on the mitochondrial DNA control region. *Environ. Biol. Fish*, 100: 1355-1375. https://doi.org/10.1007/s10641-017-0648-4

Schroeter J. C., Maloy A. P., Rees C. B. & Bartron M. L., 2020. Fish mitochondrial genome sequencing: expanding genetic resources to support species detection and biodiversity monitoring using environmental DNA. *Conserv. Genet. Resour.*, 12: 433-446. https://doi.org/10.1007/s12686-019-01111-0

Scott W. B. & Crossman E. J., 1973. Freshwater Fishes of Canada. Ottawa, Fisheries Research Board of Canada: 966 p.

Sepulveda A. J., Nelson N. M., Jerde C. L. & Luikart G., 2020. Are environmental DNA methods ready for aquatic invasive species management? *Trends Ecol. Evol.*, 35: 668-678. https://doi.org/10.1016/j.tree.2020.03.011

Shedko S. V., Miroschnichenko I. L. & Namkova G. A., 2012. Phylogeny of Salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of nuclear RAG1 gene. *Rus. J. Genet.*, 48: 575-579. https://doi.org/10.1134/S1022795412050201

Shedko S. V., Miroschnichenko I. L. & Namkova G. A., 2013. Phylogeny of Salmonids (Salmoniformes: Salmonidae) and its molecular dating: analysis of mtDNA data. *Rus. J. Genet.*, 49: 718-734. https://doi.org/10.1134/S1022795413060112

Stamatakis A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9): 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Strohm J. H. T., Gwiazdowski R. A. & Hanner R., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mitochondrial DNA*, 27: 3263-3269. https://doi.org/10.3109/19401736.2015.1015003

Taberlet P., Bonin A., Coissac E. & Zinger L., 2018. Environmental DNA: For Biodiversity Research and Monitoring. Oxford, Oxford University Press: 253 p. https://doi.org/10.1093/oso/9780198767220.001.0001

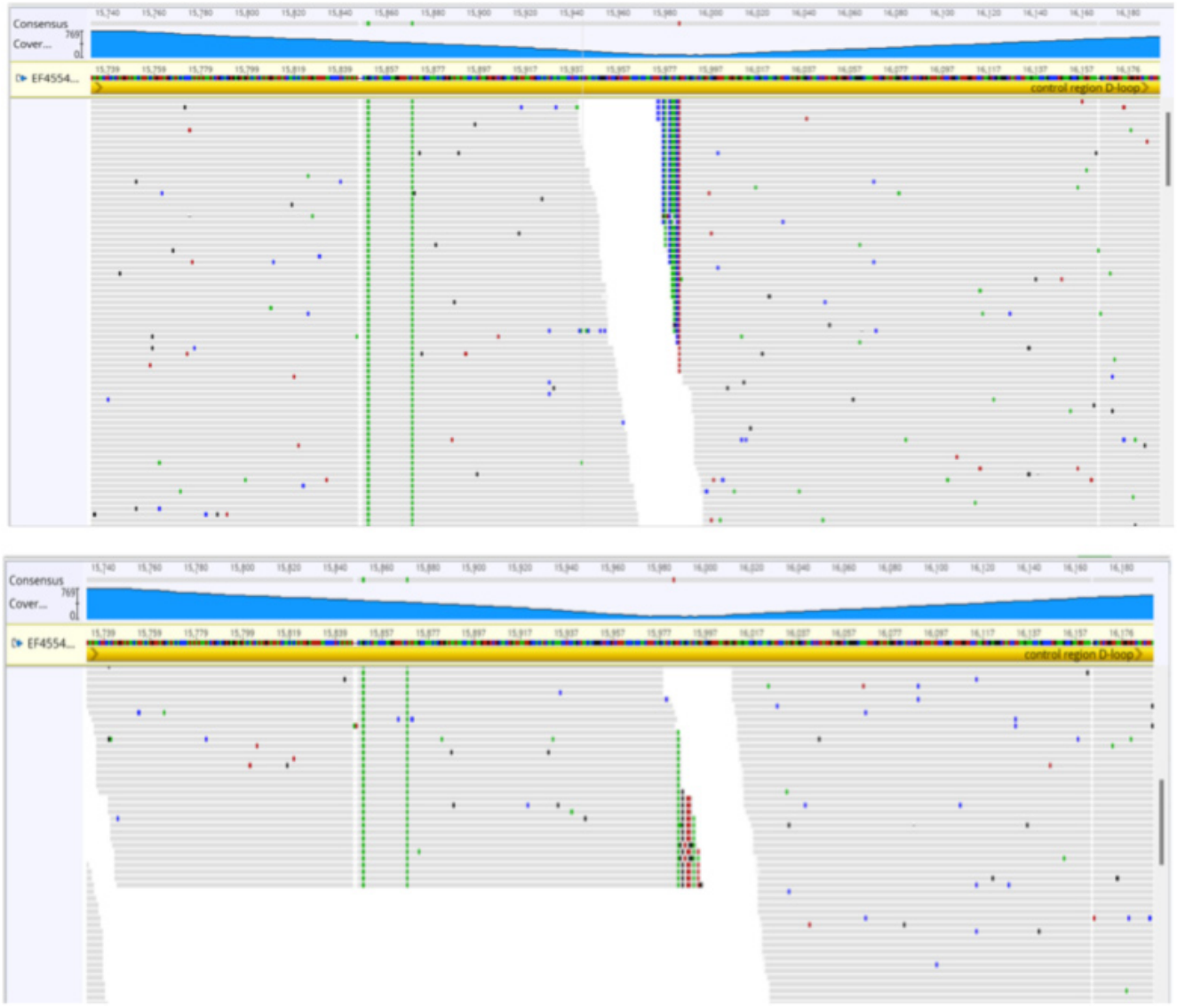
Valentini A., Taberlet P., Miaud C., Civade R., Herder J., Thomsen P. F., Bellemain E., Besnard A., Coissac E., Boyer F., Gaboriaud C., Jean P., Poulet N., Roset N., Copp G. H., Geniez P., Pont D., Argillier C., Baudoin J. M., Peroux T., Crivelli A. J., Olivier A., Acqueberge M., Le Brun M., Møller P. R., Willerslev E. & Dejean T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.*, 25(4): 929-942. https://doi.org/10.1111/mec.13428

Van Der Veer G. & Nentwig W., 2015. Environmental and economic impact assessment of alien and invasive fish species in Europe using the generic impact scoring system. *Ecol. Freshw. Fish*, 24: 646-656. https://doi.org/10.1111/eff.12181

Wang N., Xing R.-R., Zhou M.-Y., Sun R.-X., Han J.-X., Zhang J.-K., Zheng W.-J. & Chen Y., 2021. Application of DNA barcoding and metabarcoding for species identification in salmon products. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.*, 38(5): 754-768. https://doi.org/10.1080/19440049.2020.1869324

Ward R. D., Zemlak T. S., Innes B. H., Last P. R. & Hebert P. D. N., 2005. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B Biol. Sci.*, 360: 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Appendix 1. – Screen captures of the problematic map to reference mapping around position 15985 of sequence EF455489. Two screen captures of reads of the same area are presented (second is simply scrolled down from the first), as the high number of reads did not allow for a clear single picture. No read overlaps both sides at once without mismatch, causing coverage to drop for this mapping. Allowing free reconstruction using elongation from one of the sides recovers a different sequence with high, unambiguous coverage.



Appendix 2. – GenBank accession numbers for the sequences used as comparison material for the four loci used in metabarcode studies (12S, 16S, COI, CytB) for *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* and *O. tshawytscha*.
O. gorbuscha: PP272106, CM029873, EF455489; *O. kisutch*: EF126369, MF621749, MF621751, MH003640; *O. mykiss*: AF392054, DQ288268 to DQ288271, HQ167664, HQ167694, HQ167682, KP013084, KP085590, KY798500, MF621750, MT410879, MT667254, MZ2567214; *O. tshawytscha*: AF392054, HQ167665

Appendix 3. – Gene composition of the complete mitochondrial genome of *Oncorhynchus gorbuscha* (GenBank Accession Number PP272106) detailing position, length and direction.

Name	Type	First nucleotide	Last nucleotide	Length (bp)	Direction
tRNA-Phe	tRNA	1	68	68	Forward
12S rRNA	rRNA	69	1015	947	Forward
tRNA-Val	tRNA	1016	1087	72	Forward
16S rRNA	rRNA	1088	2765	1678	Forward
tRNA-Leu	tRNA	2766	2840	75	Forward
NADH1 gene	gene	2841	3815	975	Forward
tRNA-Ile	tRNA	3819	3890	72	Forward
tRNA-Gln	tRNA	3888	3958	71	Reverse
tRNA-Met	tRNA	3958	4026	69	Forward
NADH2 gene	gene	4027	5076	1050	Forward
tRNA-Trp	tRNA	5078	5149	72	Forward
tRNA-Ala	tRNA	5151	5219	69	Reverse
tRNA-Asn	tRNA	5221	5293	73	Reverse
tRNA-Cys	tRNA	5328	5393	66	Reverse
tRNA-Tyr	tRNA	5394	5464	71	Reverse
COI gene	gene	5466	7016	1551	Forward
tRNA-Ser	tRNA	7017	7087	71	Reverse
tRNA-Asp	tRNA	7092	7165	74	Forward
COII gene	gene	7180	7870	691	Forward
tRNA-Lys	tRNA	7871	7944	74	Forward
ATP8 gene	gene	7946	8113	168	Forward
ATP6 gene	gene	8114	8786	673	Forward
COIII gene	gene	8787	9551	765	Forward
tRNA-Gly	tRNA	9572	9641	70	Forward
NADH3 gene	gene	9642	9990	349	Forward
tRNA-Arg	tRNA	9991	10060	70	Forward
NADH4L gene	gene	10061	10357	297	Forward
NADH4 gene	gene	10351	11731	1381	Forward
tRNA-His	tRNA	11732	11800	69	Forward
tRNA-Ser	tRNA	11801	11869	69	Forward
tRNA-Leu	tRNA	11871	11943	73	Forward
NADH5 gene	gene	11944	13782	1839	Forward
NADH6 gene	gene	13779	14300	522	Reverse
tRNA-Glu	tRNA	14301	14369	69	Reverse
CYTB gene	gene	14373	15513	1141	Forward
tRNA-Thr	tRNA	15514	15585	72	Forward
tRNA-Pro	tRNA	15585	15654	70	Reverse
control region D-loop	D-loop	15655	16695	1041	Forward