Comparison of whole-genome assemblies of European river lamprey (*Lampetra fluviatilis*) and brook lamprey (*Lampetra planeri*)

Authors: Ole K. Tørresen[1*], Benedicte Garmann-Aarhus[1,2*], Siv Nam Khang Hoff[1], Sissel Jentoft[1], Mikael Svensson[3], Eivind Schartum[4], Ave Tooming-Klunderud[1], Morten Skage[1], Anders Krabberød[1,5], Leif Asbjørn Vøllestad[1], Kjetill S. Jakobsen[1,§]
Affiliations:
[1]Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of Oslo

[2]Current address: Natural History Museum, University of Oslo, Oslo, 0562, Norway

[3]SLU Artdatabanken, SLU - Swedish Species Information Centre, Box 7007, SE-750 07 Uppsala, Sweden

[4]Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Bø i Telemark, Norway

[5]Section for Genetics and Evolutionary Biology, Department of Biosciences, University of Oslo, Norway

*equal contributions

§corresponding author

Abstract
We present haplotype-resolved whole-genome assemblies from two individuals of the sister species the European river lamprey (*Lampetra fluviatilis*) and the brook lamprey (*Lampetra planeri*). The genome assemblies for *L. fluviatilis* consists of pseudo-haplotype one, spanning 1073 megabases and 963 megabases for pseudo-haplotype two. For *L. planeri*, the genome assemblies span 1049 megabases and 960 megabases for pseudo-haplotypes one and two, respectively. The river lamprey assemblies have been scaffolded into 82 pseudochromsomes for both pseudo-haplotypes, with the same number for *L. planeri*. All four haplotype assemblies were annotated, identifying 21,479 and 16,973 genes in pseudo-haplotypes one and two for *L. fluviatilis*, and 24,961 and 21,668 genes in pseudo-haplotypes one and two for *L. planeri*. A comparison of the genomes of *L. fluviatilis* and *L. planeri*, alongside a separate chromosome level assembly of *L. fluviatilis* from the UK, indicates that they form a species complex, potentially representing distinct ecotypes. This is further supported by phylogenetic analyses of the three reference *Lampetra* genomes in addition to sea lamprey.

Keywords: *Lampetra fluviatilis*, *Lampetra planeri*, European river lamprey, brook lamprey, genome sequence, chromosomal assemblies

Introduction
Freshwater fish reside in lakes, rivers, and streams and often migrate between different habitats, such as between rivers and lakes, and sometimes also to marine environments (called diadromous fishes). In particular, in postglacial lakes many species show large phenotypic plasticity and also possess many morphotypes - sometimes regarded as different species. Determining what constitutes a species has been challenging for many freshwater groups, particularly within the Salmoniformes, such as trout, charr, and whitefish (Whiteley et al., 2019). However, this is clearly not restricted to freshwater fishes; there are also

numerous examples of sister species or complexes in purely marine habitats that are difficult to distinguish. Gauging what constitutes a species has also been difficult in the lamprey family (Petromyzontidae) since many taxa form species pairs (Docker 2009). The pairs often consist of a non-parasitic freshwater-resident species, which matures at a smaller size, alongside a larger migratory (diadromous), parasitic species. The migratory and parasitic European river lamprey (*Lampetra fluviatilis)* and the non-migratory and non-parasitic brook lamprey (*Lampetra planeri*) are regarded as sister species. Despite these two species having been the subject of several genetic studies, using mtDNA (mitochondrial DNA) (Bracken et al., 2015; Cahsan et al., 2020), RADseq (restriction-site associated DNA sequencing) (Hume et al., 2018; Mateus et al., 2013; Rougemont et al., 2017), and microsatellite markers (Rougemont et al., 2015), there is no definitive consensus if these two taxa are separate species, or merely ecotypes, with different life-history traits. While *L. fluviatilis* and *L. planeri* are morphologically and behaviourally similar in their larval stages, sustaining themselves through filter feeding at the bottom of freshwater streams for the first five to seven years of their lives (Potter et al., 2015; Rougemont et al., 2015), they differ greatly upon entering maturity. *L. planeri* develops eyes and the characteristic lamprey sucker mouth, degenerating its gut and stops feeding, only to mate and die in the freshwater where it has spent its entire life (Rougemont et al., 2015). In contrast, *L. fluviatilis,* following metamorphosis, enters a migratory and often anadromous, parasitic juvenile life stage, where it migrates to lakes or the sea to feed on larger fish. For up to three years, the juvenile *L. fluviatilis* lives as a parasite (Kelly and King, 2001; Rougemont et al., 2016) and returns at sexual maturity to running water to mate and die (Kelly and King, 2001; Rougemont et al., 2016). A central unanswered question is whether the morphological and life-history differences between the two species are due to genetics or phenotypic plasticity.

Genetic studies to date have not clearly identified any distinctions that would suggest two separate species or morphological and behavioral diverged ecotypes. It is thus suggested that the *L. fluviatilis/L. planeri* species pair is at different stages of speciation in different locations (Mateus et al., 2016). Therefore, whole genome sequencing at the population level needs to be performed in order to capture not only SNP (single nucleotide polymorphism) variation but also structural variation (such as chromosomal rearrangements, inversions, CNVs [copy-number variations] and STR [short tandem repeat] length variations). Investigations of structural variation in addition to SNPs require high-quality reference genomes for the two sister species. Here, we report two chromosome-level reference genomes of *L. fluviatilis* and *L. planeri*, using long-read PacBio HiFi sequencing and scaffolding with Hi-C to achieve the standards of the Earth BioGenome Project (Lewin et al., 2022) generating pseudo-haplotype-resolved assemblies. Furthermore, we compare the genome assemblies for the two species to each other and to separate chromosome-level assemblies of *L. fluviatilis* as well as the sea lamprey (*Petromyzon marinus*) to shed light on the species versus ecotype discussion.

Methods

Sample acquisition and DNA extraction

In this study, two lamprey specimens, the *L. fluviatilis* and the *L. planeri*, were collected from different locations in Scandinavia. *L. fluviatilis* was caught in Åsdalsåa, Telemark, Norway (59.410917, 9.305889) on 21.04.2021 using electrofishing and transported alive to the University of Oslo. The individual was euthanized in the laboratory using an overdose of methanesulfonate (MS-222) and decapitation. The fish was 170 mm long, and muscle and heart tissues were extracted and snap-frozen in individual Eppendorf tubes using liquid nitrogen. Similarly, *L. planeri* was caught in Hunserödsbäcken, Skåne, Sweden (56.250944,

13.001400) on 27.10.2020 using electrofishing and was euthanized on-site. The whole individual was then stored on 96% ethanol and shipped to Oslo. The fish was 122 mm long, and muscle, skin tissue, gill filaments, and the entire heart were dissected. All tissues from both lampreys were transferred to the Norwegian Sequencing Centre for library preparation and stored at -80 degrees C.

Library preparation and sequencing for *de-novo* assembly

For PacBio HiFi sequencing, DNA was isolated from *L. fluviatilis*'s blood and from *L. planeri*'s muscle and skin tissue. For *L. fluviatilis*, 10-20 µl of fresh blood was used per reaction, and the Circulomics Nanobind CBB Big DNA kit was employed with the blood and tissue protocol, following the manufacturer's guidelines. The high molecular weight DNA was eluted from the Nanodisk with 150µl Tris-Cl buffer and incubated overnight at room temperature. The resulting DNA was then quality-checked for its amount, purity, and integrity using UV-absorbance ratios, Qubit BR DNA quantification assay kit, and Fragment Analyzer with the DNA HS 50kb large fragment kit. In contrast, for *L. planeri*, 30 mg of dry-blotted, EtOH-stored muscle and skin tissue was used per reaction. The same isolation protocol was followed as for *L. fluviatilis* with an additional step of incubation with proteinase K for two hours at room temperature, followed by RNAse for an additional 30 minutes of incubation. The quality of the isolated DNA was then assessed using the same methods as for *L. fluviatilis*.

Both *L. fluviatilis* and *L. planeri* underwent PacBio HiFi sequencing. The Norwegian Sequencing Centre conducted the sequencing protocols for both species. For *L. fluviatilis*, two libraries from muscle tissue were prepared following the Pacific Biosciences protocol "Preparing HiFi SMRTbell® Libraries using the SMRTbell Express Template Prep Kit 2.0". The size selection for the final libraries, involving the removal of suboptimal nucleic fragments, was determined using BluePippin with an 11 kb cut-off (Wang et al., 2021) and was sequenced on three 8M SMRTcells on Sequel II. Similarly, for *L. planeri*, two libraries were prepared from muscle and skin tissues using the Pacific Biosciences protocol mentioned earlier. BluePippin with an 11 kb cut-off determined the size selection for the final libraries before being sequenced on three M SMRT cells in the PacBio Sequel II.

Both the *L. fluviatilis* and *L. planeri* underwent Hi-C sequencing to capture their three-dimensional chromatin structures. For *L. fluviatilis,* the library preparation followed the "Omni-C Proximity Ligation assay for Non-mammalian samples, version 1.0" protocol. This involved grinding 20 mg of fresh, snap-frozen heart tissue to a fine powder, followed by lysis and proximity ligation. The prepared library was then sequenced on the NovaSeq 6000 Sequencing System at the Norwegian Sequencing Centre, using one full S Prime NovaSeq Flow Cell for 2 x 150 bp paired-end sequencing.

Similarly, for the *L. planeri*, 100 mg of gill tissue stored in ethanol was utilized, and the library was prepared using the "Arima Genome-Wide HiC+ Kit" and the "Arima-HiC 2.0 kit standard user guide for Animal tissues"-protocol. The sequencing was carried out on the NovaSeq 6000 at the Norwegian Sequencing Centre, utilizing one quarter of a NovaSeq Flow Cell for 2 x 150 bp paired-end sequencing.

Genome assembly and curation, annotation, and evaluation

A full list of relevant software tools and versions is presented in Table 1. *KMC* (Kokot et al., 2017) was used to count k-mers of size 21 in the PacBio HiFi reads, excluding k-mers occurring more than 10,000 times. *GenomeScope* (Ranallo-Benavidez et al., 2020) was run

on the k-mer histogram output from *KMC* to estimate genome size, heterozygosity, and repetitiveness, while ploidy level was investigated using *Smudgeplot* (Ranallo-Benavidez et al., 2020). *HiFiAdapterFilt* (Sim et al., 2022) was applied on the HiFi reads to remove possible remnant PacBio adapter sequences. The filtered HiFi reads were assembled using *hifiasm* (Cheng et al., 2021) with Hi-C integration resulting in a pair of haplotype-resolved assemblies, pseudo-haplotype one (hap1) and pseudo-haplotype two (hap2) for each species. Unique k-mers in each assembly/pseudo-haplotype were identified using *meryl* (Rhie et al., 2020) and used to create two sets of Hi-C reads, one without any k-mers occurring uniquely in hap1 and the other without k-mers occurring uniquely in hap2. K-mer filtered Hi-C reads were aligned to each scaffolded assembly using *BWA-MEM* (Li, 2013) with -5SPM options. The alignments were sorted based on name using samtools (Li et al., 2009) before applying *samtools fixmate* to remove unmapped reads and secondary alignments and to add mate score, and *samtools markdup* to remove duplicates. The resulting BAM files were used to scaffold the two assemblies using *YaHS* (Zhou et al., 2022) with default options. *FCS-GX* (Astashyn et al., 2023) was used to search for contamination. Contaminated sequences were removed. If a contaminant was detected at the start or end of a sequence, the sequence was trimmed using a combination of *samtools faidx*, *bedtools* (Quinlan and Hall, 2010) *complement*, and *bedtools getfasta*. If the contaminant was internal, it was masked using *bedtools maskfasta*. The mitochondrion was searched for in contigs and reads using *MitoHiFi* (Uliano-Silva et al., 2023). *Merqury* (Rhie et al., 2020) was used to assess the completeness and quality of the genome assemblies by comparing them to the k-mer content of the Hi-C reads. *BUSCO* (Manni et al., 2021) was used to assess the completeness of the genome assemblies by comparing against the expected gene content in the metazoa lineage. We also ran *BUSCO* on sea lamprey (kPetMar1; *P. marinus*; GCA_010993605.1) and another river lamprey (kcLamFluv1; *L. fluviatilis;* GCA_964198585.1). *Gfastats* (Formenti et al., 2022) was used to output different assembly statistics of the assemblies, including kPetMar1 and kcLamFluv1. The assemblies were manually curated using *PretextView*. Chromosomes were identified by inspecting the Hi-C contact map in *PretextView* and named according to homology to kcLamFluv1. *BlobToolKit* and *BlobTools2* (Laetsch and Blaxter, 2017), in addition to *blobtk* were used to visualize assembly statistics. To generate the Hi-C contact map image, the Hi-C reads were mapped to the assemblies using *BWA-MEM* (Li, 2013) using the same approach as above, before *PretextMap* was used to create a contact map which was visualized using *PretextSnapshot*.

Table 1. Software tools: versions and sources

Software tool	**Version**	**Source**
BlobToolKit	4.1.7	https://github.com/blobtoolkit/blobtoolkit
blobtk	0.5.1	https://github.com/blobtoolkit/blobtk
BUSCO	v5.4.7	https://gitlab.com/ezlab/busco
hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm

KMC	v3.1.2rc1	https://github.com/refresh-bio/KMC
GenomeScope	v2.0	https://github.com/tbenavi1/genomescope2.0
HiFiAdapterFilt	v2.0.0	https://github.com/sheinasim/HiFiAdapterFilt
PretextView	0.2.5	https://github.com/wtsi-hpag/PretextView
PretextMap	0.1.9	https://github.com/wtsi-hpag/PretextMap
PretextSnapshot	commit 16b42f2	https://github.com/wtsi-hpag/PretextSnapshot
meryl	1.3.0	https://github.com/marbl/meryl
BWA-MEM	v0.7.17	https://github.com/lh3/bwa
samtools	1.17	https://github.com/samtools/samtools
YaHS	yahs-1.1.91eebc2	https://github.com/c-zhou/yahs
FCS-GX	0.3.0	https://github.com/ncbi/fcs
Merqury	v1.3	https://github.com/marbl/merqury
AGAT	v1.0	https://github.com/NBISweden/AGAT
MitoHiFi	v2.2	https://github.com/marcelauliano/MitoHiFi
miniprot	0.11-r234	https://github.com/lh3/miniprot
GALBA	1.0.6	https://github.com/Gaius-Augustus/GALBA
RED	v2018.09.10	http://toolsmith.ens.utulsa.edu/
Funannotate	v1.8.13	https://github.com/nextgenusfs/funannotate
EvidenceModeler	v1.1.1	https://github.com/EVidenceModeler/EVidenceModeler

DIAMOND	v2.0.15 v2.1.6*	https://github.com/bbuchfink/diamond
InterProScan	v5.47-82	https://www.ebi.ac.uk/interpro/search/sequence/
EMBLmyGFF3	v2.2	https://github.com/NBISweden/EMBLmyGFF3
Flagger	v0.3.2	https://github.com/mobinasri/flagger
winnowmap	2.03	https://github.com/marbl/Winnowmap
Secphase	v0.4.3	https://github.com/mobinasri/secphase
DeepVariant	1.4.0	https://github.com/google/deepvariant
MUMmer	v4.0.0rc1	https://github.com/mummer4/mummer
EMBOSS	6.6.0	https://emboss.sourceforge.net/
OrthoFinder	2.5.5	https://github.com/davidemms/OrthoFinder
MAFFT	7.526	https://mafft.cbrc.jp/alignment/software/
IQ-TREE	2.3.6	http://www.iqtree.org/
ASTRAL-Pro3	1.16.2.4	https://github.com/chaoszhang/ASTER
MCscanX	commit b1ca533	https://github.com/wyp1125/MCScanX
Synvisio	commit 3415935	https://synvisio.usask.ca/#/

We annotated the genome assemblies using a pre-release version of the EBP-Nor genome annotation pipeline (https://github.com/ebp-nor/GenomeAnnotation). First, *AGAT* (https://zenodo.org/record/7255559) *agat_sp_keep_longest_isoform.pl* and *agat_sp_extract_sequences.pl* were used on the sea lamprey (GCA_010993605.1) genome assembly and annotation to generate one protein (the longest isoform) per gene. *Miniprot* (Li, 2023) was used to align the proteins to the curated assemblies. UniProtKB/Swiss-Prot (Consortium et al., 2022) release 2022_03 in addition to the Vertebrata part of OrthoDB v11 (Kuznetsov et al., 2022) were also aligned separately to the assemblies. *Red* (Girgis, 2015) was run via *redmask* (https://github.com/nextgenusfs/redmask) on the assemblies to mask repetitive areas. *GALBA* (Brůna et al., 2023; Buchfink et al., 2015; Hoff and Stanke, 2018; Li, 2023; Stanke et al., 2006) was run with the sea lamprey proteins using the miniprot mode on the masked assemblies. The *funannotate-runEVM.py* script from *Funannotate* was used to run *EvidenceModeler* (Haas et al., 2008) on the alignments of sea lamprey proteins, UniProtKB/Swiss-Prot proteins, Vertebrata proteins and the predicted genes from *GALBA*. The resulting predicted proteins were compared to the protein repeats that *Funannotate* distributes using *DIAMOND blastp*, and the predicted genes were filtered based on this

comparison using *AGAT*. The filtered proteins were compared to the UniProtKB/Swiss-Prot release 2022_03 using *DIAMOND* (Buchfink et al., 2015) *blastp* to find gene names, and *InterProScan* was used to discover functional domains. *AGATs agat_sp_manage_functional_annotation.pl* was used to attach the gene names and functional annotations to the predicted genes. *EMBLmyGFF3* (Norling et al., 2018) was used to combine the fasta files and GFF3 files into an EMBL format for submission to ENA. We also annotated the sea lamprey (kPetMar1; GCA_010993605.1) and another river lamprey (kcLamFluv1; GCA_964198585.1) using the same approach as described here.

To evaluate the diploid assembly, we ran *Flagger* (Liao et al., 2023) to detect possible mis-assemblies. The HiFi reads were mapped to the diploid assembly (created by concatenating the two pseudo-haplotypes) using *winnowmap* (Jain et al., 2022). *Secphase* (Liao et al., 2023) was run on the BAM file produced by *winnowmap* to correct the alignments of the reads by scoring them based on marker consistency and selecting the alignment with the highest score as primary. SNPs were called from the corrected BAM file by *DeepVariant* (Poplin et al., 2018) using default parameters for PacBio HiFi data and filtered to keep only biallelic SNPs. *Flagger* (Liao et al., 2023) was then run on the corrected BAM file together with the filtered VCF and categorized the diploid assembly into erroneous, duplicated, haploid, collapsed, and unknown regions.

To characterize the differences between the different assemblies (both pseudo-haplotypes of both species, in addition to kcLamFluv1), we ran *nucmer* from the *MUMmer* (Marçais et al., 2018) genome alignment system on the homologous chromosomes from the assemblies. The resulting alignments were processed with *dnadiff*, also from *MUMmer*, producing reports listing the number of insertions, SNPs, and indels between the different assemblies. *EMBOSS* (Rice et al., 2000) *infoseq* was used to calculate GC content of the different sequences.

We ran *OrthoFinder* (Buchfink et al., 2015; Emms and Kelly, 2019, 2018, 2017) on the predicted proteins for all the assemblies to infer multiple sequence alignment gene trees. *OrthoFinder* was run with the option msa using *MAFFT* (Katoh and Standley, 2013) as the multiple alignment tool and *IQ-TREE* (Minh et al., 2020) for gene tree inference. We obtained the species tree from the gene trees using *ASTRAL-Pro3* (Zhang and Mirarab, 2022) by optimizing the objective function of *ASTRAL-Pro* (Zhang et al., 2020).

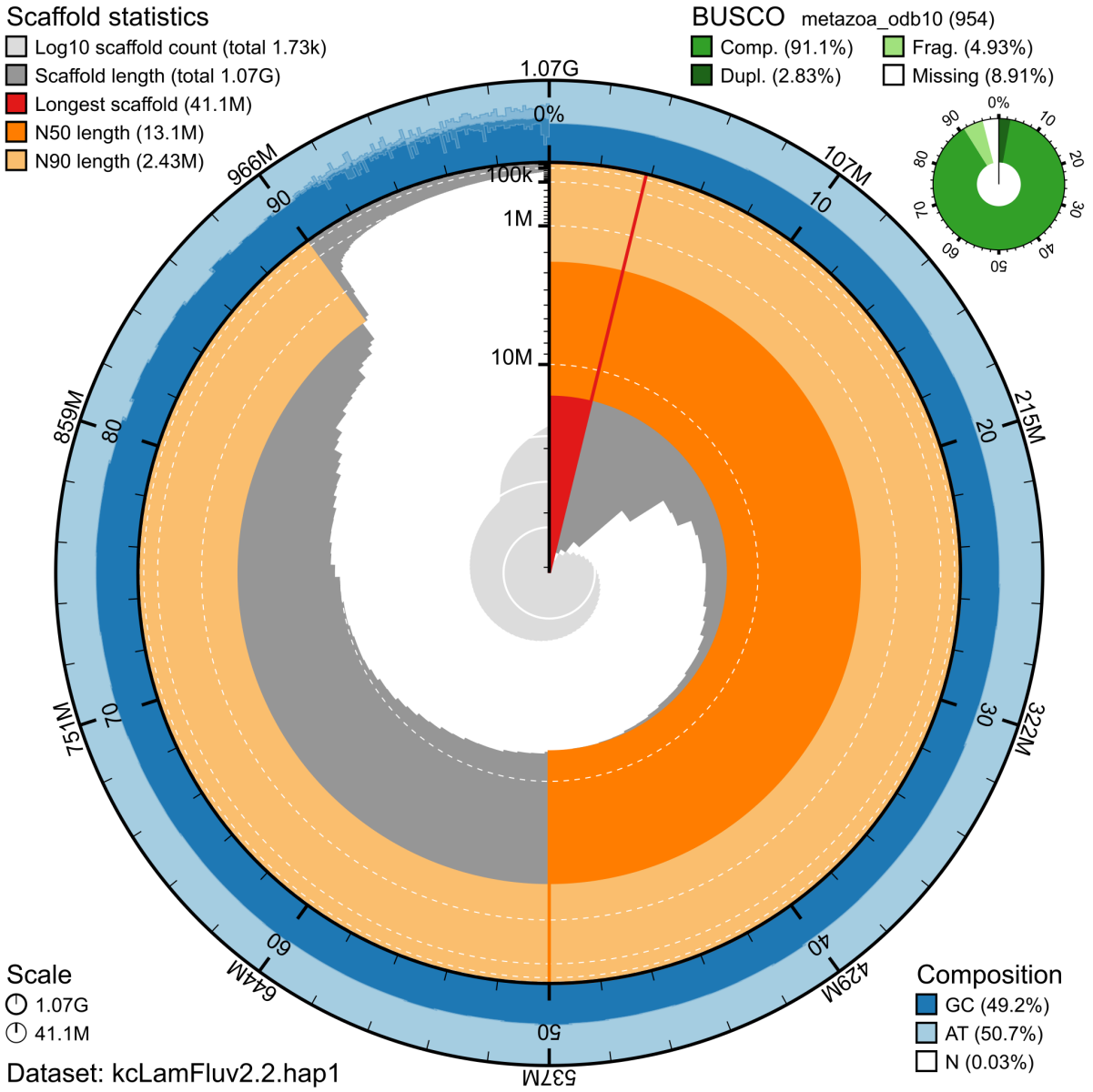
To inspect the syntenic relationship among the genomes between the different species, we ran *MCScanX* (Wang et al., 2012) and visualized the results using *Synvisio (Bandi and Gutwin, 2020)*. First, we used *DIAMOND* blastp* with the options -q ${IN_PROT} -p 16 -e 1e-10 --max-hsps 5 with annotated proteins for kcLamPlan1.1.hap1, kcLamPlan1.1.hap2, kcLamFluv2.1.hap1, kcLamFluv2.1.hap2, kcLamFluv1, and kPetMar1 as input data. Subsequently, *MCScanX* was run with default settings, and results visualized using the online interactive platform *Synvisio*.

Results

De novo genome assembly and annotation

The genome from the European river lamprey (*L. fluviatilis*) had an estimated genome size of 742 Mb, with 1.09% heterozygosity and a bimodal distribution based on the k-mer spectrum (Supplementary Figure 1). The genome from European brook lamprey (*L. planeri*) had an estimated genome size of 720 Mb, with 1.1% heterozygosity and a bimodal distribution based on the k-mer spectrum (Supplementary Figure 2.). A total of 38-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 100-fold coverage in Arima Hi-C reads

resulted in two haplotype-separated assemblies for *L. fluviatilis*, while for *L. planeri* the amounts were 44-fold and 120-fold, respectively.



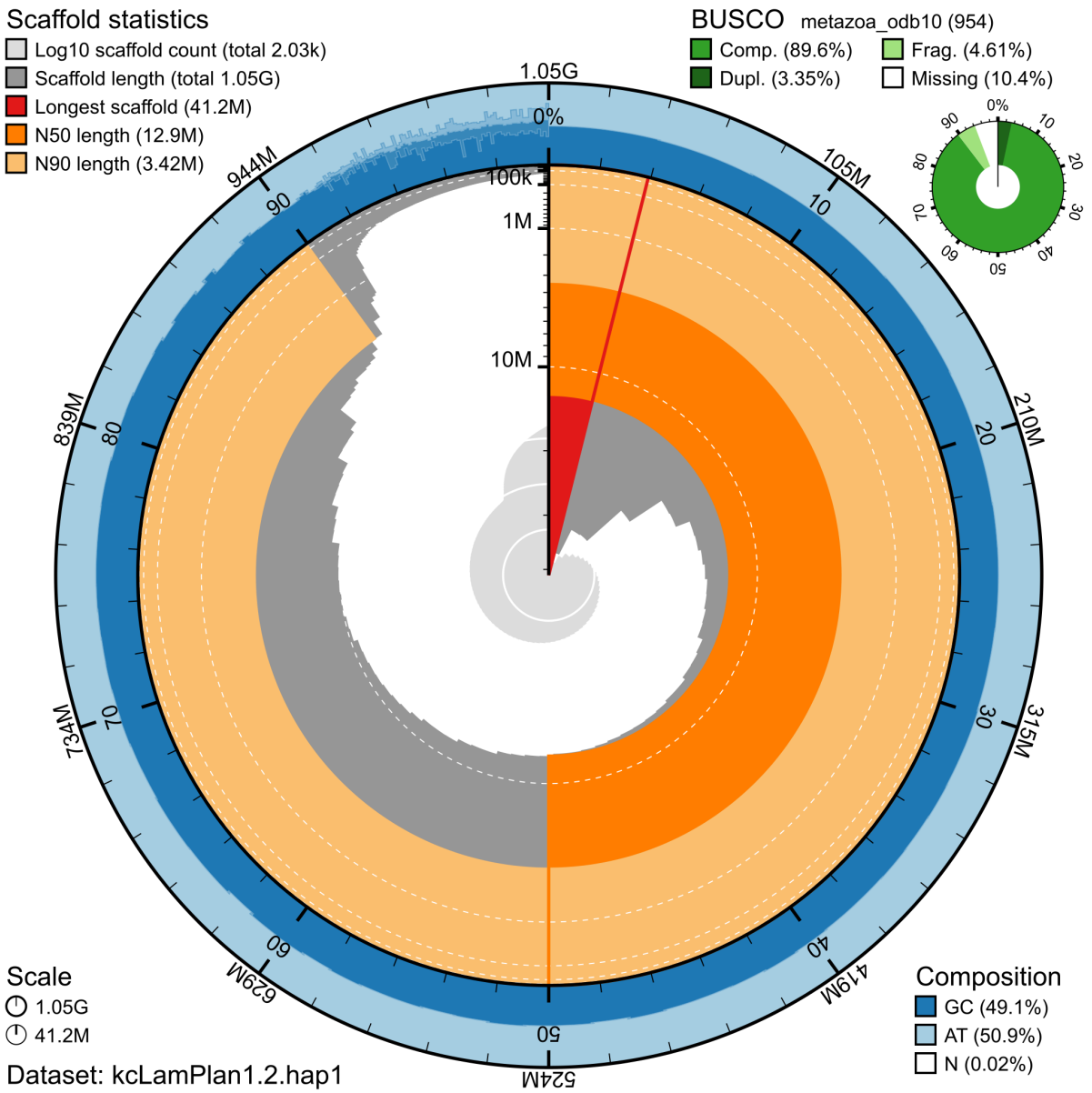


Figure 1: Metrics of the genome assemblies of *Lampetra fluviatilis* and *Lampetra planeri*, pseudo-haplotype one for both species. The BlobToolKit Snailplots show N50 metrics and BUSCO gene completeness. The two outermost bands of the circle signify GC versus AT composition at 0.1% intervals. Light orange shows the N90 scaffold length, while the deeper orange is N50 scaffold length. The red line shows the size of the largest scaffold. All the scaffolds are arranged in a clockwise manner from the largest to the smallest and are shown in darker gray with white lines at different orders of magnitude, while the light gray shows the cumulative count of scaffolds.

The final assemblies have total lengths of 1073 Mb (Figure 1) and 963 Mb (Table 2) for pseudo-haplotypes one and two for *L. fluviatilis,* respectively. For *L. planeri,* the pseudo-haplotypes one and two have total lengths of 1049 Mb (Figure 1) and 960 Mb, respectively. Pseudo-haplotypes one and two for *L. fluviatilis* have scaffold N50 sizes of 13.1 Mb and 13.4 Mb, respectively, and contig N50 of 2.7 Mb and 2.9 Mb, respectively (Table 2). *L. planeri* have scaffold N50 sizes of 12.9 Mb and 12.9 Mb in pseudo-haplotype one and two, respectively, and contig N50 sizes of 2.8 Mb and 3.0 Mb, respectively. 82 automosomes were identified in both pseudo-haplotypes for *L. fluviatilis* (chromosomes named after

kcLamFluv1) and 82 in both pseudo-haplotypes in *L. planeri* (chromosomes also named after kcLamFluc1).

Table 2: Genome data for *Lampetra fluviatilis,* kcLamFluv2 and *Lampetra planeri,* kcLamPlan1.

Project accession data				
Species	*Lampetra fluviatilis*		*Lampetra planeri*	
Specimen	kcLamFluv2		kcLamPlan1	
NCBI taxonomy ID	7748		7750	
BioProject	PRJEB77187		PRJEB77192	
BioSample ID	SAMEA115797768		SAMEA115802553	
Isolate information	Male, fin		Sex not provided, fin	
Raw data accessions				
PacBio HiFi reads	ERX12712303, ERX12712308, ERX12712309	3 PACBIO_SMRT (Sequel II) runs: 2.5 M reads, 38.5 Gbp	ERX12713797, ERX12713780, ERX12713807	3 PACBIO_SMRT (Sequel II) runs: 3.2 M reads, 44.0 Gbp
Hi-C Illumina reads	ERX12712501	1 ILLUMINA (Illumina NovaSeq S4) run: 334 M pairs of reads, 100.8 Gbp	ERX12714064	1 ILLUMINA (Illumina NovaSeq S4) run: 407 M pairs of reads, 122.9 Gbp
Genome assembly metrics				
HiFi read coverage	38		44	
Assembly accession	ERZ24889083	ERZ24889084	ERZ24889000	ERZ24889001
Assembly identifier	kcLamFluv2.2.hap1	kcLamFluv2.2.hap2	kcLamPlan1.2.hap1	kcLamPlan1.2.hap 2
Span (Mb)	1073	963	1049	960
Number of contigs	3060	1828	3142	3066
Contig N50 length (Mb)	2.7	2.9	2.8	3.0
Longest contig (Mb)	22.2	21.8	26.2	16.7

Number of gaps	1327	942	1113	873
Number of scaffolds	1733	886	2029	2193
Scaffold N50 length (Mb)	13.1	13.4	12.9	12.9
Longest scaffold (Mb)	41.1	41.3	41.1	41.0
Consensus quality (QV) compared to Hi-C (compared to HiFi)	38.6766 (54.8795)	40.4312 (56.0416)	34.9015 (52.7149)	28.9161 (52.4613)
Both assemblies	39.4197 (55.3907)		31.0849 (52.5919)	
k-mer completeness (percentage; compared to HiFi)	83.8059 (89.3051)	80.3495 (86.2251)	89.6766 (91.5393)	84.8223 (87.4288)
Both assemblies	92.194 (98.4232)		96.4739 (98.2448)	
BUSCO*	C:91.4%[S:88.5%,D:2.9%],F:4.6%,M:4.0%,n:954	C:83.9%[S:82.4%,D:1.5%],F:3.5%,M:12.6%,n:954	C:89.8%[S:86.4%,D:3.4%],F:4.4%,M:5.8%,n:954	C:83.5%[S:77.5%,D:6.0%],F:3.1%,M:13.4%,n:954
Percentage of assembly mapped to chromosomes	90.44	95.90	91.43	91.21
Flagger**	H: 79.33%, D: 20.35%, E:0.0%, C:0.03%	H: 82.20%, D: 17.54%, E:0.0%, C:0.03%	H: 75.27%, D: 21.22%, E:3.2%, C:0.03%	H: 75.57%, D: 18.69%, E:5.5%, C:0.02%
Organelles	MT		MT	
Genome annotation				
Number of protein-coding genes	21,479	16,973	24,691	2,1668
Number of protein-coding genes with functional domain***	20,126	7875	12,006	20,026

Number of protein-coding genes with gene names****	13,217	11,576	13,227	13,244
BUSCO*	C:89.0%[S:84.6%,D:4.4%],F:3.2%,M:7.8%,n:954	C:82.4%[S:79.8%,D:2.6%],F:2.5%,M:15.1%,n:954	C:89.2%[S:85.5%,D:3.7%],F:3.1%,M:7.7%,n:954	C:82.6%[S:76.8%,D:5.8%],F:2.5%,M:14.9%,n:954

* BUSCO scores based on the metazoa BUSCO set using v5.4.7. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison.
**Flagger scores H = haploid, D = duplicated, E = error, C = collapsed
***Number of genes annotated with a functional domain as found by InterProScan
****Number of genes that had a match against a named protein in UniProtKB/Swiss-Prot

For *L. fluviatilis,* pseudo-haplotype one had 91.4%, and pseudo-haplotype two had 83.9% complete BUSCO genes using the metazoa lineage set. *L. planeri* pseudo-haplotype one had 89.8% and pseudo-haplotype two 83.5%. When compared to a k-mer database of the Hi-C reads, pseudo-haplotype one in *L. fluviatilis* had a k-mer completeness of 83.8%, pseudo-haplotype two of 80.3%, and combined they have a completeness of 92.2%. For *L. planeri*, the equivalent numbers were 89.7%, 84.8%, and 96.5% for pseudo-haplotype one, pseudo-haplotype two and combined, respectively. Further, pseudo-haplotype one for *L. fluviatilis* has an assembly consensus quality value (QV) of 38.9 and pseudo-haplotype two of 40.4, where a QV of 40 corresponds to one error every 10,000 bp, or 99.99% accuracy compared to a k-mer database of the Hi-C reads (QV 54.9 and 56.0, respectively, compared to a k-mer database of the HiFi reads). For *L. planeri*, the equivalent numbers are 34.9 and 28.9 compared to a Hi-C k-mer database for pseudo-haplotype one and two respectively, and 52.2 and 52.5 against a k-mer database of HiFi reads.

The Hi-C contact maps for the assemblies are shown in Supplementary Figure 6, and show a clear separation of the different chromosomes.

For *L, fluviatilis,* Flagger identified 79.33% of pseudo-haplotype one as haploid, 20.35% as duplicated, 0.0% as error regions, and 0.03% as collapsed. The respective percentages for pseudo-haplotype two are 82.20% haploid, 17.54% duplicated, 0.0% error, and 0.03% collapsed (Table 1). For *L. planeri,* Flagger identified 75.27% of pseudo-haplotype one as haploid, 21.22% as duplicated, 3.2% as error regions, and 0.03% as collapsed. The respective percentages for pseudo-haplotype two are 75.57% haploid, 18.69% duplicated, 5.5% error, and 0.02% collapsed (Table 1)

We also aligned the pseudo-haplotypes of *L. fluviatilis* and *L. planeri* to each other and to another *L. fluviatilis* individual from the United Kingdom (kcLamFluv1; GCA_964198585.1) (Table 3 and Supplementary table 1). It was not possible to get an alignment toward the sea lamprey based on the settings we used, it was too divergent.

Table 3: Different metrics based on alignment of pseudo-haplotype one of *L. fluviatilis* and *L. planeri* to each other and to a *L. fluviatilis* individual from the United Kingdom. See Supplementary Table 1 for the inclusion of pseudo-haplotype two.

Aligned bases:

	kcLamFluv2.2.h1	kcLamPlan1.2.h1	kcLamFluv1.1
kcLamFluv2.2.h1		949,977,127 (90.5964%)	927,780,578 (88.9979%)
kcLamPlan1.2.h1	958,093,979 (89.2691%)		923,055,679 (88.5446%)
kcLamFluv1.1	952,584,705 (88.7558%)	940,046,391 (89.6493%)	

Insertions (sum in bp):

	kcLamFluv2.2.h1	kcLamPlan1.2.h1	kcLamFluv1.1
kcLamFluv2.2.h1		71,559 (179,071,556)	68,502 (176,805,416)
kcLamPlan1.2.h1	74,939 (208,858,838)		68,711 (179,916,524)
kcLamFluv1.1	77,251 (216,215,970)	74,769 (190,651,971)	

SNPs:

	kcLamFluv2.2.h1	kcLamPlan1.2.h1	kcLamFluv1.1
kcLamFluv2.2.h1		4,547,241	4,604,025
kcLamPlan1.2.h1	4,547,241		4,539,518
kcLamFluv1.1	4,604,025	4,539,518	

Indels:

	kcLamFluv2.2.h1	kcLamPlan1.2.h1	kcLamFluv1.1
kcLamFluv2.2.h1		5,879,800	5,949,776
kcLamPlan1.2.h1	5,879,800		5,885,164
kcLamFluv1.1	5,949,776	5,885,164	

We also ran OrthoFinder on all the predicted proteins of the different assemblies and used ASTRAL-Pro3 to generate quartet scores based on the gene trees from OrthoFinder (Figure 2). 40.1 % of the gene trees placed *L. planeri* from Sweden (kcLamPlan1.2.h1) as sister clade to *L. fluviatilis* from UK (kcLamFluv1.1) and *L. fluviatilis* from Norway (kcLamFluv2.2.h1) as sister clade to sea lamprey. 35.2 % of the gene trees supported the sea lamprey as sister clade to *Lampetra fluviatilis* from UK (kcLamFluv1.1) and *L. fluviatilis* from Norway (kcLamFluv2.2.h1) as sister clade to *L. planeri* from Sweden (kcLamPlan1.2.h1), while 24.7 % of the gene trees supported the last possible tree topology; *L. planeri* from Sweden (kcLamPlan1.2.h1) as sister clade to sea lamprey, and *L. fluviatilis* from Norway (kcLamFluv2.2.h1) as sister clade to *L. fluviatilis* from UK (kcLamFluv1.1).

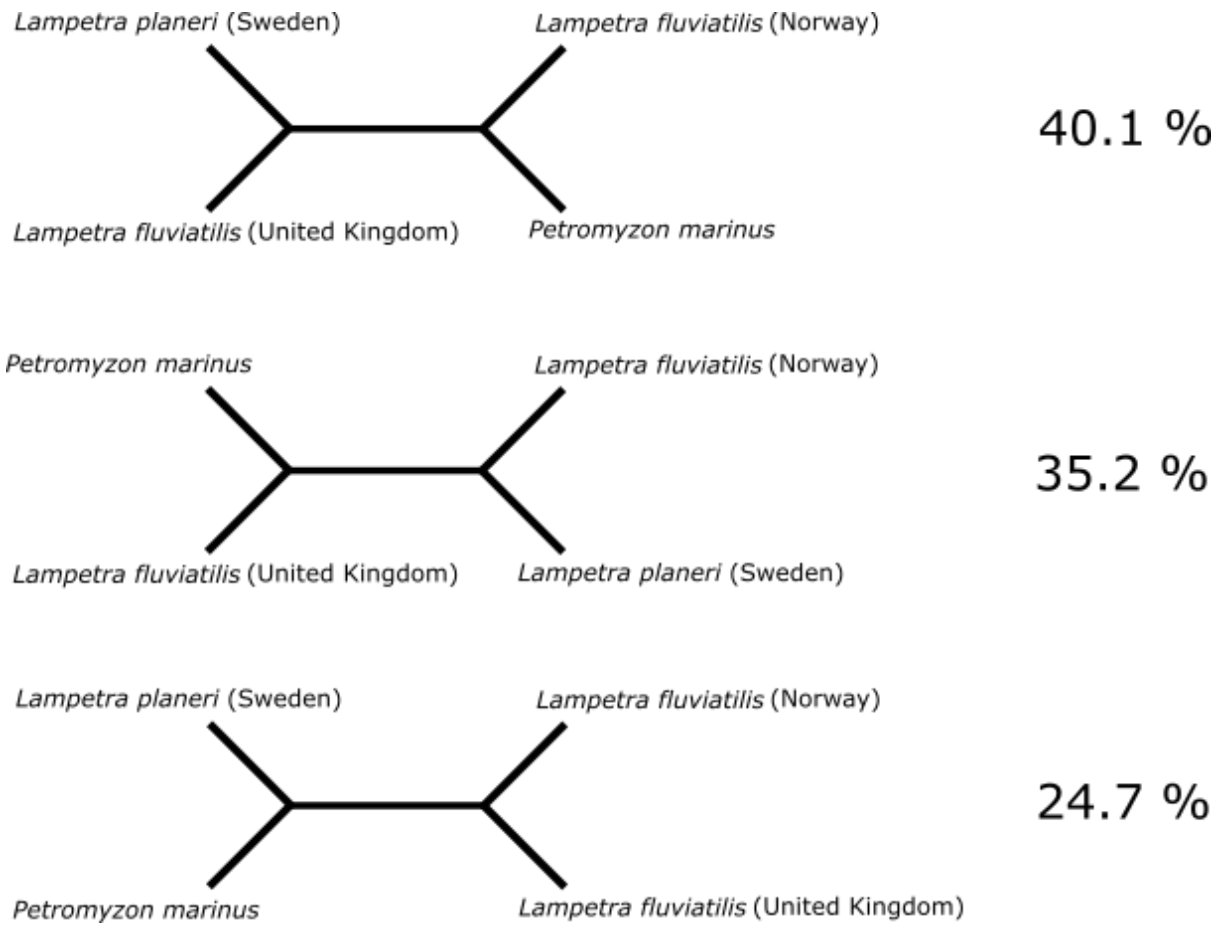


Figure 2: Different tree topologies and their support. The most complete pseudo-haplotype of *L. planeri* (hap1; called *Lampetra planeri* (Sweden) in the figure) and of *L. fluviatilis* (hap1; called *Lampetra fluviatilis* (Norway) in the figure) were used and compared with *L. fluviatilis* (UK) (kcLamFluv1; GCA_964198585.1) and *Petromyzon marinus* (sea lamprey; kPetMar1; GCA_010993605.1). ASTRAL-Pro3 was used to infer the species tree based on all gene trees from OrthoFinder and in addition, to calculate the different quartet scores.

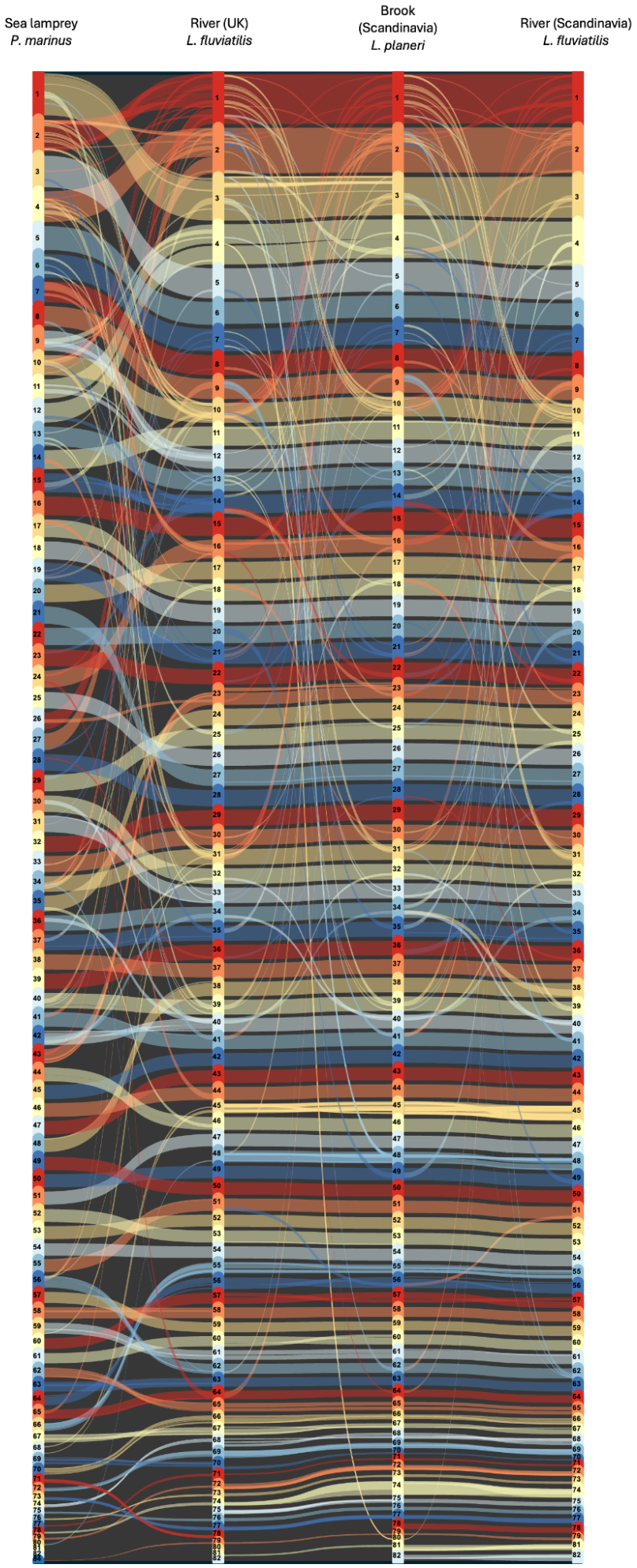

Sea lamprey
P. marinus
River (UK)
L. fluviatilis
Brook
(Scandinavia)
L. planeri
River (Scandinavia)
L. fluviatilis

Figure 3: Chromosomal synteny between sea lamprey (*P. marinus*), river (UK;* L. fluviatilis*), rrook (Scandinavia;* L. planeri*) and river (Scandinavia;* L. fluviatilis*). *Chromosomal synteny of the most complete pseudo-haplotype of kcLamPlan1 and kcLamFluv2 (hap1 in both cases), kcLamFluv1 and kPetMar. Plots generated by MCScanX and SynVisio include chromosomes 1-82 for* L. fluviatilis *individuals and* L. planeri*, and 1-84 for P. marinus. Syntenic blocks are visualized as connection ribbons between individuals.*

Gene order comparisons between the three different *Lampetra* individuals revealed conserved synteny among the genomes, with few chromosomal rearrangements (Figure 3). An increased number of reorganizations was observed when compared with the more distantly related sea lamprey (Figure 3 and Supplementary Figure 5). In particular, chromosome 1 among the *Lampetra* individuals seems to be homologous across their length, while when compared to sea lamprey, they are homologous to sea lamprey chromosome 2 and chromosome 26. The same pattern can be observed with chromosome 2 among the *Lampetra* individuals, which were found to be homologous to chromosome 4 and chromosome 27 in comparison to sea lamprey (Supplementary Figure 5). Moreover, chromosome 1 in the *Lampetra* individuals displays substantial connections between chromosome 1 as well as chromosome 10 when compared to other *Lampetra* individuals, indicating that these share shorter syntenic blocks along their length (Figure 3 and Supplementary Figure 5). This is also the case with chromosome 2 (homologous to chromosome 1 in *Lampetra*) and chromosome 10 in sea lamprey.

Discussion

Here, we have sequenced, assembled, and annotated chromosome-level genomes from *L. planeri* and *L. fluviatilis*, resulting in two pseudo-haplotype separated assemblies. These have comparative N50 statistics for both contigs (2.7-3.0 Mb here vs. 1.3 Mb for kcLamFluv1 and 2.5 Mb for kPetMar1) and scaffolds (all around 13 Mb N50) as previously released lamprey genome assemblies (kcLamFluv1 and kPetMar1) (Table 2 and Supplementary Figure 2). With regards to BUSCO scores, these are also comparable with 91.4% complete *BUSCO* genes in hap1 for *L. fluvialitis* (83.9% in hap2), 89.8% complete in hap1 for *L. planeri* (83.5% in hap2) and 91.8% in kcLamFluv1 and 92.5% in kPetMar1 (Table 2 and Supplementary Figure 2).

Flagger indicates that around 20% of the assemblies are duplicated. The BUSCO results do not support this (around 2-3% duplicated genes), however, we used the Metazoa marker gene set, with only 954 genes which could be too few to discover a putative duplication (Table 2). *GenomeScope* also only estimates 720-740 Mb genome sizes (about 20% less than the final assemblies) (Supplementary Figures 1 and 2). The common ancestor of lampreys and hagfish likely went through a triplication event of its genome (Yu et al., 2024), and this is likely reflected in the *Flagger* statistics and *GenomeScope* output as well as in the synteny plots. Interestingly, chromosomes 2 and 10 in sea lamprey (1 and 10 in *Lampetra*) contain the two (of six in total) Hox clusters which do not have a clear ortholog relationship to the Hox clusters found in jawed vertebrates (Marlétaz et al., 2024). Our synteny analysis shows that there is collinearity between these chromosomes also in the *Lampetra* individuals, which shows that the pattern extends to multiple lamprey species (Figure 3).

If *L. fluviatilis* and *L. planeri* were two clearly differentiated species, we would expect more differences between the species than in a species. Based on the alignments between the different *Lampetra* individuals (Table 3), there is no clear separation between the two species. Rather, there are more differences between the two *L. fluviatilis* individuals with regards to indels and SNPs, than between either of the *L. fluviatilis* individuals and *L. planeri,* while

there are no clear structure in insertions (depending on which assembly is query and target). Further, most of the gene trees (40.1%) support *L. fluviatilis* (UK) and *L. planeri* as phylogenetic sister species, while only 24.7 % support the two *L. fluviatilis* as sister species (Figure 2). Based on synteny, there are only minor differences, with the three *Lampetra* individuals - representing two *L. fluvatilis* from Norway and UK, respectively and an *L. planeri* from Sweden (fairly close to Norway: see details in Methods). With regards to chromosomal architecture the results show that the genomes display conserved synteny with few large rearrangements (Figure 3). The rearrangements that have taken place, when comparing the *Lampetra* individuals to the sea lamprey, are particularly involving chromosomes 1 and 2 (in *Lampetra*), which could be the result of lineage-specific fusions or fissions. Most notably, the differences between *L. fluvatilis* and *L. planeri* are little compared to the geographical separation (Norway/Sweden and UK).

This study, based on two new high-quality reference genomes (*L planeri* and *L. fluvatilis*) and comparison with *L. fluvatilis* reference genome from the UK suggests that the two species rather is a species complex representing two ecotypes. However, our study is only represented by 4 individuals (including the *P. marinus* outgroup individual). Even though the *L. fluviatilis* individual from Scandinavia robustly looks as different from *L. planeri* from Scandinavia as *L. fluviatilis* from the UK, the ultimate test for this conclusion would be to include whole genome sequenced individuals from multiple geographical locations across Europe - from the Mediterranean/South Atlantic oceans to the northern Atlantic. Ideally, such a study should also include spawning individuals to properly untangle the question of how the two putative ecotypes relate to each other.

Funding
This research was funded by the Research Council of Norway (# 326819) to KSJ (Earth BioGenome Project Norway (EBP-Nor)) and Nansen Legacy (RCN # 276730).

Acknowledgements
This project received data management and infrastructure support from ELIXIR Norway, supported by the Research Council of Norway grant 270068, the University of Bergen, the University of Oslo, the Arctic University of Norway in Tromsø, the Norwegian University of Science and Technology and the Norwegian University of Life Sciences: NMBU. The authors acknowledge support from the National Infrastructure for High Performance Computing and resources provided by Sigma2 as well as Data Storage in Norway (project NN8013K) for computational work. The Norwegian Sequencing Centre generated the sequencing data used in this project (http://sequencing.uio.no).

Data Availability
Data generated for this study are available under ENA BioProject PRJEB77187 and PRJEB77192 for EBP-Nor. Raw PacBio sequencing data for *L. fluviatilis* (ENA BioSample: SAMEA115797768) are deposited in ENA under ERX12712303, ERX12712308 and ERX12712309, while Illumina Hi-C sequencing data is deposited in ENA under ERX12712501. Pseudo-haplotype one can be found in ENA at PRJEB77117 while pseudo-haplotype two is PRJEB77186. Raw PacBio sequencing data for *L. planeri* (ENA BioSample: SAMEA115802553) are deposited in ENA under ERX12713780, ERX12713797 and ERX12713807, while Illumina Hi-C sequencing data is deposited in ENA under ERX12714064. Pseudo-haplotype one can be found in ENA at PRJEB77190 while pseudo-haplotype two is PRJEB77191.

The gene annotations are available at https://zenodo.org/records/14288109 (DOI:10.5281/zenodo.11159637).

Authors' contributions':
Ole K. Tørresen: Writing - original draft, Formal analysis, Visualization, Writing - review and editing. **Benedicte Garmann-Aarhus:** Writing - original draft, Investigation, Formal analysis, Visualization. **Siv Nam Khang Hoff:** Writing - original draft, Formal analysis, Visualization. **Sissel Jentoft:** Writing - review and editing. **Mikael Svensson:** Resources. **Eivind Schartum:** Resources. **Ave Tooming-Klunderud:** Investigation. **Morten Skage:** Investigation. **Anders Krabberød:** Formal analysis. **Leif Asbjørn Vøllestad:** Project Writing - original draft, Writing - review and editing, administration. **Kjetill S. Jakobsen:** Project administration, Writing - original draft, Writing - review and editing, Funding acquisition.

References
Astashyn A, Tvedte ES, Sweeney D, Sapojnikov V, Bouk N, Joukov V, et al. Rapid and sensitive detection of genome contamination at scale with FCS-GX. BioRxiv 2023:2023.06.02.543519. https://doi.org/10.1101/2023.06.02.543519.

Bandi V, Gutwin C. Interactive Exploration of Genomic Conservation. Proceedings of Graphics Interface 2020, Canadian Human-Computer Communications Society / Société canadienne du dialogue humain-machine; 2020, p. 74–83.

Bracken FSA, Hoelzel AR, Hume JB, Lucas MC. Contrasting population genetic structure among freshwater‐resident and anadromous lampreys: the role of demographic history, differential dispersal and anthropogenic barriers to movement. Mol Ecol 2015;24:1188–204. https://doi.org/10.1111/mec.13112.

Brůna T, Li H, Guhlin J, Honsel D, Herbold S, Stanke M, et al. Galba: genome annotation with miniprot and AUGUSTUS. BMC Bioinform 2023;24:327. https://doi.org/10.1186/s12859-023-05449-z.

Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nat Methods 2015;12:59–60. https://doi.org/10.1038/nmeth.3176.

Cahsan BD, Nagel R, Schedina I, King JJ, Bianco PG, Tiedemann R, et al. Phylogeography of the European brook lamprey (Lampetra planeri) and the European river lamprey (Lampetra fluviatilis) species pair based on mitochondrial data. J Fish Biol 2020;96:905–12. https://doi.org/10.1111/jfb.14279.

Cheng H, Concepcion GT, Feng X, Zhang H, Li H. Haplotype-resolved de novo assembly using phased assembly graphs with hifiasm. Nat Methods 2021;18:170–5. https://doi.org/10.1038/s41592-020-01056-5.

Consortium TU, Bateman A, Martin M-J, Orchard S, Magrane M, Ahmad S, et al. UniProt: the Universal Protein Knowledgebase in 2023. Nucleic Acids Res 2022;51:D523–31. https://doi.org/10.1093/nar/gkac1052.

Docker, M. F. (2009). A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. American Fisheries Society Symposium, 72, 71-114.

Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biology 2019;20:1–14. https://doi.org/10.1186/s13059-019-1832-y.
Emms DM, Kelly S. STAG: Species Tree Inference from All Genes. BioRxiv 2018:267914. https://doi.org/10.1101/267914.

Emms DM, Kelly S. STRIDE: Species Tree Root Inference from Gene Duplication Events. Mol Biol Evol 2017;34:3267–78. https://doi.org/10.1093/molbev/msx259.

Formenti G, Abueg L, Brajuka A, Brajuka N, Gallardo-Alba C, Giani A, et al. Gfastats: conversion, evaluation and manipulation of genome sequences using assembly graphs. Bioinformatics 2022;38:4214–6. https://doi.org/10.1093/bioinformatics/btac460.

Girgis HZ. Red: an intelligent, rapid, accurate tool for detecting repeats de-novo on the genomic scale. BMC Bioinform 2015;16:227. https://doi.org/10.1186/s12859-015-0654-5.

Haas BJ, Salzberg SL, Zhu W, Pertea M, Allen JE, Orvis J, et al. Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biology 2008;9:R7. https://doi.org/10.1186/gb-2008-9-1-r7.

Hoff KJ, Stanke M. Predicting genes in single genomes with AUGUSTUS. Current Protocols in Bioinformatics / Editoral Board, Andreas D Baxevanis . [et Al] 2018;28:e57. https://doi.org/10.1002/cpbi.57.

Hume JB, Recknagel H, Bean CW, Adams CE, Mable BK. RADseq and mate choice assays reveal unidirectional gene flow among three lamprey ecotypes despite weak assortative mating: Insights into the formation and stability of multiple ecotypes in sympatry. Mol Ecol 2018;27:4572–90. https://doi.org/10.1111/mec.14881.

Jain C, Rhie A, Hansen NF, Koren S, Phillippy AM. Long-read mapping to repetitive reference sequences using Winnowmap2. Nat Methods 2022;19:705–10. https://doi.org/10.1038/s41592-022-01457-8.

Katoh K, Standley DM. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol 2013;30:772–80. https://doi.org/10.1093/molbev/mst010.

Kelly FL, King JJ. A Review of the Ecology and Distribution of Three Lamprey Species,

Lampetra fluviatilis (L.), Lampetra planeri (Bloch) and Petromyzon marinus (L.): A Context for Conservation and Biodiversity Considerations in Ireland. Biology and Environment: Proceedings of the Royal Irish Academy 2001;101B:165–85.

Kokot M, Dlugosz M, Deorowicz S. KMC 3: counting and manipulating k-mer statistics. Bioinform (Oxf, Engl) 2017;33:2759–61. https://doi.org/10.1093/bioinformatics/btx304.

Kuznetsov D, Tegenfeldt F, Manni M, Seppey M, Berkeley M, Kriventseva EV, et al. OrthoDB v11: annotation of orthologs in the widest sampling of organismal diversity. Nucleic Acids Res 2022;51:D445–51. https://doi.org/10.1093/nar/gkac998.

Laetsch DR, Blaxter ML. BlobTools: Interrogation of genome assemblies. F1000Research 2017;6:1287. https://doi.org/10.12688/f1000research.12232.1.

Lewin HA, Richards S, Aiden EL, Allende ML, Archibald JM, Bálint M, et al. The Earth BioGenome Project 2020: Starting the clock. Proc National Acad Sci 2022;119:e2115635118. https://doi.org/10.1073/pnas.2115635118.

Li H. Protein-to-genome alignment with miniprot. Bioinformatics 2023;39:btad014. https://doi.org/10.1093/bioinformatics/btad014.

Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. ArXivOrg 2013.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078–9. https://doi.org/10.1093/bioinformatics/btp352.

Liao W-W, Asri M, Ebler J, Doerr D, Haukness M, Hickey G, et al. A draft human pangenome reference. Nature 2023;617:312–24. https://doi.org/10.1038/s41586-023-05896-x.

Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 2021;38:msab199-. https://doi.org/10.1093/molbev/msab199.

Marçais G, Delcher AL, Phillippy AM, Coston R, Salzberg SL, Zimin A. MUMmer4: A fast and versatile genome alignment system. PLOS Comput Biol 2018;14:e1005944. https://doi.org/10.1371/journal.pcbi.1005944.

Marlétaz F, Timoshevskaya N, Timoshevskiy VA, Parey E, Simakov O, Gavriouchkina D, et al. The hagfish genome and the evolution of vertebrates. Nature 2024;627:811–20. https://doi.org/10.1038/s41586-024-07070-3.

Mateus CS, Almeida PR, Mesquita N, Quintella BR, Alves MJ. European Lampreys: New Insights on Postglacial Colonization, Gene Flow and Speciation. PLoS ONE 2016;11:e0148107. https://doi.org/10.1371/journal.pone.0148107.

Mateus CS, Stange M, Berner D, Roesti M, Quintella BR, Alves MJ, et al. Strong genome-wide divergence between sympatric European river and brook lampreys. Curr Biol 2013;23:R649–50. https://doi.org/10.1016/j.cub.2013.06.026.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Haeseler A von, et al. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol Biol Evol 2020;37:1530–4. https://doi.org/10.1093/molbev/msaa015.

Norling M, Jareborg N, Dainat J. EMBLmyGFF3: a converter facilitating genome annotation submission to European Nucleotide Archive. BMC Res Notes 2018;11:584. https://doi.org/10.1186/s13104-018-3686-x.

Poplin R, Chang P-C, Alexander D, Schwartz S, Colthurst T, Ku A, et al. A universal SNP and small-indel variant caller using deep neural networks. Nat Biotechnol 2018;36:983–7. https://doi.org/10.1038/nbt.4235.

Potter IC, Gill HS, Renaud CB, Haoucher D. The Taxonomy, Phylogeny, and Distribution of Lampreys 2015:35–73. https://doi.org/10.1007/978-94-017-9306-3_2.

Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 2010;26:841–2. https://doi.org/10.1093/bioinformatics/btq033.

Ranallo-Benavidez TR, Jaron KS, Schatz MC. GenomeScope 2.0 and Smudgeplot for reference-free profiling of polyploid genomes. Nat Commun 2020;11:1432. https://doi.org/10.1038/s41467-020-14998-3.

Rhie A, Walenz BP, Koren S, Phillippy AM. Merqury: reference-free quality, completeness, and phasing assessment for genome assemblies. Genome Biol 2020;21:245. https://doi.org/10.1186/s13059-020-02134-9.

Rice P, Longden I, Bleasby A. EMBOSS: The European Molecular Biology Open Software Suite. Trends in Genetics : TIG 2000;16:276–7. https://doi.org/10.1016/s0168-9525(00)02024-2.

Rougemont Q, Gagnaire P, Perrier C, Genthon C, Besnard A, Launey S, et al. Inferring the demographic history underlying parallel genomic divergence among pairs of parasitic and nonparasitic lamprey ecotypes. Mol Ecol 2017;26:142–62. https://doi.org/10.1111/mec.13664.

Rougemont Q, Gaigher A, Lasne E, Côte J, Coke M, Besnard A‐L., et al. Low reproductive isolation and highly variable levels of gene flow reveal limited progress towards speciation between European river and brook lampreys. J Evol Biol 2015;28:2248–63. https://doi.org/10.1111/jeb.12750.

Rougemont Q, Roux C, Neuenschwander S, Goudet J, Launey S, Evanno G. Reconstructing the demographic history of divergence between European river and brook lampreys using approximate Bayesian computations. PeerJ 2016;4:e1910. https://doi.org/10.7717/peerj.1910.

Sim SB, Corpuz RL, Simmonds TJ, Geib SM. HiFiAdapterFilt, a memory efficient read processing pipeline, prevents occurrence of adapter sequence in PacBio HiFi reads and their negative impacts on genome assembly. BMC Genom 2022;23:157. https://doi.org/10.1186/s12864-022-08375-1.

Stanke M, Schöffmann O, Morgenstern B, Waack S. Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. BMC Bioinform 2006;7:62. https://doi.org/10.1186/1471-2105-7-62.

Uliano-Silva M, Ferreira JGRN, Krasheninnikova K, Consortium DT of L, Blaxter M, Mieszkowska N, et al. MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads. BMC Bioinform 2023;24:288. https://doi.org/10.1186/s12859-023-05385-y.

Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Research 2012;40:e49–e49. https://doi.org/10.1093/nar/gkr1293.

Whiteley, A. R., Penaluna, B. E., Taylor, E. R., Weiss, S., Abadia-Cardoso, A., Gomez-Uchida, D., Koizumi, I., & Trotter, P. (2019). Trout and char: Taxonomy, systematics, and phylogeography. In J. L. Kershner, J. E. Williams, R. E. Gresswell, & J. Lobón-Cerviá (Eds.), Trout and char of the world. (pp. 95-140). American Fisheries Society.

Yu D, Ren Y, Uesaka M, Beavan AJS, Muffato M, Shen J, et al. Hagfish genome elucidates vertebrate whole-genome duplication events and their evolutionary consequences. Nat Ecol Evol 2024;8:519–35. https://doi.org/10.1038/s41559-023-02299-z.

Zhang C, Mirarab S. ASTRAL-Pro 2: ultrafast species tree reconstruction from multi-copy gene family trees. Bioinformatics 2022;38:4949–50. https://doi.org/10.1093/bioinformatics/btac620.

Zhang C, Scornavacca C, Molloy EK, Mirarab S. ASTRAL-Pro: Quartet-Based Species-Tree Inference despite Paralogy. Mol Biol Evol 2020;37:3292–307. https://doi.org/10.1093/molbev/msaa139.

Zhou C, McCarthy SA, Durbin R. YaHS: yet another Hi-C scaffolding tool. Bioinformatics 2022;39:btac808. https://doi.org/10.1093/bioinformatics/btac808.