

1 **Genome assembly, transcriptome and SNP database for chum salmon**

2 **(*Oncorhynchus keta*)**

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32 Abstract

33 Chum salmon (*Oncorhynchus keta*) is the species with the widest geographic range of
 34 the anadromous Pacific salmonids,. Chum salmon is the second largest of the Pacific salmon,
 35 behind Chinook salmon, and considered the most plentiful Pacific salmon by overall biomass.
 36 This species is of significant commercial and economic importance: on average the commercial
 37 chum salmon fishery has the second highest processed value of the Pacific salmon within British
 38 Columbia. The aim of this work was to establish genomic baseline resources for this species.
 39 Our first step to accomplish this goal was to generate a chum salmon reference genome
 40 assembly from a doubled-haploid chum salmon. Gene annotation of this genome was
 41 facilitated by an extensive RNA-seq database we were able to create from multiple tissues.
 42 Range-wide resequencing of chum salmon genomes allowed us to categorize genome-wide
 43 geographic variation, which in turn reinforced the idea that genetic differentiation was best
 44 described on a regional, rather than at a stock-specific, level. Within British Columbia, chum
 45 salmon regional groupings were described at the conservation unit (CU) level, and there may be
 46 substructure within particular CUs. Genome wide associations of phenotypic sex to SNP genetic
 47 markers identified two clear peaks, a very strong peak on Linkage Group 15, and another on
 48 Linkage Group 3. With these new resources, we were better able to characterize the sex-
 49 determining region and gain further insights into sex determination in chum salmon and the
 50 general biology of this species.

51

52 Keywords

53 Chum salmon, Genome assembly, *Oncorhynchus keta*, RNA-seq, Resequencing, SNP database.

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57 **Background**

58 Pacific salmon of the genus *Oncorhynchus* are iconic, culturally important keystone
59 species spawning across freshwater watersheds that feed the Northern Pacific Ocean.
60 Predominately anadromous, members of most species spend years at sea, consuming marine
61 nutrients that are eventually deposited into coastal ecosystems where they provide a valuable
62 source of food to numerous marine and terrestrial species as the salmon spawn and then die
63 [1].

64 Chum salmon (*Oncorhynchus keta*) are the second largest of the Pacific salmonids and
65 may have historically represented up to 50% of the salmonid biomass in the Pacific Ocean [2]. It
66 is the most widely distributed of the Pacific salmonid species [3, 4], with spawning grounds
67 ranging from Japan and the eastern coast of the Korean Peninsula through to Northern Russia,
68 and from the Mackenzie River south through Central California in North America [5]. Among
69 the most significant species of Pacific salmon in commercial fisheries – in an analysis of British
70 Columbian commercial fisheries 2012-2015, chum salmon was the most plentiful species by
71 weight in 3 out of 4 years analyzed, and second most valuable by processed value when
72 averaged across the four year period (\$31 million per year) [6].

73 A key and fascinating biological feature in salmonids is homing, whereby adults
74 demonstrate an ability to return to the same riverine sites where they were spawned, although
75 not all species show the same degree of site fidelity (reviewed in [7]). Some species, such as
76 Sockeye, have been observed to return to within metres of where they were hatched (e.g., [8]),
77 but other species vary in their fidelity to site of return and stray rate. Reasons for straying are
78 likely varied (reviewed in [7]), but significant factors are thought to be juvenile freshwater
79 residence time and freshwater migration distance, both of which lead to reduced imprinting.
80 With chum salmon having relatively short freshwater residence (they migrate to sea as fry) and
81 short migration distances (on average), it is perhaps not surprising that chum tend to have
82 higher than average stray rates among the Pacific salmonids [7]. The consequences of such
83 straying are that while regional-level differentiation (e.g., [9, 10]) and run-timing differentiation
84 between summer and fall runs (e.g., [11–13]) can be observed, population-level genetic
85 differentiation is not often seen within chum salmon.

86 The genomes of salmonids, including chum salmon, possess a key feature shared by all
87 salmonid genomes, a salmon-lineage specific whole-genome duplications (WGD). WGDs very
88 likely play one of the more significant roles in evolutionary innovation [14–17] and are found in
89 plants (reviewed in [18]), fungi [19, 20] , arthropods [21, 22], basal vertebrates ~500 million
90 years ago (mya) [15, 23, 24], fishes ~300 mya [25–27], and more recently in ancestral salmonids
91 ~90 mya [28, 29] . These major genome expansions have been proposed to allow for
92 adaptations to new niches or conditions, particularly in times of major environmental change
93 (reviewed in [30]). The occurrence of over 70 different salmonid species lineages stemming
94 from the relatively recent ancestral WGD [29] offers a valuable system to i) observe
95 evolutionary consequences of a relatively recent autopolyploid WGD, ii) identify ensuing
96 mechanisms for regaining stable meiosis and cell division by regaining a functional diploid state
97 through re-diploidization, and iii) draw associations between mechanisms of re-diploidization to
98 potential genetic specialization that allow for species adaptation such as disease resistance.
99 Additionally, each species has evolved unique morphology, life history strategies, and responses
100 to common salmon pathogens (e.g., varied resistance to salmon aquaculture from pathogens
101 such as the sea louse [31, 32]). This phenotypic variety provides future opportunities for
102 exploring the biology and genetics behind the genomic architecture of whole-genome
103 duplication have shaped these unique species.

104 The presence of these duplications, however, can present major technological
105 challenges to genome assembly, due to limited differentiation between duplicated portions of
106 the genome. Salmonids offer additional hurdles in that a significant portion of the genome still
107 remains in a tetraploid-like state [33, 34], and may show lineage-specific re-diploidization
108 patterns [35], or chromosome architecture through species-specific fusions [36]. While many
109 challenges remain, the technological barriers to assembly of salmonid genomes are beginning
110 to fall, as evidenced by the relatively rapid recent release of salmonid genomes [37–44]. A
111 fully-annotated reference chum salmon genome will enhance development of genomics-based
112 technologies to improve the effectiveness of fisheries management of the wild chum salmon
113 fishery. This has already been performed for other Pacific salmon species in British Columbia

(e.g., [45, 46]), and a genome assembly for chum would provide the ability to adopt similar management tools based on emerging high-throughput sequencing technologies.

Genetic resources in chum salmon have, as in many other species, been in a state of transition as genetic tools have advanced and become more widespread. Early work on population genetic structure in chum salmon utilized allozymes [47, 48] and microsatellite markers [9, 49] and provided the first range-wide studies on genetic diversity [10]. Recently, genetic stock identification tools have been shifting from microsatellites to single-nucleotide polymorphisms (SNPs), providing increased accuracy of genetic discrimination with increasing marker numbers [50]. Early identification of SNPs in chum salmon [51–55] led to the development of a SNP panel for assessing genetic diversity and population structures in chum salmon [13]; development of expanded SNP panels for fisheries management continues to occur with increased marker density and improving genetic baselines allow for increased power ([56]; Beacham T.D. and Sutherland B.J.G, Personal Communication). Restriction-site Associated DNA sequencing (RADseq) has recently enabled a much more rapid throughput for SNP discovery [57, 58], and studies in chum have utilized this technique to enable researchers to develop linkage maps to explore regions of residual inheritance associated with the aforementioned genome duplication event [34]. This advance in technology has further allowed for the identification of extended patterns of linkage disequilibrium, demonstrating the power of increased marker density on the identification of genomic features of large effect [59]. Despite this significant effort, unlike in other *Oncorhynchus* species (e.g., Rainbow trout [60]; Chinook salmon [41], sockeye salmon [61]), neither a whole-genome catalog of SNP markers nor whole-genome resequencing data has been available as a resource for chum salmon to date. The development of such a resource will further allow genetic resources, such as SNP panels, to be placed in context relative to genes or other annotated genomic features.

In this work, we have sequenced and assembled the genome of a mitotic gynogen doubled haploid chum salmon to eliminate allelic variation but retain paralog differences. Extensive multi-tissue RNA-seq was generated to provide the base for annotation of the genome as well as a tissue-specific expression atlas for future comparative studies. Finally, whole-genome resequencing was performed across 59 individual chum salmon from a select

143 distribution of the species' range to catalogue genome-wide diversity in this species. The utility
144 of the dataset is further demonstrated by the genetic association of the sex phenotype onto the
145 expected chromosome in a narrow window of elevated linkage disequilibrium.

146

147 **Methods**

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149 **Data availability**

150 All raw sequencing reads and the assembled genome described in this project have
151 been submitted to NCBI under BioProject PRJNA556729. SNP variant sets described below are
152 available through [Dryad repository](#).

Comment [RE1]: TBD on acceptance

153

154 **Animal care and sample collection**

155 All animals were reared in compliance with Canadian Council on Animal Care Guidelines, under
156 oversight from the Fisheries and Oceans Canada Pacific Region Animal Care Committee
157 (PRACC). Chum salmon for genome sequencing and assembly and for transcriptome assembly
158 were from Chehalis River Hatchery parents and reared at Fisheries and Oceans Canada in West
159 Vancouver. Chum salmon mitotic gynogen doubled haploids were produced following
160 procedures described by [62]. Briefly, eggs were fertilized with UV-irradiated sperm and
161 pressure shocked (10,000 psi for 5 minutes) in batches at 30 min intervals between 4 and 7
162 hours post-fertilization. One individual from the 7h pressure shock group (Oke142-1, NCBI
163 BioSample: SAMN12367893; Supplementary Table 1) was confirmed to be homozygous for
164 maternal alleles using a panel of 14 microsatellites [49], and was used for genome sequencing
165 and assembly (see below). The individual was euthanized in a bath of 200 mg/L tricaine
166 methanesulfonate (TMS) buffered in 400 mg/L sodium bicarbonate prior to first feeding stage,
167 and stored in ethanol before DNA extraction and whole genome sequencing.

168

For transcriptomic data, control Chehalis River Hatchery chum salmon produced from the same parents as Oke142-1 but without UV milt treatment or pressure shock were grown in aerated fresh well water in 200–3700 L tanks and fed hourly as fry and to satiation 3 times daily as parr with stage-appropriate manufactured salmon feed (Skretting Canada Ltd.). At approximately 7 months post-ponding, a single selected chum female (86.9g with a 19.3cm fork length) was euthanized with TMS as above, then rapidly (< three min, PRACC management procedure 3.7) team dissected to harvest 18 tissues (see Supplementary Table 2) for RNA extraction, with an additional tissue (testes) sampled from an juvenile male. All tissues were stored in RNAlater at -20°C until extraction. RNA extractions were performed using the Qiagen RNeasy Mini Kit following the manufacturer’s protocol.

For individuals used in resequencing, samples were obtained primarily through non-lethal sampling of fin clips or operculum punches from Fisheries and Oceans Canada hatchery brood programs. Additional samples were obtained from archived tissue sets used for genetic stock ID baseline development to supplement the dataset. In total, 59 individuals were utilized in this assessment, with DNA obtained via Qiagen DNeasy Animal tissue kit’s following manufacturer’s protocol) or phenol/chloroform extractions (following Thermo Fisher Scientific’s protocol for genomic DNA preparation [63]. Tissue types, sex, collection dates and locations are summarized in Supplementary Table 3.

Genome sequencing and Assembly

DNA was isolated from RNAlater or ethanol preserved tissues using a phenol/chloroform extraction as per Thermo Fisher Scientific’s protocol for genomic DNA preparation [63]. Extracted DNA was submitted for genome sequencing across multiple library types, using both Illumina and PacBio sequencing instruments (summarized in Supplementary Table 1: SRA chum Gynogen). Extracted DNA was submitted to the McGill University and Génome Québec Innovation Centre (now the Centre d'expertise et de services Génome Québec) for construction of overlapping (library size estimate = 497 base pairs (bp) and non-overlapping (library size estimate = 620bp) IDT dual-indexed Illumina Shotgun libraries. Each library was sequenced twice on an Illumina HiSeq2500 on RAPID mode PE250. Extracted DNA

was also submitted to the McGill University and Génome Québec Innovation Centre for construction of a single library of 10X Chromium linked-reads. Following library construction, the library was sequenced across three lanes of Illumina HiSeqX PE150. Extracted DNA was also submitted to the National Research Council Plant Biotechnology Institute Genome Core for Illumina mate-pair library construction and sequencing. Mate-pair libraries targeting 2-3kb, 4-6kb and 7-12kb were constructed, and sequenced on a lane each of Illumina HiSeq2500 PE125. Finally, extracted DNA was submitted to McGill University and Génome Québec Innovation Centre for construction of a Pacific Biosciences SMRT library using a sheared large insert library type, and the MagBead OneCellPerWell v1 collection protocol. The library was ultimately sequenced across 16 total SMRT cells.

Assembly protocols followed successful strategies utilized for Northern Pike e.g., [40, 61, 64, 65]. See Supplementary Table 4 for specific parameters to assembly and trimming that were tested. Reads were first trimmed for quality, adapters and minimum length using Trimmomatic [66], and BBmap's FilterByTile was utilized to remove poorly performing portions of the Illumina reads (<https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/bbmap-guide/>; [67]). Allpaths-LG v52488 [68] was utilized with overlapping Illumina overlapping PE250 and Illumina mate-pair libraries using a 3.0 TB memory node on the Compute Canada cluster Cedar. Non-overlapping libraries were also included in two assembly attempts, but ultimately exceeded the memory availability on the node in the MergeNeighbourhoods2 module and were dropped in successful assemblies. Assembly parameters were primarily adjusted for coverage of each of the library types as had been performed in other species; additional modifications were made to read filtering to improve the assemblies.

220 Following Allpaths-LG assembly, scaffolds were passed into PB Jelly 2 v 15.8.24 [69]
221 along with all subreads produced in PacBio sequencing. Nodes on Compute Canada's Cedar
222 cluster were used for all stages, with on-node temp directory and 48 cores used in all steps
223 where allowed. Blasr parameters were ``-minMatch 8 -sdpTupleSize 8 -minPctIdentity 75 -bestn`
224 `1 -nCandidates 10 -nproc 48 -maxScore -500 -noSplitSubreads``. Extraction.py was modified to
225 ``MAXGAPHOLD= 1000000`` to take advantage of memory available. Collection.py was run with
226 ``-m 3``. All other parameters remained default. Finally, the assembly was polished with Pilon

227 [70] using the trimmed paired-end data, aligned to the genome utilizing `bwa mem -M` and
228 default parameters.

229 Scaffolds were ordered and oriented into chromosome representations (i.e.,
230 Pseudomolecules) predominately following the methods described in Christensen et al. (2018)
231 [40]. The sequences underlying the markers for the published chum linkage map from Waples
232 et al. (2016) [34] were aligned to the scaffold assembly utilizing BLAST (-outfmt 6, -word_size
233 48, perc_identity 94, -max_hsps 100, -max_target_seqs 10 -evaluate 1E-16). All scaffolds with a
234 link to at least one marker on the map were retained for subsequent pseudomolecule inclusion.
235 Scaffolds were ordered and oriented to the extent allowed by the linkage map, although
236 regions of low recombination limited the effectiveness of the maps alone at this task.
237 Therefore, the sequences underlying the markers for the linkage map were also aligned to a
238 higher contiguity genome of a related species (coho; GCF_002021735.2), and ordering and
239 orientation was further refined based on the conserved synteny between the two species via
240 manual review. Where discrepancies were observed, the chum linkage map was taken as
241 correct to ensure major species-specific rearrangements were captured. Finally,
242 pseudomolecules were aligned to genomes of additional salmonids rainbow trout
243 GCF_002163495.1 [39], Atlantic salmon (GCF_000233375.1) [38], Chinook salmon
244 (GCF_002872995.1) [40] and the non-duplicated outgroup to the salmonids, northern pike
245 (GCF_000721915.3) [71] using Symap v4.2 [72] to ensure linearity was generally conserved, and
246 where it was not, was supported by rearrangements observed in the linkage map.

247 A BUSCO v4.0.2 [73] analysis utilizing the actinopterygii_odb10 dataset and `-m geno -c
248 10 -sp zebrafish` was used to analyze the gene representation within the assembly utilizing the
249 RefSeq maintained assembly: GCF_012931545.1.

250

251 **Gene Annotation**

252 Raw reads for RNA-seq libraries were uploaded into NCBI under BioProject
253 PRJNA556729 for inclusion in the Eukaryotic Genome Annotation pipeline. NEBNext dual-
254 indexed mRNA stranded libraries were constructed from tissues described above by the McGill
255 University and G  nome Qu  bec Innovation Centre, and sequenced on a half lane of NovaSeq

6000 S4 PE150 (additional libraries in the lane consisted primarily of RNA-seq of Pink and Chinook salmon from related projects). Sequences were uploaded under: SRP216443, with individual accessions: SRR9841162 (Adipose), SRR9841163 (Brain), SRR9841160 (Gill), SRR9841161 (Head Kidney), SRR9841166 (Heart), SRR9841167 (Hindgut), SRR9841164 (Left Eye), SRR9841165 (Liver), SRR9841168 (Lower Jaw), SRR9841169 (Midgut), SRR9841171 (Ovary), SRR9841172 (Pituitary), SRR9841170 (Pyloric Caeca), SRR9841174 (Red Muscle Skin), SRR9841176 (Spleen), SRR9841177 (Stomach), SRR9841173 (Testes), SRR9841175 (Upper Jaw Nares), and SRR9841178 (White Muscle).

265 Variant Calling

All individuals sequenced for variant calling (Supplementary Table 3) used Shotgun PCR Free IDT dual-indexed Illumina libraries, produced on a quarter lane of Illumina HiSeqX – library construction and sequencing were performed at the McGill University and G  nome Qu  bec Innovation Centre. Raw reads were uploaded to the NCBI BioProject PRJNA556729, with individual accessions listed in the supplementary table.

Variant calling followed the best practices pipeline of GATK 3.8 [74–76], and generally followed the methods previously outlined in Christensen et al. (2020) [61]. Raw paired-end reads were aligned to the scaffold-version of the genome (pre-pseudomolecule construction) using bwa (v0.7.17) mem [77] and the `-M` option. Samtools (v1.9) [78] was used to sort and index the alignment files, while Picard (v2.18.9) [79] was utilized with the MarkDuplicates option to identify likely PCR duplicates, and with ReplaceSamHeader to add read group information to the alignment files. GATK’s HaplotypeCaller was then used `--genotyping_mode DISCOVERY --emitRefConfidence GVCF` to generate gvcf files, GenotypeGVCFs was used to generate vcf on intervals, and CatVariants was used to concatenate interval files into a single vcf. A training variant set was generated using a hard-filtered subset of the first round of genotyping, utilizing VariantFiltration and the parameters `--filterExpression "QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0"` as well as the VCFtools (v0.1.14) [80] parameters `--maf0.1 --hwe0.01`. A truth set was generated by overlapping the linkage map SNPs with the hard-filtered training set to obtain SNPs found in both methods.

VariantRecalibrator was applied using these sets ``-mode SNP -an QD -an MQ -an MQRankSum -an ReadPosRankSum -an FS -an SOR -an InbreedingCoeff``, and ApplyRecalibration run to generate a final SNP set ``--ts_filter_level 99.0``. Finally, as SNP calling began pre-pseudomolecule construction, vcfChromTransfer in the Genomics General repository (https://github.com/simonhmartin/genomics_general; commit: 9d12505) was used to lift over the VCF file based on the NCBI submission AGP file. This lifted-over VCF is included in the accompanying dataset as the “raw SNP” set (referred to as set 1 below).

VCFtools v1.14 [80] ``--maf 0.05 --max-alleles 2 -- min-alleles 2 --max-missing 0.9 --remove-filtered-all --remove-indels`` was used to retain only bi-allelic markers with little missing data and remove the rarest variants (referred to as set 2). The next filter utilized the VCF.Filter.v1.0.py script [61] to remove variants with allelic imbalance ``-ab 0.2``, followed by VCFtools to select only the 37 pseudomolecules ``--chr`` (referred to as set 3). The final filter utilized BCFtools v.1.9 to filter variants for LD in a 20kb window ``+prune, -w 20kb, -l 0.4, -n 2`` (referred to as set 4). Finally, VCFtools ``-relatedness2`` was run to detect closely related individuals. In light of the results, individuals ``Oke180104-Fert164`` and ``Oke171107-D`` are recommended to be used cautiously in further analysis as they were deemed most likely to be haploid progeny (expected) and sibling (unexpected) respectively of other individuals in the analysis (can be applied to all sets prior to further analysis using ``vcftools --remove``; removed for Figure 3 below, not removed in Figure 2).

SNP dataset analyses

SNPhylo [81] was run on the “set 4” dataset, using additional options ``-m 0.05 -P Oket_chroms_37_Id0.2 -b -B 1000 -a 37`` in order to generate a bootstrapped phylogenetic tree of the chum salmon. Visualization was performed using the Figtree V1.4.4 package (<http://tree.bio.ed.ac.uk/software/figtree/>). PCA analyses were performed on the same dataset using the R package ``SNPrelate``, with full and Canadian-only sample sets plotted– the set 3 is visualized in this work, with all visualization performed using the ggplot2 package [82].

The sex phenotypes associated with re-sequencing samples (Supplementary Table 3) were utilized as the basis for a genome-wide association analysis for sex. Utilizing the allele

314 balanced SNP set (“set 2” above), VCFtools v1.14 was used to generate input for plink
315 (chromosomes only). An association test was run in PLINK 1.9 [83] using the formatted output
316 data, and resulting Manhattan plot visualized in R [84] using the qqman package [85]. Further
317 visualization of identified SNPs were performed using the Adegenet package [86]. Counts of
318 coverage utilized samtools v1.9 depth, using default parameters to calculate genome wide
319 coverage over each individual *.bam alignment file, and using the ‘-b’ option to restrict the
320 calculation to only the region of the growth hormone 2 gene (*GH2*) demonstrating elevated
321 coverage in the males following a manual review of the alignments using IGV viewer 2.9.4 [87].
322 Duplicated regions, presumably from the Salmon specific 4R duplication event, were
323 identified by alignments using the default settings of SyMap v4.2 [72], using a repeat-masked
324 version of the genome following prior methods [61], by masking WindowMasker-based
325 repetitive regions using ‘sed -e ‘/^>/! s/[[:lower:]]/N/g’ from the RefSeq genome. Summary
326 tracks were predominately generated using scripts from [40]: Orientation of the blocks were
327 generated using Analyze_Symap_Block_Orientation.py; percent identity was determined using
328 Analyze_Symap_Linear_Alignments.py; percent identify of repetitive regions identified using
329 Percent_Repeat_Genome_Fasta.py. Linkage map markers from “Map 1” in [34] were aligned to
330 the genome as previously described above using BLAST. Linkage disequilibrium (LD) was
331 interpreted using the ‘--geno-r2’ option in VCFtools [80], and outputting only for those
332 comparisons exceeding ‘--min-r2 0.5’ in order to identify the most highly linked SNPs –
333 summaries were further limited to single chromosomes using the ‘--chr’ option. LD calculations
334 utilized the allele balanced set (set 3) described above. LD track utilized counts of markers in
335 linkage disequilibrium across at least 100kb, and summarized as a log sum per 1 million base
336 pairs. Circos v0.69.9 [88] was utilized to visualize the data tracks described.
337 Heterozygosity analyses followed the same parameters and method as in [61]. Runs of
338 homozygosity were identified from the variants that had been filtered for allele balance using
339 PLINK v1.9 (parameters:—homozyg) [83]. The number of heterozygous genotypes and
340 alternative homozygous genotypes per individual were counted using the same custom script
341 described in the supplementary data of the sockeye genome [61]. Heterozygotes per kbp was
342 calculated as the number of heterozygous genotypes divided by the total nucleotides in the

343 genome (1,853,104,330) multiplied by 1 kbp. The heterozygosity ratio was calculated as the
344 number of heterozygous genotypes divided by the number of alternative homozygous
345 genotypes.

346

347 **Results and Discussion**

348 **Genome Assembly and Annotation**

349 From a raw data set consisting of 59X coverage (110 billion bp) of overlapping 250bp
350 Illumina reads and 60X coverage (114 billion bp) of total mate-pair Illumina reads of three insert
351 sizes (2, 5 and 8kb mean), multiple assembly attempts were performed varying the parameters
352 on read depth as well as read-trimming . Ultimately, three of the attempts resulted in a
353 completed assembly (see Table 1), with the final attempt being the most successful, with a
354 contig N50 of 13.1 kb and a scaffold N50 of 653 kbp. Following AllPaths-LG assembly, contig
355 gaps were filled utilizing PB Suite and 53 billion bp of Pacific Biosciences Sequel long-reads.
356 Following Pilon polishing, utilizing the short insert Illumina libraries, scaffolds were organized
357 into pseudomolecules representing the 37 chromosomes in chum salmon, predominately
358 guided by the publicly available linkage map [34]; ultimately, the linkage map allowed for 70%
359 of the genome assembly to be assigned to a linkage group, slightly lower but approximately
360 equivalent to prior attempts in salmonids using equivalent techniques (e.g., [40, 61]). The final
361 assembly was uploaded to NCBI under BioProject PRJNA556729 and ultimately was included in
362 the RefSeq database as GCF_012931545.1.

363 Busco scores indicate that most of the genome is represented within the family, with
364 results similar to what has been seen in Sockeye, with 85.0% complete (25.1% duplicated), 3.2%
365 fragmented and 11.8% missing. This likely reflects the slightly more fragmented nature of the
366 genome as compared to prior attempts using the same technology in other species. We believe
367 this is most likely due to some minor shearing observed in the DNA utilized for library
368 preparation. We did attempt to use 10X chromium data as part of this assembly process, but,
369 our scaffolding power was negligible – after review, it is likely that DNA shearing noted in the
370 bioanalyzer trace prior to library construction limited the size of the fragments from which to

371 generate the linked reads, thus limiting scaffolding power. The raw data from this attempt is
 372 included under the BioProject (see Supplementary Table 1), but further attempts would need to
 373 use a separate individual in order to increase length of the starting material. Given that
 374 sequencing and assembly technology has advanced rapidly since we began this project, it is
 375 likely further efforts to improve the genome may benefit from the use of long-read
 376 technologies, where incredible advances in contiguity have already been demonstrated in
 377 salmonids [44, 89]. Indeed, a long-read assembly for chum salmon is planned by the authors,
 378 and will eventually replace this reference, in due course.

379 Following inclusion in the RefSeq database, the genome was annotated utilizing the
 380 NCBI Eukaryotic Annotation pipeline, ultimately yielding Annotation Release 100
 381 (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Oncorhynchus_keta/100/) – see
 382 Table 2 for a summary. Gene annotation, via chum-specific reads, primarily utilized the 19
 383 tissue RNA-seq dataset sequenced as part of this work (see Supplementary Table 2), with
 384 additional contribution of sequences from two additional datasets with publicly accessible RNA-
 385 seq data [90, 91]. Overall, gene numbers are comparable to other salmonid genomes, and thus
 386 likely reflect a relatively complete representation of the coding sequence. It is likely that a
 387 future genome that utilized long-reads would result in a slightly increased number of genes (as
 388 observed between *Oncorhynchus kisutch* Annotation releases 100 and 101, for example).

389 The resulting assembly is summarized visually in Figure 1. Duplicated regions, as
 390 identified via self-alignment using Mummer [92] reflect re-diploidized segments of the genome
 391 from the salmon-specific 4R duplication event. There are observations of elevated percent
 392 identity on the ends of some chromosomes (Figure 1) that demonstrate partial re-diploidization
 393 as in Waples et al. (2016) [34] (e.g., LG05 and LG32), but the effect is not nearly as extensive as
 394 that observed in other species. The repetitive elements identified by Window Masker were
 395 elevated in regions likely overlapping with centromeres based on synteny with other species for
 396 which chromosome arms have been described (Figure 1). Figure 1 also shows Map 1 from
 397 Waples et al. (2016) [34] (which can be further visualized in more detail in Supplementary
 398 Figure 1 and map 2 in Supplementary Figure 2), and demonstrates the co-linearity of the map
 399 with the pseudomolecules. As the maps do contain regions of low-recombination, much of the

400 ordering and orientation of the scaffolds into pseudomolecules (but crucially, not the
401 assignment to the pseudomolecule itself) relies heavily in some positions on the long-read and
402 Hi-C based assembly of coho salmon (GCF_002021735.2). Given the extensive conserved
403 synteny and co-linearity between orthologous salmonid chromosome arms demonstrated
404 elsewhere (e.g., [36, 40]), this would appear to be a reasonable approach, and has been part of
405 the development of pseudomolecules for short-read assemblies in salmonids previously (eg.
406 [40]). Regions of the genome with high LD generally overlap with regions of reduced
407 recombination as observed in the linkage map (Figure 1). Further exploration of regions of high
408 LD can be observed in Supplementary Figures 3 and 4.

409 As a final clarification on the assembly presented, we note that pseudomolecules have
410 been named within the publicly available assembly based on the linkage group naming
411 mechanism in Waples et al. (2016), [34] to allow for direct comparison between the two works.
412 However, the authors also note, and are enthusiastic about, the naming convention suggested
413 by Sutherland et. al., [36] to describe chromosomal arms, and indeed the adoption of the
414 system into the grayling genome assembly [42]. We provide here in Table 3 the naming for the
415 pseudomolecules that could be suggested by such a system. While the pattern of fusions do
416 make this system less than ideal, and the resulting chromosome names are somewhat
417 unwieldy, we provide them here as a quick reference and potential guide to re-naming of the
418 linkage groups should such a system continue to prove popular as future assemblies are
419 released. Presenting both names here will hopefully ease future reference, whichever naming
420 scheme ends up being formally adopted in future works.

421

422 **Population level variation**

423 Given the extensive distribution of chum salmon, attempts were made to maximize
424 geographic distribution of the samples selected within the study. We were able to take
425 advantage of an extensive collection of samples [10] in the archive of the Molecular Genetics
426 Lab (Pacific Biological Station, Fisheries and Oceans Canada), combined with more recent
427 contributions from various Fisheries and Oceans Canada hatchery staff for recent brood. While
428 the collection is focused on British Columbia, the addition of the Japanese samples originating

429 from the Tokushibetsu River on the Island of Hokkaido give a glance at the degree of variation
 430 expected across the Pacific. Samples and available metadata are summarized in Supplementary
 431 Table 3. In total, 15,372,999 nucleotide variants have been described with this data in the raw
 432 dataset, with described filters leaving 8,868,081 in set 2, 2,135,295 in set 3, and 94,080 in set 4.
 433 A summary of statistics by individual is given in supplementary table 5 [61]. On average (and
 434 ignoring the haploid individual), total lengths of runs-of-homozygosity (ROH) averaged 12.4
 435 Mbp [0 - 40.8 Mbp as determined using default parameters]. Heterozygous SNPs per 1kbp
 436 averaged 1.47 (Standard Deviation = 0.15), while the heterozygosity ratio averaged 2.23
 437 (Standard Deviation = 0.45). Overall, results are relatively similar to what was observed utilizing
 438 a parallel analysis in Sockeye salmon, although the overall length of ROH is lower (12.4 Mbp in
 439 chum salmon vs. 35.5 Mbp in Sockeye salmon), whereas heterozygous SNPs per 1kbp are
 440 increased (1.47 in chum salmon vs. 0.67 in Sockeye salmon), and the heterozygous ratio was
 441 approximately equivalent (2.23 in chum salmon vs. 2.21 in sockeye salmon after removing
 442 outliers). Deviations below the mean for both heterozygosity calculations were predominately
 443 associated with average coverage, implying that depth of sequencing likely impacted to some
 444 extent these calculations. Regardless, we demonstrate in chum salmon that there is a general
 445 increase in heterozygosity as compared to sockeye salmon, and establishes a comparative
 446 metric to be carried through to future comparative analyses in other Pacific salmonids.

447 Analyses of the SNP set resulting from whole genome resequencing (targeted coverage
 448 of 15X) should be considered exploratory, as collections were focused on geographic coverage
 449 to maximize variants within the catalogues rather than addressing additional questions.
 450 Nevertheless, the geographic variation explored allowed us to better understand differentiation
 451 among British Columbia locations. To this end, a bootstrapped maximum likelihood tree was
 452 constructed using a linkage-disequilibrium thinned SNP-set using SNPhylo [81]. As can be seen
 453 in Figure 2, the dendrogram clusters samples by regions similar to past analyses with more
 454 comprehensive sampling but using older marker technologies (see above). Samples can be
 455 resolved into regions corresponding to descriptions from the comprehensive sampling of
 456 Beacham et al. (2009) [10], with individual samples resolvable into Japan – Hokkaido; BC
 457 Central Coast (Snootli and Kitimat); BC- Haida Gwaii (Deena Creek); BC – West Coast Vancouver

458 Island (Nitinat); BC – Strait of Georgia (Tenderfoot, Big Qualicum, Puntledge); and BC – Lower
459 Fraser (Chilliwack, Inch, Chehalis). However, within clusters from multiple regions, we see a
460 relative lack of resolution to the riverine level. Such observations are supported by Principal
461 Component Analysis (PCA) as well (Figure 3); however, we do begin to see stronger
462 delineation, possibly from the increased number of variant and dimensions in the PCA analysis.
463 In Figure 3A, we observe differentiation across the Pacific Ocean (best described along PC1),
464 and to a lesser degree geographically across the British Columbia coastline (along PC2). When
465 described regionally, individuals from most populations can easily be resolved when focusing
466 on the British Columbia coastline (Figure 3B), and we are able to see delineation among all
467 collections, except those in the Fraser River Basin. Focusing on the Fraser River Basin sites
468 alone, the pattern is less clustered (Figure 3C), although we do see some separation from
469 salmon collected in different river systems of the Fraser River drainage.

470 Clustering techniques show that river-level resolution is not always observed. Such
471 results have been noted in the past when considering fishery mixture resolution and describing
472 assignments to region only (for example [9, 93]), but it is worth emphasizing that incomplete
473 resolution among collected populations remains true when considering a relatively
474 comprehensive genome-wide representation of variation. As part of the thinning procedure for
475 SNPhylo, however, by default a significant number of SNPs are removed to increase the speed
476 of the calculation. Alternatively, in the analysis of principal components, with just an LD
477 threshold applied (0.2), a much greater number of SNPs were input into the resulting analysis,
478 and it is likely that the number of SNPs in the end analysis played at least a partial role in the
479 reduced delineation observed in the dendrogram relative to the PCA. While collection level
480 differentiation does emerge in the PCA result, observations on reduced datasets (e.g., by
481 chromosome) greatly inhibited the resolving power of the analysis (supplementary figure 5).
482 Based on the results presented here, it is likely that collection level-specific SNPs could be
483 identified in this dataset that maximize the population differentiation observed genome-wide,
484 and that would further drive differentiation observed in the PCA. However, with such a small
485 sampling size, it is likely that any such discovery would be more a representation of sampling

486 depth, and the noise within a set would be high. However, this dataset is now available, should
487 future researchers need to draw on a pool of potential SNPs from which to develop such assays.

488 Within BC, chum salmon regional groupings are described at the conservation unit (CU)
489 level [94], and it is intriguing to note that there may be substructure to the results observed
490 along those lines in the present analysis. For example, the Tenderfoot hatchery samples in the
491 Howe Sound-Burrard Inlet CU do tend to cluster more strongly, relative to the other collection
492 sites in the adjacent Georgia Strait CU suggesting that a greater sample size may allow recovery
493 of further groupings. However, it is likely that straying, generally described as high in chum
494 salmon, is playing a role in limiting genetic distinctiveness to the level of the CU (or higher)
495 regional groupings. While sampling within the study focused primarily on large hatchery
496 operations, it is also possible we are simply revealing a high degree of variation within each
497 population due to a large effective population size, in which case sufficient additional sampling
498 may coalesce around a mean per population. Still, even within the dataset here, the
499 observation remains that individual population level resolution within a region may begin to be
500 demonstrated with genome-wide representation.

501

502 **Mapping the sex-determining region**

503 Although limited metadata was collected for individuals sampled beyond geographic
504 locations sampled, we were able to collect phenotypic sex information on hatchery brood
505 samples. Thus, we were able to explore genome wide associations (GWAs) of phenotypic sex.
506 As demonstrated in Figure 4A, two clear peaks were observed with the GWAS: a very strong
507 peak on Linkage Group 15, and another, albeit somewhat weaker, association on Linkage Group
508 3. As shown in Figure 4B, the specific region overlaps with an area of increased linkage
509 disequilibrium on the distal end of LG15. In Figure 4C, the genotypes for each individual is
510 displayed for the 20 SNPs seen as most associated with sex within the GWAS analysis. LG15 has
511 been previously identified by McKinney et al. (2020), [59] as linked to sex during a RAD-seq
512 based study of chum salmon populations within Alaska. In this prior work, linkage of sex to a
513 particular region of the genome was complicated by two potential factors – a lack of a
514 chromosome-level assembly for chum salmon, and the identification of a putative inversion

515 along the chromosome that resulted in significant patterns of linkage. We utilized the sex-
516 linked RAD loci to position the markers onto the new genome assembly and observed that
517 while all were indeed placed along Oket_LG15, they appeared to be more dispersed along the
518 chromosome, and were not strongly linked to sex within our geographically distinct sample set
519 (Supplementary Table 7). Within the present study, we observed sex linked to a very narrow
520 region along Oket_LG15; while some noise is observed, the peak is approximately in the 30.8
521 Mbp to 31 Mbp region and encompasses four annotated genes: potassium/sodium
522 hyperpolarization-activated cyclic nucleotide-gated channel 2-like; E3 ubiquitin-protein ligase
523 RNF126-like; SURP and G-patch domain-containing protein 1-like; and serine/threonine-protein
524 kinase STK11-like. While we do not suggest any of these are the sex-determination gene – as
525 with other Pacific salmonids it is presumed to be sdY [95] – given that the underlying genome
526 assembly is female, this likely represents the approximate region where sdY is inserted on the
527 Y-chromosome, and limited recombination surrounding the region has led to sex-specific
528 markers extending to autosomal-like sequence flanking the insertion. This region (on
529 chromosome 3.2 based on the naming scheme in Sutherland et al., 2016 [36] and Table 3)
530 would appear to be a unique placement thus far in sdY mapping – however, the relatively
531 common observation of sdY on chromosome arm 3.1 (sockeye salmon, coho salmon, lake
532 whitefish; [96] and references therein) does suggest that inter-homeologue transfer between
533 chromosome arms arising from the most recent salmon-specific duplication could be a
534 mechanism for this transfer.

535 The strong secondary peak observed on Linkage group 3 is slightly more confounding
536 and intriguing, as it does not appear to be linked to a known sex-determination orthologue in
537 salmonids [96], and because potential sex-markers appear linked to those on LG-15. While it
538 could be linked to a misplaced contig within the assembly, comparative mapping between
539 additional species did not suggest anything was misplaced based on conserved synteny (data
540 not shown; performed within Symap using default parameters) – if this is the case, it is likely
541 that a future long-read based assembly will correct such a matter. It seems most likely in this
542 case that it represents a repetitive element or otherwise duplicated sequence that is prominent
543 in the Y-specific region but is not present in this female genome; thus, mis-mapping appears to

544 occur elsewhere in the genome. A manual review of the region does imply a highly repetitive
545 region, with great differentiation in depths indicative of collapsed repeats. Such mismapping
546 based on collapsed repeats or a lack of sex-specific reference is not uncommon (e.g., as
547 demonstrated in Chinook salmon by mapping of the Y-specific growth hormone pseudogene to
548 the GH2 locus on a different chromosome [97]) and it may be that assembly of a male genome
549 will reveal repeat patterns underlying this unexpected result observed here. There may be
550 additional, more complex reasons based on the observance of multiple sdY regions seen in
551 other species (e.g., Atlantic salmon [98]), although other explanations may be equally likely
552 here. Observations have been made elsewhere that GH-Y, a commonly used proxy for genetic
553 sex in salmonids [99], was found to be missing in males or present in females in some chum
554 salmon populations [100]. While the presented genome is female-based (and thus not
555 predicted to contain GH-Y), observation of relative coverage at the most closely related gene in
556 the genome – GH2 – indeed implied that between 0-5 copies of GH-Y are observed in male
557 individuals, with those males observed to be missing GH-Y being from Kitimat (2x), Snootli (1x)
558 and Tenderfoot (1x): see supplementary table 6. These data do not suggest the phenotypes are
559 mis-identified, however, as inclusion of a Rainbow trout sdY into the alignment phase
560 demonstrated that the presence of sdY matches the phenotype, as would be predicted [95]. No
561 copy-number differences could be interpreted from the sdY alignment unfortunately, as the
562 underlying sequence from trout appeared too differentiated to obtain a reliable estimate of
563 coverage; however, reads were observed aligned to the sequence in all male individuals and
564 not in female individuals in a manual review utilizing IGV viewer. Still, the GH-Y results do
565 indicate that there is variability in the genomic architecture surrounding sdY, and perhaps may
566 indicate that alternate locations within the genome could be influential. Whatever the
567 underlying genomic architecture of the sex-determination region may be in chum salmon, the
568 result presented here underlines the usefulness and ease of use of the presented SNP dataset
569 and reference genome in mapping a trait of interest to the appropriate chromosome and
570 chromosomal region within the genome.

571

572 **Conclusions**

573 The genome assembly for chum salmon represents a relatively complete representation
574 of the chum salmon genome: the first such resource for the species. Contiguity and
575 completeness is likely most affected in regions with high residual tetraploidy or incomplete re-
576 diploidization. While long-read based assemblies (and future sequencing technologies) are
577 likely to generate a more complete picture, the current genome assembly represents a valuable
578 resource for chum salmon on par with those available for Chinook, sockeye, and longstanding
579 assemblies for Atlantic salmon and rainbow trout that allowed a transformation in genomic
580 understanding of these commercially and culturally specific species (e.g., [101]).
581 Complementing the presented genome is a pilot-scale catalogue of variation that provides a
582 genome-wide resource for British Columbian chum salmon populations, and allows for
583 contrasting variation in Western and Eastern Pacific lineages. Such a dataset will be explored
584 further as a resource for SNP genotyping panel expansion, structural variation discovery, or as
585 demonstrated here, in identification of the chromosome and position most likely to contain the
586 sex-determination gene in chum salmon.
587

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597

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853

854 **Tables**

855

856 Table 1: Assembly results for Allpaths and PBSuite based assemblies performed.

857

858 Table 2: Summary of Annotation Release 100 from the NCBI Eukaryotic Annotation pipeline.

859 See https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Oncorhynchus_keta/100/ for

860 more details.

861

862 Table 3: Pike-like chromosome naming for the chum salmon pseudomolecules described in this

863 work, based on Sutherland et al. (2016) [36]

864

865 **Figures**

866

867 Figure 1: Circos plot of the chum salmon genome GCF_012931545.1. Inner ribbons demonstrate

868 ohnologous regions (regions duplicated at the salmon-specific genome duplication event).

869 Working in to out, Track A describes the average percent identity between the duplicated

870 regions, in 1 Mbp bins. Track B describes the average percent identity in the chromosomes, in 1

871 Mbp bins. Track C describes the relationship to “Map 1” chum linkage map from Waples et al.

872 (2016) [34]. Track D describes SNPs demonstrating elevated LD ($R^2 \geq 0.5$) and ≥ 100

873 kb apart, demonstrated as a log10 based count, in 1Mbp bins.

874

875 Figure 2: Dendrogram produced by SNPhylo, utilizing set 4 SNP data described in the text.

876 Values at nodes indicate bootstrapping. Samples are coloured by geographic region.

877

878 Figure 3: Principal component analyses performed on set 3 SNPs described in the text, using

SNPrelate and plotted in ggplot2. Samples are coloured by collection and displayed in the legend. A) the full dataset (all samples) are presented. B), Japanese samples are removed from the analysis. C), the collections are reduced solely to the collections within the Fraser River drainage.

883

Figure 4: Association of the phenotypic sex to the genome utilizing SNP variant set 1. A) the results of the GWAS are presented, with Bonferroni-adjusted p-values shown at the 5% level (blue line) and 1% (orange line) levels. B) The SNPs with R-squared greater than 0.5 are counted, and plotted to show relationship of distance between SNPs being measured, for the region flanking the signal on Oket_LG15. C) The genotypes for each individual is displayed for the 20 SNPs seen as most associated within the GWAS analysis, with homozygous reference in blue, heterozygous in purple, homozygous alternate in red, and missing genotypes in white. Samples are sorted to group males, females and unknowns (Japanese samples—most likely females).

893

894 **Supplementary Data**

895

Supplementary Table 1: Biosample and SRA data for individual chum used in generating the genome assembly.

898

Supplementary Table 2: Biosample and SRA data for individual chum used in generating the Illumina RNA-seq data.

901

902 Supplementary Table 3: Biosample and SRA data for individual chum used in generating the Re-
903 sequencing data.

904

905 Supplementary Table 4: Allpaths-LG parameters explored in attempting to obtain the highest
906 contiguity assemblies.

907

908 Supplementary Table 5: Heterozygosity metrics by individual are described. Includes counts of
909 missing genotypes, Homozygous Reference and Alternate genotypes, Heterozygous genotypes,
910 average depth at called sites, the mean count of heterozygous SNPs per kbp, the ratio of
911 Heterozygous genotypes to Homozygous alternate, and the total length of runs-of
912 homozygosity as determined from PLINK using default parameters.

913

914 Supplementary Table 6: Depth of coverage across the alignments, and at the GH2 locus to
915 approximate the count of GH-Y copies in each individual. GH2 is used for this calculation due to
916 the lack of GH-Y in the reference genome, and therefore the alignment of GH-Y to the closest
917 homologue.

918

919 Supplementary Table 7: Placement of SNPs associated with phenotypic sex from McKinney et
920 al. [59] in Alaskan chum populations onto the current reference genome.

921

922 Supplementary Figure 1: Plotting the association between Linkage groups in Waples et al.
923 (2016), [34] map 1, and the reference genome assembly presented in this work.

924

925 Supplementary Figure 2: Plotting the association between Linkage groups in Waples et al.
926 (2016), [34] map 2, and the reference genome assembly presented in this work.

927

928 Supplementary Figure 3: Plotting the linkage disequilibrium along each chromosome. SNPs are
929 only displayed if R-squared is greater than 0.5, and is plotted as a count of SNPs.

930

931 Supplementary Figure 4: Plotting the linkage disequilibrium along each chromosome. SNPs are
932 only displayed if R-squared is greater than 0.5, and each SNP is plotted by R-squared value.

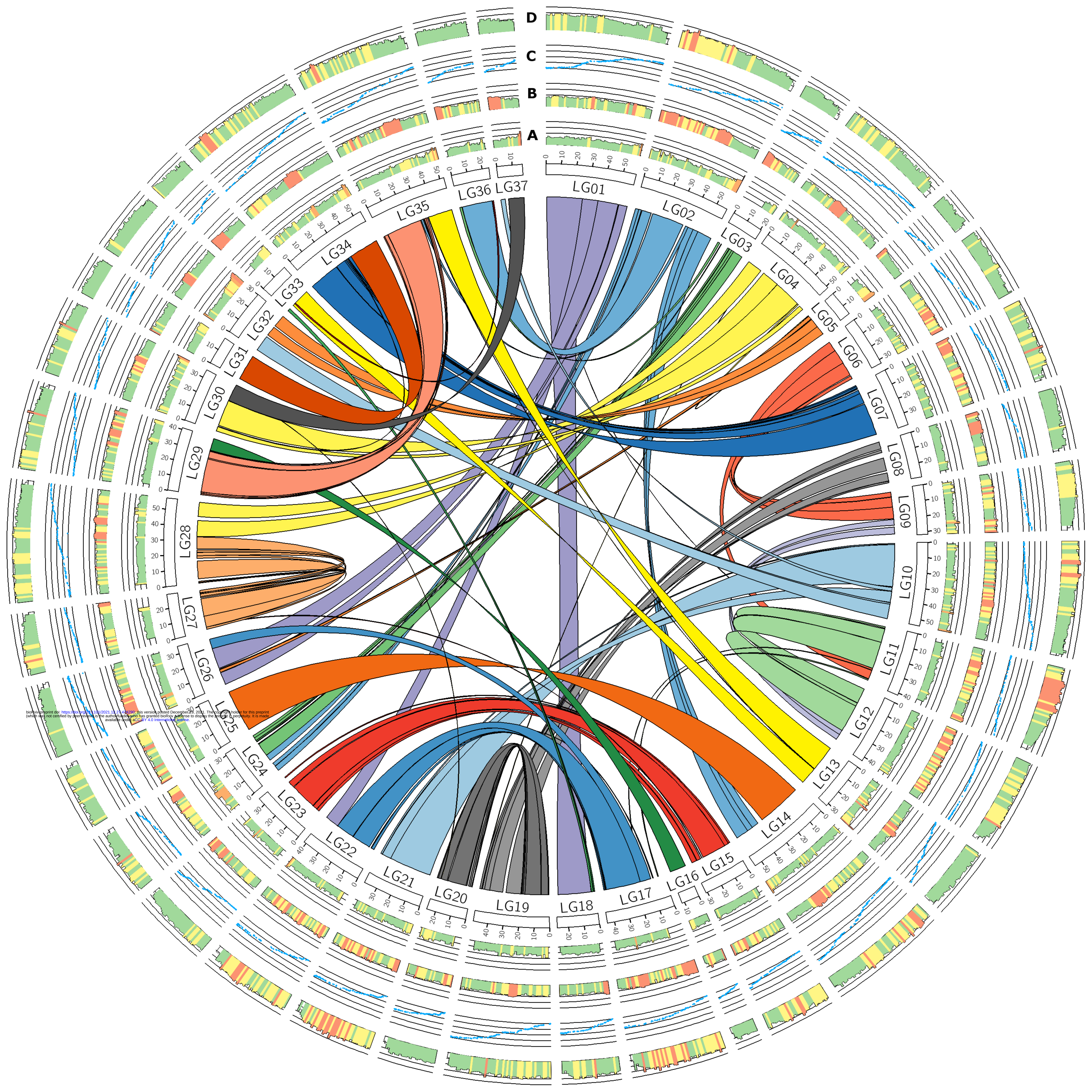
933

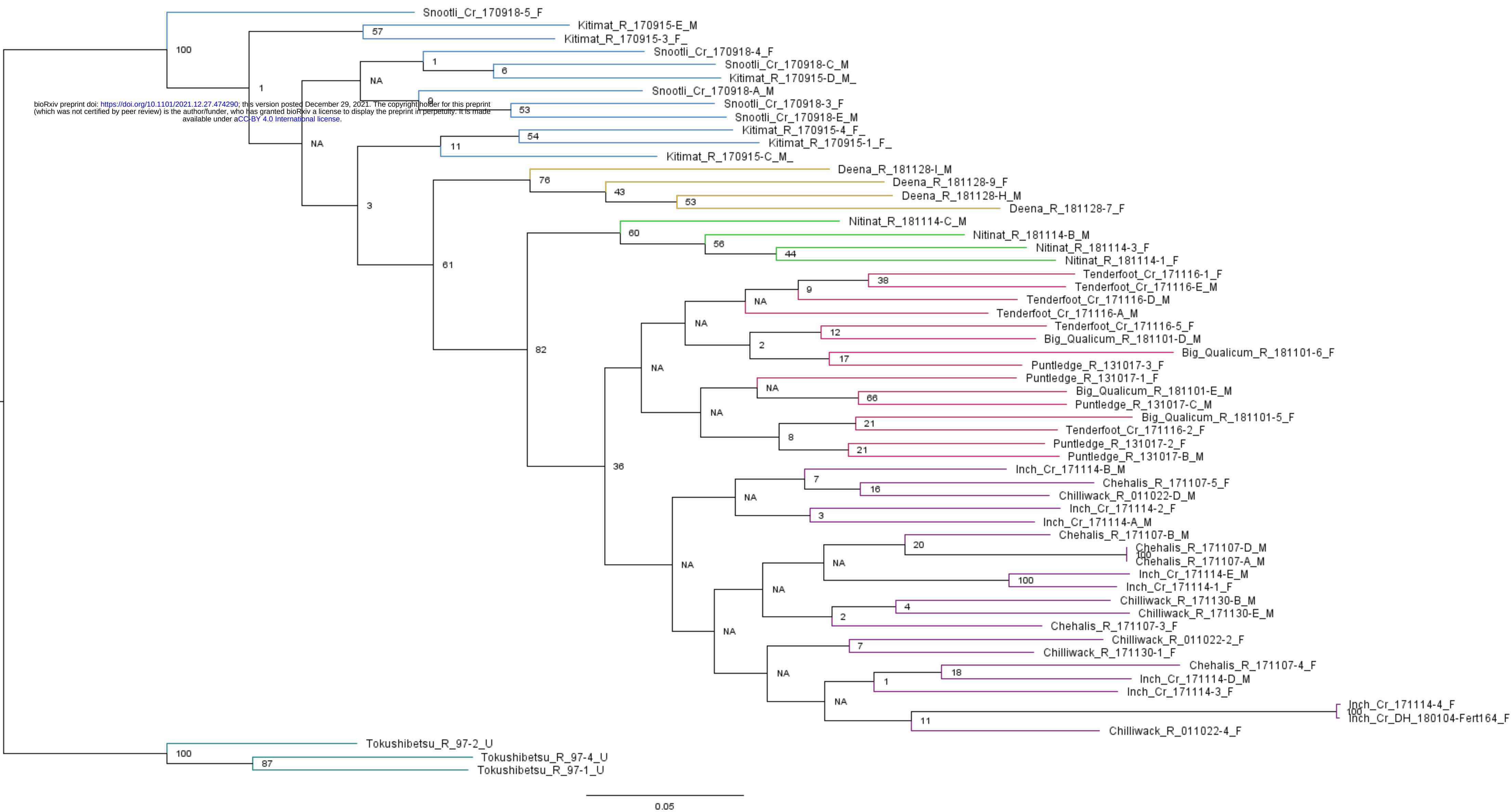
934 Supplementary Figure 5: Principal component analyses performed on set 3 SNPs described in
935 the text, using SNPrelate and plotted in ggplot2 and reduced to only query LG15. Samples are
936 coloured by collection, displayed in the legend. In panel A, the full dataset (all samples) are
937 presented. In panel B, Japanese samples are removed from the analysis. In panel C, the samples
938 are coloured by collection site rather than by region.

	Assembly size (Contigs)	Assembly size (Scaffolds)	Scaffold N50	Contig N50
Allpaths	1471097779	1813373414	653	13.1
PBSuite	1,766,907,823	1,852,809,593	665,581	52,191

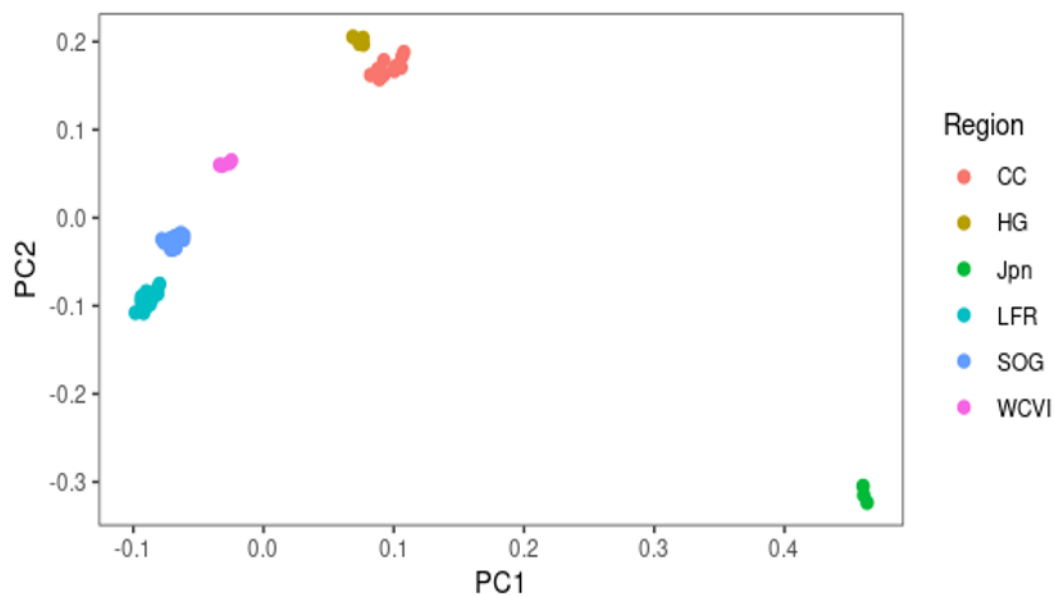
Feature	Annotation Release 100
Genes and pseudogenes	45643
protein-coding	36325
non-coding	6205
transcribed pseudogenes	222
non-transcribed pseudogenes	2821
genes with variants	14102
immunoglobulin/T-cell receptor gene segments	70

Linkage Group	Accession	Alternate Naming
Oket_LG01	NC_050106	1.2-8.2
Oket_LG02	NC_050107	6.1-2.2
Oket_LG03	NC_050108	5.1
Oket_LG04	NC_050109	21.1-4.2
Oket_LG05	NC_050110	11.2
Oket_LG06	NC_050111	14.2
Oket_LG07	NC_050112	16.1
Oket_LG08	NC_050113	18.2
Oket_LG09	NC_050114	14.1
Oket_LG10	NC_050115	17.1-9.2
Oket_LG11	NC_050116	7.2
Oket_LG12	NC_050117	7.1
Oket_LG13	NC_050118	24.1-23.1
Oket_LG14	NC_050119	13.1-2.1
Oket_LG15	NC_050120	3.2
Oket_LG16	NC_050121	25.1
Oket_LG17	NC_050122	19.1
Oket_LG18	NC_050123	1.1
Oket_LG19	NC_050124	10.2-18.1
Oket_LG20	NC_050125	10.1
Oket_LG21	NC_050126	17.2
Oket_LG22	NC_050127	19.2
Oket_LG23	NC_050128	3.1
Oket_LG24	NC_050129	5.2
Oket_LG25	NC_050130	13.2
Oket_LG26	NC_050131	8.1
Oket_LG27	NC_050132	12.1
Oket_LG28	NC_050133	12.2-21.2
Oket_LG29	NC_050134	15.2-25.2
Oket_LG30	NC_050135	4.1-22.2
Oket_LG31	NC_050136	20.1
Oket_LG32	NC_050137	9.1-11.1
Oket_LG33	NC_050138	23.2
Oket_LG34	NC_050139	16.2-20.2
Oket_LG35	NC_050140	15.1-24.2
Oket_LG36	NC_050141	6.2
Oket_LG37	NC_050142	22.1

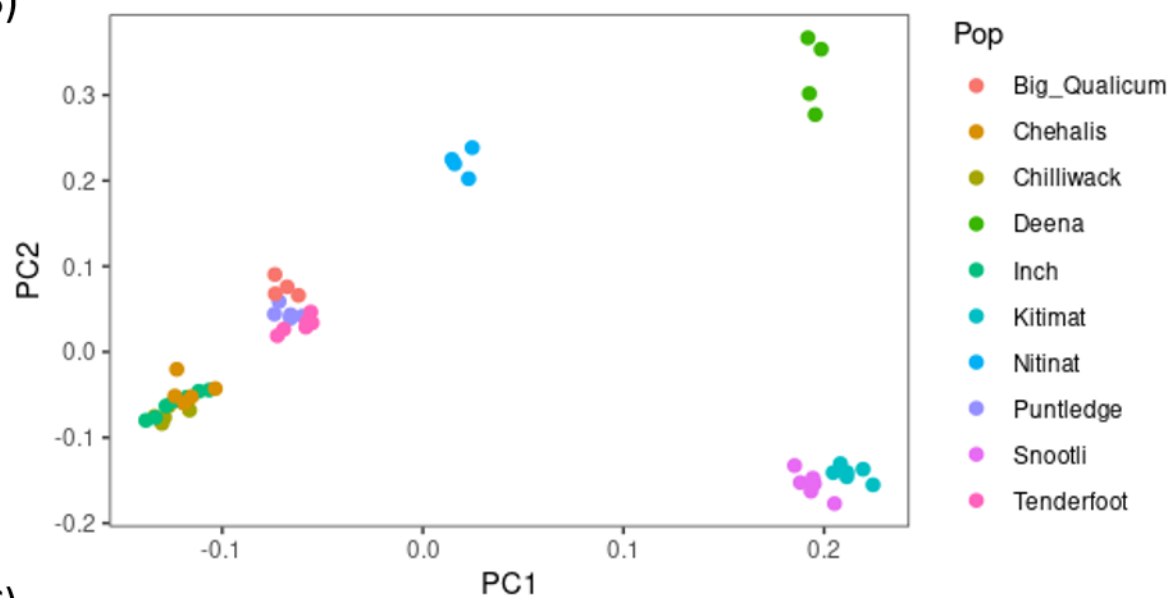




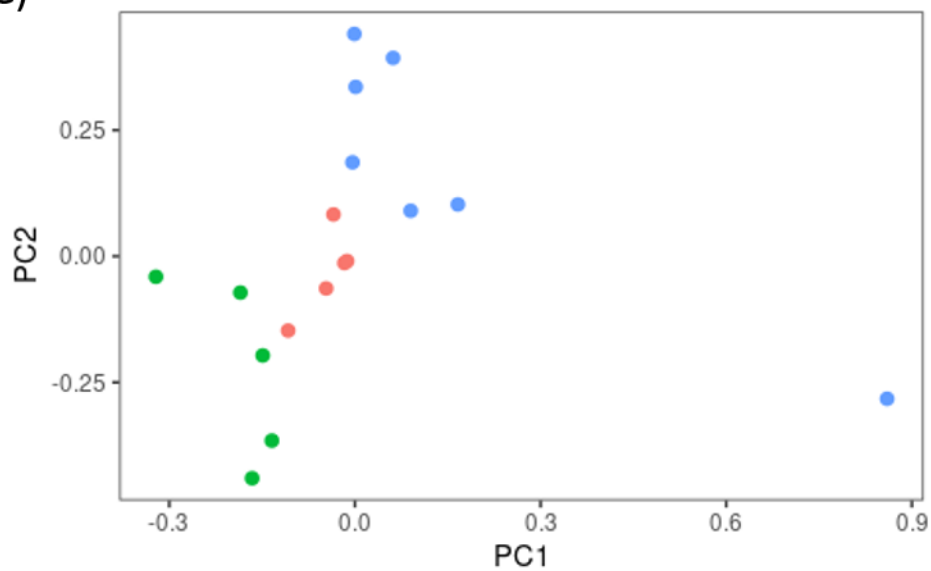
A)



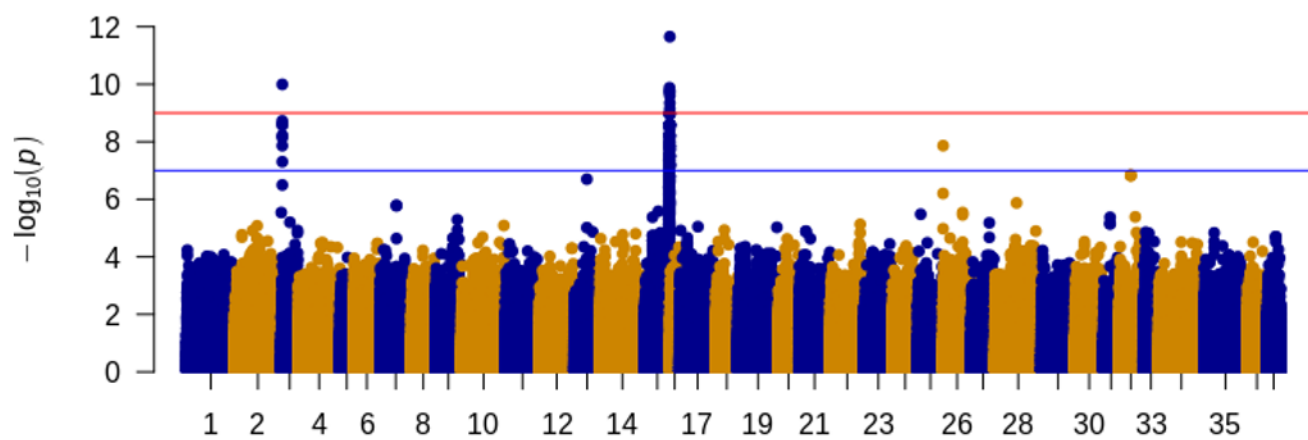
B)



C)

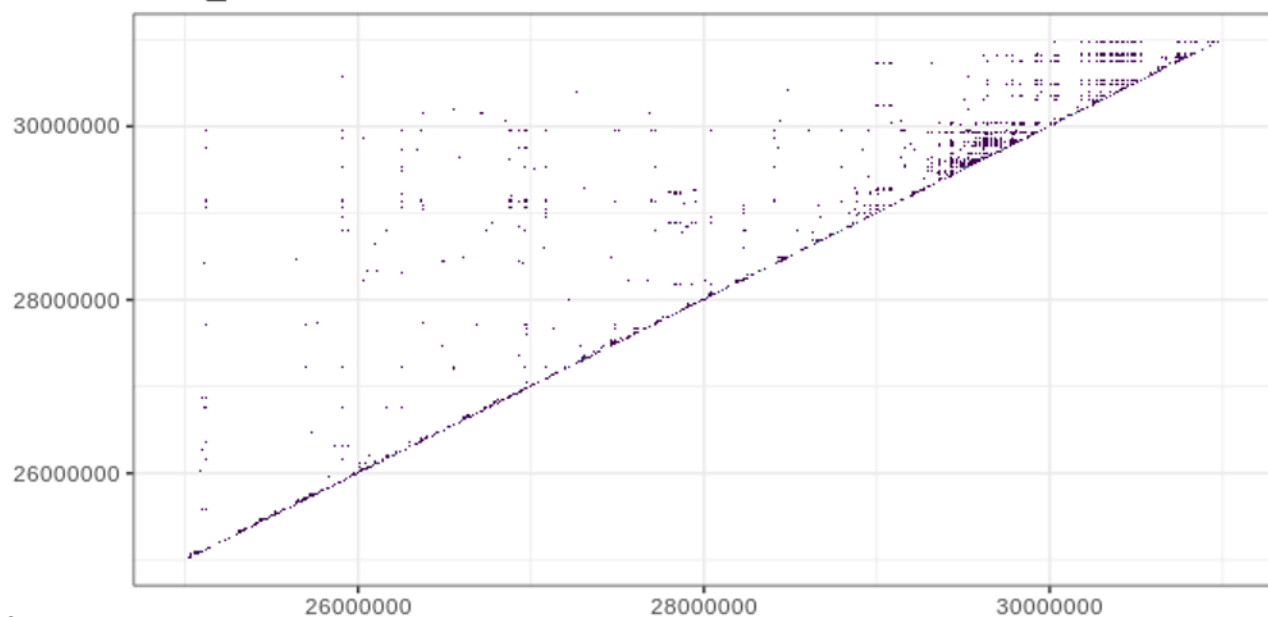


A)



B)

Oket_LG15



C)

