The giant sequoia genome and proliferation of disease resistance genes

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A high quality reference genome for giant sequoia

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ABSTRACT

The giant sequoia (*Sequoiadendron giganteum*) of California are massive, long-lived trees that grow along the U.S. Sierra Nevada mountains. As they grow primarily in isolated groves within a narrow range, conservation of existing trees has been a national goal for over 150 years. Genomic data are limited in giant sequoia, and the assembly and annotation of the first giant sequoia genome has been an important goal to allow marker development for restoration and management. Using Illumina and Oxford Nanopore sequencing combined with Dovetail chromosome conformation capture libraries, 8.125 Gbp of sequence was assembled into eleven chromosome-scale scaffolds. This giant sequoia assembly represents the first genome sequenced in the Cupressaceae family, and lays a foundation for using genomic tools to aid in giant sequoia conservation and management. Beyond conservation and management applications, the giant sequoia assembly is a resource for answering questions about the life history of this enigmatic and robust species. Here we provide an example by taking an inventory of the large and complex family of NLR type disease resistance genes.

INTRODUCTION

Giant sequoia, *Sequoiadendron giganteum* (Lindl.) J.Buchh., is a California endemic conifer found in fragmented groves throughout the U.S. Sierra Nevada mountain range. Giant sequoias are known for their substantial size; individual specimens can reach over 90 m in height, more than 10 m in diameter, and may exceed 1000 m^3 of wood volume (Sillett et al., 2015). In addition to their considerable proportions, giant sequoias are among the oldest tree species, as individuals can live for over 3,200 years (Douglass, 1919). Giant sequoias are one of

the two redwood species in California, where they share the title of state tree with their closest relative, the coast redwood (*Sequoia sempervirens* Endl.).

Though they have occupied their current range for millennia and were known by indigenous people for centuries before colonizers arrived, giant sequoias became icons of the American west beginning with the exploitation of the Discovery Tree in 1853 (Cook, 1961). Despite the brittle nature of their wood, historical research indicates a third of groves were either completely or partially logged (Elliot-Fisk et al., 1997, cited by Burns et al., 2018). Giant sequoias were first protected in 1864 (Cook, 1961), and have remained a cornerstone of the American conservation movement ever since.

While the majority (98%) of remaining giant sequoia groves are now protected (Burns et al., 2018), the species is listed as endangered (IUCN) and is overall experiencing a decline (Schmid & Farjon, 2013). The dwindling numbers of giant sequoia are largely attributed to a lack of reproductive success due in part to fire suppression over the last century (Stephenson, 1994), as giant sequoia trees rely on extreme heat to open their cones and release seeds in addition to preparing the understory for germination. Mature giant sequoias in natural stands appear to withstand most pests and diseases, but relatively little is documented about the potential impact of insects and pathogens on younger trees. Recent research suggests giant sequoias are potentially susceptible to bark beetles, which can exacerbate the impacts of drought (Stephenson et al., 2018).

In plants, disease resistance is typically conferred by genes encoding nucleotide binding leucine-rich repeat (NLR) proteins that individually mediate responses to different pathogens. In crop species, NLR genes have demonstrated contributions to resistance against insects (Stahl et

al., 2018), and a recent examination of transcriptome data from several conifer species showed that many conifer NLRs were induced following drought stress (Van Ghelder et al., 2019), suggesting an even broader role. Their importance in resilience to disease and abiotic stress makes cataloging NLR genes of particular interest for conservation and management. Notably, however, across species and even among plant populations, NLR genes account for the majority of copy-number and presence/absence polymorphisms (Yu et al., 2011; Zheng et al., 2011; Xu et al., 2012; Bush et al., 2013; Schatz et al., 2014), and this complexity makes accurate inventory challenging in the absence of a high quality genome assembly.

More broadly, a whole genome reference assembly provides a foundation for understanding the distribution of genetic variation in a species, which is critical for conservation and management. Though studies of population genetics and phylogenetics of giant sequoia have been conducted using isozymes, microsatellites, RADseq, and transcriptomic data (Fins and Libby, 1982; DeSilva & Dodd, 2014; Dodd & DeSilva, 2016; Scott et al., 2016) there is a dearth of robust genomic resources in this species. The closest species with fully sequenced genomes exist entirely in the family Pinaceae, which last shared a common ancestor with giant sequoia (Cupressaceae) more than 300 million years ago (Leslie et al., 2018).

A combination of short-read Illumina data, long-read Oxford Nanopore data, and Dovetail proximity ligation libraries produced a highly contiguous assembly with chromosome-scale scaffolds, many of which are telomere-to-telomere. This assembly also includes the largest scaffolds assembled to date in any organism. The genome was found to contain over 900 complete or partial NLR genes, of which over 250 are in consensus with annotation derived from protein evidence and gene modeling. The giant sequoia genome

assembly and annotation presented here is an unprecedented resource in conifer genomics, both for the quality of the assembly and because it represents an understudied branch of the gymnosperm tree of life.

MATERIALS AND METHODS

Sequencing and assembly

Megagametophyte DNA extraction and sequencing

Cones were collected from a 1,360-year-old giant sequoia (SEGI21, Sillett et al., 2015) in Sequoia/Kings Canyon National Park in 2012. As in previous conifer genome sequencing projects (e.g. Zimin et al., 2014), the megagametophyte from a single fertilized seed was dissected out and its haploid DNA extracted with a Qiagen DNeasy Plant Kit (Hilden, Germany), followed by library preparation with an Illumina TruSeq Nano kit using the low throughput protocol. This megagametophyte library was then sequenced on 10 lanes of an Illumina HiSeq 4000 with 150 bp paired-end reads at the UC Davis Genome Center DNA Technologies Core facility.

Foliage DNA extraction and Nanopore sequencing

In 2017 foliage was collected from the upper canopy of the same giant sequoia tree (SEGI21). From this foliage, high molecular weight DNA was extracted following the protocol described here (dx.doi.org/10.17504/protocols.io.4vbgw2n) . Briefly, purified genomic DNA was isolated through a nuclei extraction and lysis protocol. First, mature leaf tissue was homogenized in liquid nitrogen until well-ground, then added to a gentle lysis buffer (after Zhang et al., 2016,

containing spermine, spermidine, triton, and β-mercaptoethanol) and stirred at 4°C for ten minutes. Cellular homogenate was filtered through five layers of Miracloth into a 50mL Falcon tube, then centrifuged at 4°C for 20 minutes at 1900 x g, which was selected based on the estimated giant sequoia genome size of around 9 Gb (Zhang et al., 2012; Hizume et al., 2001). Extracted nuclei were then lysed and gDNA precipitated using the Circulomics Nanobind Plant Nuclei Big DNA kit - alpha version (SKU NB-900-801-01). Then 1 µg of purified genomic DNA was input into the Ligation sequencing kit (LSK108-LSK109, Oxford Nanopore), according to protocol, with the exception of end repair optimization (100 µL sample, 14 µL enzyme, 6 µL enzyme at 20°C for 20 minutes, then 65°C for 20 minutes). Samples were sequenced on R9.4 minION flowcells using either the minION or GridION for 48 hours, then raw fast5 data was basecalled with Albacore version 2.13.

Hi-C and Chicago library preparation and sequencing

Additional foliage from SEGI21 was submitted to Dovetail Genomics (Scotts Valley, CA) for Hi-C and Chicago library preparation as described by Putnam et al., 2016. Hi-C libraries preserve *in vivo* chromatin structures while Chicago libraries are based on *in vitro* reconstituted chromatin; the combination of these two approaches allows for marked improvement in contiguity for genome assemblies. Three Hi-C libraries and two Chicago libraries passed QC for sequencing and were sent to the UC San Francisco Center for Advanced Technology where they were pooled and sequenced on an Illumina Novaseq 6000 in a single lane of an S4 flowcell (PE 150 bp).

Genome assembly

Assembly of the giant sequoia genome involved two major steps: contig assembly from Illumina and Oxford Nanopore reads and scaffolding with Chicago and Hi-C data by Dovetail Genomics. Contigs were produced using MaSuRCA assembler version 3.2.4 (Zimin et al, 2013, Zimin et al, 2017) with the default parameters. Then the sequence data from the two Chicago libraries were used to scaffold the initial contig assembly using Dovetail's HiRise software (Putnam et al., 2016). Following this step, the output assembly comprised of Illumina, Oxford Nanopore, and Chicago data plus the Hi-C data was used as input for a second run of HiRise re-scaffolding software. The initial contig assembly was named giant sequoia 1.0 and the final scaffolded assembly giant sequoia 2.0.

Identification of centromeric and telomeric repeats

Tandem repeat elements up to 500 bp long were identified with the tandem repeat finder program (trf v4.09; Benson, 1999) with the recommended parameters (max mismatch delta PM PI minscore maxperiod, 2 7 7 80 10 50 500 resp.). A histogram of repeat unit lengths was then produced, which had the peaks at 7, 181, and 359 bp.

Annotation

RNA isolation and sequencing

RNA was isolated from giant sequoia roots, foliage, and cambium using a LiCl-Urea buffer followed by cleanup using Zymo columns and reagents (Zymo Research, Irvine, CA). RNA

quality was assessed using an Experion Electrophoresis System (Bio-Rad, Hercules, CA) and Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA).

Double-stranded cDNA was generated from total RNA (2 µg per tissue) using the Lexogen TeloTM prime Full-length cDNA Kit (Lexogen, Inc., Greenland, NH, USA). Tissue-specific cDNAs were first barcoded by PCR (16-19 cycles) using IDT barcoded primers (Integrated DNA Technologies, Inc., Coralville, Iowa), and then bead-size selected with AMPure PB beads (two different size fractions of 1X and 0.4X). The three cDNAs were pooled in equimolar ratios and used to prepare a SMRTbell™ library using the PacBio Template Prep Kit (PacBio, Menlo Park, CA). The SMRTbell™ library was then sequenced on a Sequel v2 SMRT cell with polymerase 2.1 and chemistry 2.1 (P2.1C2.1) on one PacBio Sequel v2 SMRT cell at the UC Davis Genome Center DNA Technologies Core Facility.

Processing of IsoSeq data

Raw IsoSeq subreads were processed using the PacBio IsoSeq3 v3.0 workflow (https://github.com/PacificBiosciences/IsoSeq/blob/master/README_v3.0.md). Briefly, ccs v.3.0.0 was run to merge subreads one full-length circular consensus sequence (ccs) per Zero Mode Waveguide (ZMW). Then, lima v.1.7.0 was run to remove primer artifacts and to demultiplex the ccs by library barcode. Finally, isoseq3 cluster 3.0.0 was run to cluster the demultiplexed CCS reads into transcripts.

Repetitive element library generation and masking

RepeatModeler (2.0; Smit and Hubley, 2008) was used to detect *de novo* repeats in the giant sequoia 2.0 assembly, after scaffolds shorter than 3 kbp were removed. The resulting repeat library with classification was used as input for RepeatMasker (v4.0.9, Smit, Hubley, and Green, 2013) which soft masks repetitive elements in the genome. After this initial repeat masking using the de novo giant sequoia repeat library, RepeatMasker was run using a library of conifer repeats identified in other gymnosperm species clustered at 80% to further mask repetitive elements.

Structural annotation

PacBio IsoSeq data and previously published Illumina RNAseq data (Scott et al., 2016) were mapped to the soft masked genome, using Minimap2 v.2.12 (Li, 2018) for the long-read data and HISAT2 v.2.1.0 (Kim, Langmead, and Salzberg, 2015) for short reads. The resulting alignment files were merged and sorted, then used alongside protein evidence generated with GenomeThreader (Gremme et al., 2005) as input to Braker2 v2.1.2 (Hoff et al., 2019; Hoff et al., 2015; Stanke et al., 2008; Stanke et al., 2006) to generate putative gene models.

Functional annotation

Structural gene predictions were used as input for Eukaryotic Non-Model Transcriptome Annotation Pipeline (EnTAP; Hart et al., 2019), to add functional information and to and identify improbable gene models. EnTAP was run in runP mode with taxon = Acrogymnospermae using the RefSeq Plant and SwissProt databases plus a custom conifer protein database (O'Leary et al., 2016; The Uniprot Consortium, 2019). To further filter putative gene models, gFACs (Caballero and Wegrzyn, 2019) was used, first by separating multiexonic and monoexonic models.

Multiexonics were retained after filtering out models with non-canonical splice sites, micro-introns and micro-exons (<20 bp), and in-frame premature stop codons to ensure correct geneic structure. Additionally, to control for function, genes annotating through Inteproscan (Jones et. al., 2014) as retrodomains (including gag-polypeptide, retrotransposon, reverse transcriptase, copia, gypsy, and ty1) were discarded. In addition, any multi-exonic models that lacked functional annotation either with a sequence similarity hit or gene family assignment were removed. Additionally, gffcompare (https://ccb.jhu.edu/software/stringtie/gffcompare.shtml) identified overlap between gene models and softmasked regions of the genome, and multi-exonic gene models were removed if more than 50% of their length fell in masked regions. Clustered transcriptome sequences were aligned to the genome using GMAP (v. 2018-07-04; Wu & Watanabe, 2005; Wu & Nacu, 2010) with a minimum trimmed coverage of 0.95 and a minimum identity of 0.95. To determine overlap and nesting of gene models with this high confidence transcriptomic alignment, BEDtools (Quinlan and Hall, 2010). BUSCO v.3.0.2 (Simao et al., 2015) was used to assess the completeness of the filtered gene space.

Orthogroup assignment of proteins

Translated UniGenes for all available gymnosperms were downloaded from the forest genomics database TreeGenes (https://treegenesdb.org/; Wegrzyn et al., 2019; Falk et al., 2018). The corresponding files from the *Amborella trichopoda* genome assembly were also included to provide an outgroup to the gymnosperm taxa. Each taxon was evaluated for completeness with BUSCO v4.0.2 in protein mode. All taxa with over 60% completeness were included in OrthoFinder (Emms and Kelly 2015; Emms and Kelly 2019) to identify orthogroups. The longest

sequence in each orthogroup was retained, regardless of source species. Species-specific orthogroups unique to giant sequoia were noted. The resulting nonredundant species-specific orthogroups were functionally annotated with EnTAP in runP mode with taxon = *Sequoiadendron* using the RefSeq Plant and SwissProt databases.

Gene family evolution

Following orthogroup assignment with OrthoFinder, a species tree and orthogroup statistics were used as input for CAFE v4.1 (Han et al., 2013) to assess gene family contraction and expansion dynamics, using a single birth/death parameter (λ) across the phylogeny. Gene families in the giant sequoia lineage experiencing rapid evolution were then functionally annotated using EnTAP.

Annotation and analysis of NLR genes

NLR genes were identified using the NLR-Annotator pipeline (Steuernagel et al., 2018) on the giant sequoia 2.0 assembly, then that output was cross-referenced with the genome annotation. Using the genome annotation file and the NLR gene file as input, the BEDtools intersect function (Quinlan and Hall, 2010) was used to identify putative NLRs that were also present in the annotation, requiring features in the NLR gene file to overlap with 100% of the annotation feature. NLR-gene maximum likelihood trees were generated with RAxML v8.2.12 (Stamatakis, 2014) using the amino acid sequence of the central NB-ARC [AB2] domain output by NLR-Annotator. NB-ARC domains that included greater than 50% missing data were excluded from all analyses. The best trees were visualized with the Interactive Tree of Life (iTOL) tool,

with bootstrap values shown (Letunic and Bork, 2016). Determination of TIR and CC domains was based on motif data from Jupe and colleagues (2012). RPW8-like motifs were determined by alignment to a recently described RNL motif (CFLDLGxFP) (Van Ghelder et al., 2019).

Data availability

The genome assembly of giant sequoia is available at NCBI under accession GCA_007115665.2, and raw sequence data are available under accessions SRX5827056 - SRX5827083. Annotation files are available at https://treegenesdb.org/FTP/Genomes/Segi.

RESULTS AND DISCUSSION

Sequencing and assembly

Assembly of the giant sequoia genome leveraged sequence data from four libraries (Table 1). Illumina reads (135x) from a haploid megagametophyte library combined with Oxford Nanopore sequence from foliage (21x) contributed to the contig assembly. The contig assembly was subsequently scaffolded with data from Dovetail Chicago (47x) and Hi-C libraries (76x) in succession.

Giant sequoia 1.0 assembly

Initial contig assembly of the Illumina and Oxford Nanopore sequence data yielded giant sequoia 1.0. The initial contig assembly giant sequoia 1.0 had a contig N50 of 359,531 bp and a scaffold N50 of 489,478.

Genome size was estimated by counting 31-mers (all sub-sequences of 31 bases) in the Illumina reads and computing the histogram of the kmer frequencies vs. counts using jellyfish tool version 2.0 (Marcais et al., 2011). The histogram of 31-mer frequency counts had its largest peak at 101 (see Figure 1). There was a small second peak at 204, roughly double the highest 31-mer frequency was 101, likely corresponding to 2x repeat sequences in the genome. The k-mer coverage of the genome was then estimated by computing the area under the curve for frequencies between 1 and 10000 and dividing that number by 101. This method arrived at the genome size estimate of 8,588 Gbp.

The intermediate step of correction of the Nanopore in MaSuRCA resulted in 24,279,305 mega-reads with an average read length of 6,726 bp. The consensus error rate for the assembly was estimated by aligning the Illumina reads to the contigs with bwa mem (Li, 2013) and then calling variants with freebayes (Garrison et al., 2012) software. Any site in the consensus that had no Illumina reads agreeing with the consensus and at least three Illumina reads agreeing on an alternative variant was considered an error. The total number of bases in the error variants were counted and divided by the total number of bases in the contigs. This yielded an assembly error rate of 0.3 errors per 10000 bases, or consensus quality of 99.997%.

The initial contig assembly giant sequoia 1.0 had a contig N50 of 347,954 bp and a scaffold N50 of 490,521.

Giant sequoia 2.0 assembly

The Dovetail HiRise Chicago and Hi-C assembly increased the total assembly size marginally, to 8.125 Gbp, but notably yielded a large increase in the N50 to 690.55Mb (Table 2). The overall

number of scaffolds was reduced to 8,125, and the N90 of the final assembly was 690.55Mb. It is worth noting that the largest scaffold in this assembly is 985 Mbp in length, making it the longest contig assembled to date in any organism.

The tandem repeat finder program (trf v4.09, G. Benson 1999) identified repeat elements up to 500 bp long, and those data were used to plot a histogram of repeat unit lengths which had peaks at 7, 181, and 359 bp. Based on the position and clustering along the chromosomes, the 7-mer was identified as the telomeric repeat and the 181-mer as the centromeric one.

The most common telomeric 7-mers were TTTAGGG (present in most land plants), and TTGAGGG. The two 7-mers alternate and have similar frequencies.

The 181 bp centromeric repeat unit consensus sequence was

AAAAATTGGAGTTCGCGTGACACAGATGCAACGTAGCCTTAAAATCAGGTCTTCGCCGAA
CTCGACATTAAATCGATGGAAATTCAACATTCACGAAAACTGATAGAAAATAAAGGTTCTT
AATAGTCATCTACAACACAATCTAAATCAAAGTTCTCCAAACATGGTTGATTATGGGTG.

By looking at the positions of the centromeric and telomeric repeats, a mis-assembly was identified in the original HiRise reference. Two centromeric and one telomeric region were located in the middle of the longest scaffold (1.82Gb), and subsequently this scaffold was split into chr1 (0.95Gb) and chr3 (0.84Gb).

There are 11 chromosomes in giant sequoia (Buccholz, 1939; later confirmed by Jensen and Levan, 1941 and Schlarbaum and Tschuiya, 1984), and the 11 largest scaffolds in the assembly span across the centromere (Table 3), suggesting a chromosome-level assembly. The 11 largest scaffolds range from 443 Mbp to 985 Mbp in size. Of these 11 scaffolds, seven include telomeric sequence on both ends. The remaining four scaffolds have telomeric sequence on one

end. Beyond the 11 largest scaffolds, the next largest (Sc7zsyj_3574) (171 Mb) includes telomere at one end, suggesting it is a substantial portion of a chromosome arm for one of the scaffolds with only one telomere (chromosomes 1, 3, 6, and 9).

Assessing assembly completeness

For a rough estimate of the assembly completeness, BUSCO v3.0.2 was run with the embryophyta database (Simao et al., 2015) of 1440 genes. For the complete giant sequoia 2.0 genome, the tool found 559 complete BUSCOs out of which 515 were in a single copy, 44 were duplicated, and 133 were fragmented BUSCOs (Table 4). Another 748 BUSCOs were missing. In both the full giant sequoia 2.0 assembly and the version filtered to remove all scaffolds smaller than 3 kbp, completeness was estimated at 38% using BUSCO. Assembly completeness of other conifer assemblies (Supplementary Table S1) range from 27-44%, suggesting giant sequoia 2.0 completeness is consistent with existing work. Despite the contiguity of the assembly, the BUSCO completeness of the genome appears lower than expected, likely due to the presence of very long introns in conifers, which can inhibit identification of genes.

Comparison to existing gymnosperm assemblies

The contiguity of giant sequoia 2.0 is most apparent when comparing with other gymnosperm assemblies (Table 5). Giant sequoia 2.0 has an N50 scaffold size of 690Mb, an order of magnitude larger than scaffold N50s reported in other conifers.

Annotation of giant sequoia 2.0

Repeat annotation

Using the custom repeat database created by RepeatModeler, the majority (72.85%) of the giant sequoia genome was softmasked. Subsequent masking using conifer-specific repeat libraries yielded an additional 6% of masked sequence. LTRs were the most abundant known element (28%, Supplementary Table S2) in the masked sequence. These results are comparable to observations from different conifer species, e.g. the most recent *Pinus lambertiana* assembly contained 79% repetitive sequence (Stevens et al., 2016). That our observations are consistent with the only conifer lineage sequenced until now (Pinaceae) is not surprising, as all conifers have large genome sizes, and this genomic bloat is attributed to the proliferation of repetitive elements throughout the genome (Neale et al., 2014).

Gene Annotation

Structural annotation using BRAKER2 resulted in 1,460,545 predicted gene models, with an average intron length of 2,362 bp (Table 6). The average CDS length was 613 bp, including both multi- and mono-exonic models. The initial gene set included models with long introns, with the longest measuring 385,133 bp. The number of mono-exonic genes (941,659) was almost twice as large as the total number of multi-exonic gene models (518,886). Even with reasonable filters, the number of *ab initio* prediction of mono-exonic genes was highly inflated. Therefore, the mono-exonic *ab initio* genes were removed from the gene space. The *ab initio* gene space was expanded by the addition of 14,538 well aligned unique transcriptome sequences of which 6,982 are mono-exonic and the remaining 7,556 are multi-exonic. After filtering, annotation yielded 37,936 high quality gene models. The average CDS length increased to 1,083 bp. The proportion

of mono-exonics (5,163) to multi-exonics (32,773) was drastically reduced using the transcriptome as an evidence source. Long introns were maintained, with the max intron length in the high quality set reaching nearly 1.4 Mb.

Of the 37,936 high quality gene models, 35,183 were functionally annotated by either sequence similarity search or gene family assignment with EnTAP. These functionally annotated gene models include the longest plant intron found so far, at 1.4 Mb. Large introns are characteristic of conifer genomes, with introns up to 800 Kbp observed in *Pinus taeda* (Wegrzyn et al., 2014) and introns over 500 Kbp in *Pinus lambertiana* (Stevens et al., 2016).

Functional annotation of the gene containing the 1.4 Mb long intron suggests it is a member of the WASP (Wiskott-Aldrich syndrome protein) family. Wiskott-Aldrich syndrome proteins are in turn members of the SCAR/WAVE (suppressor of cAMP receptor/WASP family verprolin homologous) gene regulatory complex, which in plants has an important role in cell morphogenesis via activation of actin filament proteins (Yanagisawa, Zhang, and Szymanski, 2013).

Distribution of the high-quality gene models spanned the length of all 11 chromosomes (Figure 2). Repeat density varied across the chromosomes, including overlap with annotated regions.

Assessing annotation completeness

Completeness of the annotation was assessed with BUSCO (Table 4). The independent transcriptome completeness of 79% represents the maximum possible BUSCO score for the gene model sets. The BUSCO completeness of the final high-quality gene set was 53%, comparable to the same metric in *Pinus taeda* (53%, Wegrzyn et al., 2014) and *Pinus lambertiana* (50%,

406 Stevens et al., 2016), suggesting the annotation of giant sequoia is on par with other conifer
407 genomes.
408
409 *Comparison to existing gymnosperm annotations*
410 While the genome size of giant sequoia is rather small for a gymnosperm (Table 5), the identified
411 repeat content of giant sequoia 2.0 (79%) is in line with observations from other taxa. The
412 number of high quality annotated genes (37,936) is higher than many gymnosperm assemblies,
413 though there is substantial variation in annotation results across the lineage. Average CDS
414 length and average intron length in giant sequoia 2.0 fall within the observed ranges for existing
415 assemblies, though notably the longest intron reported here is ~1.4 Mb, nearly 400kb longer
416 than the previous longest intron (from *Pinus taeda*, at over 800 kbp). That giant sequoia 2.0
417 contains an even longer intron is likely due to the contiguity of our assembly, which is
418 unprecedented in conifers.
419
420 *Orthology assignment and gene family evolution*
421 Using unigene sets from TreeGenes, twenty gymnosperm taxa passed the 60% threshold
422 for BUSCO completeness (Table 7). Orthogroup clustering of 695,700 protein sequences from
423 these twenty gymnosperms plus an outgroup (*Amborella trichopoda*) yielded a total of 44,797
424 orthogroups (Supplementary Table S3). Only 206 were single-copy in all species, and 5,953
425 orthogroups had representatives from each species. Overall, 6.5% of all protein sequences were
426 in species-specific orthogroups. Of the species-specific orthogroups (12,121 in total), 607 were
427 unique to giant sequoia (Table 8). Among the 607 giant sequoia-specific orthogroups, 536 were

functionally annotated with either gene family assignment (318) sequence similarity search (8) or both (536) (Supplementary Table S4).

Orthogroup assignments were used as branch labels on a rooted species tree to show gene family contraction and expansion. On branch is the number of families that experienced expansion (dark blue, above) or contraction (light blue, below) (see Figure 3). Giant sequoia (Segi) experienced an overall expansion, with 4,953 families expanding and 1,923 families contracting since the species last shared common ancestor with coast redwood (*Sequoia sempervirens*; Sese).

The expansions and contractions were further examined to identify nodes that experienced particularly rapid evolution. Many representatives of the Pinaceae have thousands of gene families that experienced rapid evolution since their lineages diverged (Figure 4). Along the branch to giant sequoia (Segi), 4,176 orthologous groups evolved rapidly. The majority of these 4,176 orthogroups are moderately represented in the giant sequoia dataset (e.g. with two to four members in an orthogroup), while others contain dozens of paralogs, up to over a hundred orthogroup members. Extracting the longest sequence from each of these yielded functional annotation with EnTAP for 3,994 of the rapidly evolving orthogroups. Rapidly expanding families were associated with primarily metabolic processes (GO:0090304, GO:0006796, GO:0044267) and macromolecule synthesis(GO:0009059, GO:0034645), in addition to molecular functions including metal-ion binding (GO:0046872), purine nucleotide (GO:0017076) and nucleoside (GO:0001883) binding, and kinase activity (GO:0016301). Rapidly contracting families were associated with biological processes such as protein (GO:0036211) and macromolecule modification (GO:0043412

and metabolic processes (GO:0044267, GO:0006796), and molecular functions including purine binding with nucleotides (GO:0017076) and nucleosides (GO:0001883), and phosphotransferase activity (GO:0016773).

NLR genes in the giant sequoia genome

NLR proteins are structurally modular, typically containing an N-terminal coiled-coil (CC) domain, a Toll/interleukin-1 receptor (TIR) domain, or more rarely an RPW8-like CC domain; a conserved nucleotide binding domain (NB-ARC); and a C-terminal region comprising a variable number of leucine-rich repeats (LRRs) (Monteiro and Nishimura, 2018). NLR genes in giant sequoia 2.0 were identified by first running the genomic sequence through the NLR-Annotator pipeline (Steuernagel et al., 2018). Importantly, this pipeline does not require masking of repetitive regions and does not rely on gene model predictions. NLR-Annotator outputs are categorized as either ‘complete’ or ‘partial’ depending on whether all canonical domains (CC/TIR, NB-ARC, LRR) are present, and then further categorized as ‘pseudo-’ if a stop codon is predicted in any domain. All categorizations should be interpreted with care because the NLR-Annotator algorithm does not take intron/exon boundaries into account.

A total of 984 NLR genes were predicted by NLR-Annotator, of which 442 were identified as complete, 332 complete pseudo-, 88 partial, and 122 partial pseudo-. Seven hundred and twelve included intact NB-ARC domains with fewer than 50% gaps in the alignment. NLR-gene coordinates of all NLR gene sequences, and the relationships of the 712 based on an NB-ARC domain maximum likelihood tree are included in Supplementary Table S5, S6, and S7 as well as Supplementary Figure S1. This number is roughly twice the number found in

cultivated rice (Zhou et al., 2004; Read et al., 2020) and is consistent with other conifers (Van Ghelder et al., 2019).

NLR-Annotator identifies all suspected NLR motif-encoding regions of the genome. This likely includes true pseudogenes or gene fragments, both of which are important from an evolutionary perspective, but do not reflect the functional NLR arsenal. The NLR-Annotator output was cross-referenced with the giant sequoia genome annotation to identify the NLR genes that are supported by the annotation and therefore likely part of this arsenal; we refer to these 315 genes as consensus NLR genes. Of these, 211 were categorized by NLR-Annotator as complete, 65 as complete pseudo-, 29 as partial, and 10 as partial pseudo-. Two hundred and fifty seven of the 315 consensus NLR genes encode NB-ARC domains that met our criteria (see Methods); a maximum likelihood tree was generated using these domains (Figure 5). Coordinates of the genes and their NB-ARC sequences are included in Supplementary Table S5 and S7. NLR-Annotator predicted, non-consensus NLR genes may represent genes missed by the annotation, pseudogenes, or false positives.

To investigate the evolution of NLR genes in giant sequoia, the list of consensus NLRs was compared with orthogroup assignments. Overall, consensus NLRs had membership in 63 orthogroups. Assessing the change in orthogroup size along each branch of the phylogeny revealed rapid expansion in NLR-associated orthogroups across the tree (Figure 6). Along the branch leading to giant sequoia (Segi), 34 NLR orthogroups expanded rapidly. The shared ancestors of giant sequoia and its closest relative, coast redwood (Sese), experienced rapid expansion in 11 NLR orthogroups. After the divergence of the California redwoods, five additional NLR orthogroups rapidly expanded in coast redwood, compared to the 34 rapidly

expanding NLR orthogroups in giant sequoia. This pattern, a larger number of NLR orthogroups rapidly expanding in giant sequoia compared to coast redwood, is consistent with the numbers of all rapidly evolving orthogroups in each lineage (Figure 4).

While the shared and unique NLR orthogroups identified in giant sequoia, coast redwood, and their common ancestors are perhaps associated with the observed pest resilience in both species, further work will be necessary to fully characterize the evolutionary patterns and functional roles of NLR gene families in redwoods and conifers as a whole.

SUMMARY AND CONCLUSIONS

The high quality of this assembly demonstrates the value of combining multiple sequencing technologies and leveraging a unique biological feature of conifers (sufficient haploid megagametophyte tissue for sequencing), along with the value of incorporating chromosome-conformation capture libraries to allow improvements in scaffolding. The giant sequoia genome assembly presented here provides a robust foundation for ongoing genomic studies to identify groves with evidence of local adaptation, with a focus on not only NLR genes but the many other genes and gene families potentially useful in conservation and management.

For the future, inferences about the evolutionary trajectory of conifers (and gymnosperms) will require a broadening of taxonomic focus. As the vast majority of conifer genomic research is centered on Pinaceae, developing resources in understudied conifer families is essential for meaningful comparative genomic work that could further inform conservation and management for iconic species..

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REFERENCES

Albert, V. A., Barbazuk, W. B., Depamphilis, C. W., Der, J. P., Leebens-Mack, J., Ma, H., ... & Soltis, D. E. (2013). The Amborella genome and the evolution of flowering plants. *Science*, *342*(6165), 1241089.

Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic acids research. 1999;27(2):573-80

Buchholz, J. T. (1939). The generic segregation of the sequoias. *American Journal of Botany*, *26*(7), 535-538.

Burns, E.B., Campbell, R., & Cowan, P.D. (2018). State of Redwoods Conservation Report.

Bush, S.J., Castillo-Morales, A., Tovar-Corona, J.M., Chen, L., Kover, P.X., and Urrutia, A.O. (2013). Presence–absence variation in *A. thaliana* is primarily associated with genomic signatures consistent with relaxed selective constraints. *Mol. Biol. Evol.* 31**,** 59-69.

553 Caballero, M., & Wegrzyn, J. (2019). gFACs: Gene Filtering, Analysis, and Conversion
554 to Unify Genome Annotations Across Alignment and Gene Prediction
555 Frameworks. *Genomics, proteomics & bioinformatics*, *17*(3), 305-310.
556
557 Cook, L.F. (1961) The Giant Sequoias of California.
558 DeSilva, R., & Dodd, R. (2014). Development and characterization of microsatellite
559 markers for giant sequoia, Sequoiadendron giganteum (Cupressaceae). *Conservation*
560 *genetics resources*, *6*(1), 173-174.
561
562 Dodd, R. S., & DeSilva, R. (2016). Long−term demographic decline and late glacial
563 divergence in a Californian paleoendemic: Sequoiadendron giganteum (giant
564 sequoia). *Ecology and evolution*, *6*(10), 3342-3355.
565
566 Douglass, A. E. (1919). *Climatic cycles and tree-growth* (Vol. 289). Carnegie Institution
567 of Washington.
568
Elliott-Fisk, D.L., Stephens, S.L., Aubert, J.E., Murphy, D., Schaber, J. "Mediated
569 Settlement Agreement for Sequoia National Forest, Section B. Giant Sequoia Groves: an
570 evaluation." In *Sierra Nevada Ecosystem Project: Final report to Congress: status of the*
571 *Sierra Nevada*. Davis, CA: Centers for Water and Wildland Resources, University of
572 California, 1997.

573 Emms, D. M., & Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole
574 genome comparisons dramatically improves orthogroup inference accuracy. *Genome*
575 *biology*, *16*(1), 157.
576
577 Emms, D. M., & Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for
578 comparative genomics. *Genome biology*, *20*(1), 1-14.
579
580 Falk T., Herndon N., Grau E., Buehler S., Richter P., Zaman S., Baker E.M., Ramnath R.,
581 Ficklin S., Staton M., Feltus F.A., Jung S., Main D., Wegrzyn J.L. (2018). Growing and
582 cultivating the forest genomics database, TreeGenes. Database, Volume 2018
583 doi:10.1093/database/bay084
584
585 Fins, L., & Libby, W. J. (1982). Population variation in Sequoiadendron: seed and
586 seedling studies, vegetative propagation, and isozyme variation. *Silvae Genet*, *31*(4),
587 102-110.
588
589 G. Gremme, V. Brendel, M.E. Sparks, and S. Kurtz. Engineering a software tool for gene
590 structure prediction in higher organisms. *Information and Software Technology*,
591 **47**(15):965-978, 2005
592
593 Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing.
594 arXiv preprint arXiv:1207.3907 [q-bio.GN] 2012

595 Guan, R., Zhao, Y., Zhang, H., Fan, G., Liu, X., Zhou, W., ... & Fu, Y. (2016). Draft
596 genome of the living fossil Ginkgo biloba. *Gigascience*, *5*(1), s13742-016.
597
598 Han, M. V., Thomas, G. W., Lugo-Martinez, J., & Hahn, M. W. (2013). Estimating gene
599 gain and loss rates in the presence of error in genome assembly and annotation using
600 CAFE 3. *Molecular biology and evolution*, *30*(8), 1987-1997.
601
602 Hart, A. J., Ginzburg, S., Xu, M., Fisher, C. R., Rahmatpour, N., Mitton, J. B., ... &
603 Wegrzyn, J. L. (2019). EnTAP: bringing faster and smarter functional annotation to
604 non−model eukaryotic transcriptomes. *Molecular Ecology Resources*.
605
606 Hizume, M., Kondo, T., Shibata, F., & Ishizuka, R. (2001). Flow cytometric
607 determination of genome size in the Taxodiaceae, Cupressaceae sensu stricto and
608 Sciadopityaceae. *Cytologia*, *66*(3), 307-311.
609
Hoff, K.J., Lange, S., Lomsadze, A., Borodovsky, M. and Stanke, M. (2015). BRAKER1:
610 unsupervised RNA-Seq-based genome annotation with GeneMark-ET and AUGUSTUS.
611 Bioinformatics, 32(5):767-769.

612 Hoff, K.J., Lomsadze, A., Borodovsky, M. and Stanke, M. (2019). Whole-Genome
613 Annotation with BRAKER. Methods Mol Biol. 1962:65-95, doi:
614 10.1007/978-1-4939-9173-0_5.

615 Howe KL, Contreras-Moreira B, De Silva N, Maslen G, Akanni W, Allen J,
616 Alvarez-Jarreta J, Barba M, Bolser DM, Cambell L, Carbajo M, Chakiachvili M,
617 Christensen M, Cummins C, Cuzick A, Davis P, Fexova S, Gall A, George N, Gil L,
618 Gupta P, Hammond-Kosack KE, Haskell E, Hunt SE, Jaiswal P, Janacek SH, Kersey PJ,
619 Langridge N, Maheswari U, Maurel T, McDowall MD, Moore B, Muffato M, Naamati G,
620 Naithani S, Olson A, Papatheodorou I, Patricio M, Paulini M, Pedro H, Perry E, Preece J,
621 Rosello M, Russell M, Sitnik V, Staines DM, Stein J, Tello-Ruiz MK, Trevanion SJ,
622 Urban M, Wei S, Ware D, Williams G, Yates AD, Flicek P. Ensembl Genomes
623 2020-enabling non-vertebrate genomic research. Nucleic Acids Research 2019
624
625 Jensen, H., & Levan, A. (1941). Colchicine−induced tetraploidy in Sequoia
626 gigantea. *Hereditas*, *27*(3−4), 220-224.
627
628 Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., ... & Pesseat, S.
629 (2014). InterProScan 5: genome-scale protein function
630 classification. *Bioinformatics*, *30*(9), 1236-1240.
631
632 Jupe, F., Pritchard, L., Etherington, G.J., Mackenzie, K., Cock, P.J., Wright, F., Sharma,
633 S.K., Bolser, D., Bryan, G.J., Jones, J.D., and Hein, I. (2012). Identification and
634 localisation of the NB-LRR gene family within the potato genome. *BMC Genomics* 13**,**
635 75. PMC3297505: 22336098.

Kim D, Langmead B, and Salzber SL. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods* 2015.

Leslie, A. B., Beaulieu, J., Holman, G., Campbell, C. S., Mei, W., Raubeson, L. R., & Mathews, S. (2018). An overview of extant conifer evolution from the perspective of the fossil record. *American journal of botany*, *105*(9), 1531-1544.

Letunic, I., and Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.* 44**,** W242-W245. PMC4987883: 27095192.

Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, **34**:3094-3100. doi:10.1093/bioinformatics/bty191

Li H. (2013) Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997v1 [q-bio.GN]

Marçais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics. 2011 Mar 15;27(6):764-70.

Monteiro, F., and Nishimura, M.T. (2018). Structural, functional, and genomic diversity of plant NLR proteins: an evolved resource for rational engineering of plant immunity. *Annu. Rev. Phytopathol.* 56**,** 243-267. 29949721.

Mosca, E., Cruz, F., Gómez-Garrido, J., Bianco, L., Rellstab, C., Brodbeck, S., ... & Gömöry, D. (2019). A reference genome sequence for the european silver fir (abies alba mill.): a community-generated genomic resource. *G3: Genes, Genomes, Genetics*, *9*(7), 2039-2049.

Neale, D. B., McGuire, P. E., Wheeler, N. C., Stevens, K. A., Crepeau, M. W., Cardeno, C., ... & Casola, C. (2017). The Douglas-fir genome sequence reveals specialization of the photosynthetic apparatus in Pinaceae. *G3: Genes, Genomes, Genetics*, *7*(9), 3157-3167.

Neale, D.B., Wegrzyn, J.L., Stevens, K.A. *et al.* Decoding the massive genome of loblolly pine using haploid DNA and novel assembly strategies. *Genome Biol* **15,** R59 (2014). https://doi.org/10.1186/gb-2014-15-3-r59

Neale DB, Wegrzyn JL, Stevens KA, Zimin AV, Puiu D, Crepeau MW, Cardeno C, Koriabine M, Holtz-Morris AE, Liechty JD, Martínez-García PJ. Decoding the massive genome of loblolly pine using haploid DNA and novel assembly strategies. Genome biology. 2014 Mar;15(3):R59.

O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova

O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD, Pruitt KD. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 2016 Jan 4;44(D1):D733-45.

Putnam NH, O'Connell BL, Stites JC, Rice BJ, Blanchette M, Calef R, Troll CJ, Fields A, Hartley PD, Sugnet CW, Haussler D. Chromosome-scale shotgun assembly using an in vitro method for long-range linkage. Genome research. 2016 Mar 1;26(3):342-50.

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841-842.

Read, A.C., Moscou, M.J., Zimin, A.V., Pertea, G., Meyer, R.S., Purugganan, M.D., Leach, J.E., Triplett, L.R., Salzberg, S.L., and Bogdanove, A.J. (2020). Genome assembly and characterization of a complex zfBED-NLR gene-containing disease resistance locus in Carolina Gold Select rice with Nanopore sequencing. *PLoS Genet.* 16**,** e1008571. 31986137.

Schatz, M.C., Maron, L.G., Stein, J.C., Wences, A.H., Gurtowski, J., Biggers, E., Lee, H., Kramer, M., Antoniou, E., Ghiban, E., Wright, M.H., Chia, J.-m., Ware, D., McCouch, S.R., and McCombie, W.R. (2014). Whole genome de novo assemblies of three divergent strains of rice, *Oryza sativa*, document novel gene space of aus and indica. *Genome Biol.* 15**,** 506.

Schlarbaum, S. E., & Tsuchiya, T. (1984). Cytotaxonomy and phylogeny in certain species of Taxodiaceae. *Plant systematics and evolution*, *147*(1-2), 29-54.

Schmid, R. & Farjon, A. 2013. *Sequoiadendron giganteum* . *The IUCN Red List of Threatened Species* 2013: e.T34023A2840676. https://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T34023A2840676.en. Downloaded on 25 January 2020.

Scott, A. D., Stenz, N. W., Ingvarsson, P. K., & Baum, D. A. (2016). Whole genome duplication in coast redwood (Sequoia sempervirens) and its implications for explaining the rarity of polyploidy in conifers. *New Phytologist*, *211*(1), 186-193.

Sillett, S. C., Van Pelt, R., Carroll, A. L., Kramer, R. D., Ambrose, A. R., & Trask, D. A. (2015). How do tree structure and old age affect growth potential of California redwoods?. Ecological Monographs, 85(2), 181-212.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015 Oct 1;31(19):3210-2.

Smit, AFA, Hubley, R. *RepeatModeler Open-1.0*. 2008-2015 <http://www.repeatmasker.org>.

Smit, AFA, Hubley, R & Green, P. *RepeatMasker Open-4.0*. 2013-2015 <http://www.repeatmasker.org>.

Stahl, E., Hilfiker, O., and Reymond, P. (2018). Plant-arthropod interactions: who is the winner? *Plant J.* 93**,** 703-728. 29160609.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30**,** 1312-1313. PMC3998144: 24451623.

Stanke, M., Diekhans, M., Baertsch, R. and Haussler, D. (2008). Using native and syntenically mapped cDNA alignments to improve de novo gene finding. Bioinformatics, doi: 10.1093/bioinformatics/btn013.

Stanke. M., Schöffmann, O., Morgenstern, B. and Waack, S. (2006). Gene prediction in eukaryotes with a generalized hidden Markov model that uses hints from external sources. BMC Bioinformatics 7, 62.

744 Stephenson, N. L., Das, A. J., Ampersee, N. J., Cahill, K. G., Caprio, A. C., Sanders, J.
745 E., & Williams, A. P. (2018). Patterns and correlates of giant sequoia foliage dieback
746 during California's 2012–2016 hotter drought. *Forest ecology and management*, *419*,
747 268-278.
748
749 Stephenson, N. L. (1994, July). Long-term dynamics of giant sequoia populations:
750 implications for managing a pioneer species. In *Proceedings of the symposium on giant*
751 *sequoias: Their place in the ecosystem and society'.(Tech. coord. P Aune) pp* (pp. 56-63).
752
753 Steuernagel, B., Witek, K., Krattinger, S.G., Ramirez-Gonzalez, R.H., Schoonbeek, H.-j.,
754 Yu, G., Baggs, E., Witek, A.I., Yadav, I., Krasileva, K.V., Jones, J.D.G., Uauy, C., Keller,
755 B., Ridout, C.J., and Wulff, B.B.H. (2018). Physical and transcriptional organisation of
756 the bread wheat intracellular immune receptor repertoire. *bioRxiv***,** 339424.
757
758 Stevens, K. A., Wegrzyn, J. L., Zimin, A., Puiu, D., Crepeau, M., Cardeno, C., ... &
759 Martínez-García, P. J. (2016). Sequence of the sugar pine megagenome. *Genetics*, *204*(4),
760 1613-1626.
761
762 The UniProt Consortium. (2019) UniProt: a worldwide hub of protein knowledge.
763 Nucleic Acids Res. 47: D506-515.
764

765 Van Ghelder, C., Parent, G.J., Rigault, P., Prunier, J., Giguere, I., Caron, S., Stival Sena,
766 J., Deslauriers, A., Bousquet, J., Esmenjaud, D., and MacKay, J. (2019). The large
767 repertoire of conifer NLR resistance genes includes drought responsive and highly
768 diversified RNLs. *Sci Rep* 9**,** 11614. PMC6691002: 31406137.
769
770 Wan, T., Liu, Z. M., Li, L. F., Leitch, A. R., Leitch, I. J., Lohaus, R., ... & Wang, W. C.
771 (2018). A genome for gnetophytes and early evolution of seed plants. *Nature Plants*, *4*(2),
772 82-89.
773
774 Warren, R. L., Keeling, C. I., Yuen, M. M. S., Raymond, A., Taylor, G. A., Vandervalk,
775 B. P., ... & Robertson, G. (2015). Improved white spruce (Picea glauca) genome
776 assemblies and annotation of large gene families of conifer terpenoid and phenolic
777 defense metabolism. *The Plant Journal*, *83*(2), 189-212.
778
779 Wegrzyn, J. L., Liechty, J. D., Stevens, K. A., Wu, L. S., Loopstra, C. A., Vasquez-Gross,
780 H. A., ... & Holt, C. (2014). Unique features of the loblolly pine (Pinus taeda L.)
781 megagenome revealed through sequence annotation. *Genetics*, *196*(3), 891-909.
782
783 Wegrzyn J.L., Staton M.A., Street N. R., Main D., Grau E., Herndon N., Buehler S., Falk
784 T., Zaman S., Ramnath R., Richter P., Sun L., Condon B., Almsaeed A., Chen
785 M.,Mannapperuma C., Jung S., Ficklin S. Cyberinfrastructure to Improve Forest Health

and Productivity: The Role of Tree Databases in Connecting Genomes, Phenomes, and the Environment, TreeGenes. Database, Volume 2019. doi:10.3389/fpls.2019.00813

Wu, T. D., & Watanabe, C. K. (2005). GMAP: a genomic mapping and alignment program for mRNA and EST sequences. *Bioinformatics*, *21*(9), 1859-1875.

Wu, T. D., & Nacu, S. (2010). Fast and SNP-tolerant detection of complex variants and splicing in short reads. *Bioinformatics*, *26*(7), 873-881.

Xu, X., Liu, X., Ge, S., Jensen, J.D., Hu, F., Li, X., Dong, Y., Gutenkunst, R.N., Fang, L., Huang, L., Li, J., He, W., Zhang, G., Zheng, X., Zhang, F., Li, Y., Yu, C., Kristiansen, K., Zhang, X., Wang, J., Wright, M., McCouch, S., Nielsen, R., Wang, J., and Wang, W. (2012). Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nat. Biotechnol.* 30**,** 105-111.

Yanagisawa, M., Zhang, C., & Szymanski, D. B. (2013). ARP2/3-dependent growth in the plant kingdom: SCARs for life. *Frontiers in Plant Science*, *4*, 166.

Y. Zhang, Y. Zhang, J. M. Burke, K. Gleitsman, S. M. Friedrich, K. J. Liu, and T. H. Wang, A Simple Thermoplastic Substrate Containing Hierarchical Silica Lamellae for High-Molecular-Weight DNA Extraction. Adv Mater (2016). PubMed PMID: 27862402

Yi, F., Ling, J., Xiao, Y., Zhang, H., Ouyang, F., & Wang, J. (2018). ConTEdb: a comprehensive database of transposable elements in conifers. Database, 2018.

Yu, P., Wang, C., Xu, Q., Feng, Y., Yuan, X., Yu, H., Wang, Y., Tang, S., and Wei, X. (2011). Detection of copy number variations in rice using array-based comparative genomic hybridization. *BMC Genomics* 12**,** 372. PMC3156786: 21771342.

Zhang M, Zhang Y, Scheuring CF, Wu CC, Dong JJ, Zhang H Bin. 2012. Preparation of megabase-sized DNA from a variety of organisms using the nuclei method for advanced genomics research. Nat Protoc **7**: 467–478.

Zheng, L.-Y., Guo, X.-S., He, B., Sun, L.-J., Peng, Y., Dong, S.-S., Liu, T.-F., Jiang, S., Ramachandran, S., and Liu, C.-M. (2011). Genome-wide patterns of genetic variation in sweet and grain sorghum (*Sorghum bicolor*). *Genome Biol.* 12**,** R114.

Zhou, T., Wang, Y., Chen, J.-Q., Araki, H., Jing, Z., Jiang, K., Shen, J., and Tian, D. (2004). Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Mol. Genet. Genomics* 271**,** 402-415.

Zimin A, Stevens KA, Crepeau MW, Holtz-Morris A, Koriabine M, Marçais G, Puiu D, Roberts M, Wegrzyn JL, de Jong PJ, Neale DB. Sequencing and assembly of the 22-Gb loblolly pine genome. Genetics. 2014 Mar 1;196(3):875-90.

Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. The MaSuRCA genome assembler. Bioinformatics. 2013 Aug 29;29(21):2669-77.

Zimin AV, Puiu D, Luo MC, Zhu T, Koren S, Marçais G, Yorke JA, Dvořák J, Salzberg SL. Hybrid assembly of the large and highly repetitive genome of Aegilops tauschii, a progenitor of bread wheat, with the MaSuRCA mega-reads algorithm. Genome research. 2017 May 1;27(5):787-92.

Table 1. Data used for the giant sequoia assemblies from four library types.

Type	Number of reads	Average read length (bp)	Estimated coverage
Illumina paired end	7,752,481,576	2x151	135x
Oxford Nanopore MinION	24,360,895	7,484	21x
Dovetail Chicago	2,592,465,290	2x151	47x
Dovetail Hi-C	4,202,954,328	2x151	76x

Table 2. Assembly statistics for the initial contig assembly giant sequoia 1.0 and the final scaffolded assembly giant sequoia 2.0.

Assembly	Sequence (bp)	N50 contig	N50 scaffold	Number of contigs	Number of scaffolds
Giant sequoia 1.0	8,122,145,191	347,954	490,521	49,651	39,821
Giant sequoia 2.0	8,125,622,286	347,954	690,549,816	52,886	8,125

Table 3. Summary of largest scaffolds in giant sequoia 2.0 and presence of centromeric and telomeric repeat regions

Scaffold ID	Length (bp)	Centromere?	Number of telomeres
chr1	986,618,365	Y	1
chr2	873,713,311	Y	2
chr3	843,110,718	Y	1
chr4	722,823,090	Y	2
chr5	690,549,816	Y	2
chr6	676,903,824	Y	1
chr7	659,235,867	Y	2
chr8	649,867,199	Y	2
chr9	641,211,466	Y	1
chr10	632,191,860	Y	2
chr11	443,565,592	Y	2
Sc7zsyj_3574	171,454,409	N	1

Table 4. Completeness of assembly and gene sets assessed with BUSCOv3.0.2.

	Giant sequoia v2.0	**Giant sequoia v2.0 (≥3kbp)**	**Transcriptome**	**High-confidence gene set**
Number of input sequences	8215	8120	25859	37936
Complete BUSCOs (C)	559	553	1139	766
Complete and single-copy BUSCOs (S)	515	508	1076	683
Complete and duplicated BUSCOs (D)	44	45	63	83
Fragmented BUSCOs (F)	133	131	66	149
Missing BUSCOs (M)	748	756	235	525
Total BUSCO groups searched	1440	1440	1440	1440
Percentage found	38.82%	38.40%	79.10%	53.19%

Table 5. Comparison of giant sequoia v2.0 assembly and annotation to selected gymnosperm genome projects. 5a shows assembly statistics as reported in referenced manuscripts. 5b shows annotation statistics as calculated using gFACs on most recent annotations available at TreeGenes. Annotation statistics for *Picea glauca* are reported as in referenced manuscript.

5a	***Sequoiadendron giganteum***	***Abies alba***	***Picea glauca***	***Pinus lambertiana***	***Pinus taeda***	***Pseudotsuga menzesii***	***Ginkgo biloba***	***Gnetum montanum***
Reference		Mosca et al., 2019	Warren et al., 2015	Stevens et al., 2016	Neale et al., 2014	Neale et al., 2017	Guan et al., 2016	Wan et al., 2018
Genome size (Mbp)	8,114	18,167	20,000	31,000	20,613	15,700	10,610	4,110
Chromosomes	11	12	12	12	12	12	12	22
TE content (%)	79	78	N/A	79	81	72	77	86
N50 scaffold size (kb)	690,549.82	14.05	71.50	246.60	107.04	340.70	1,360.00	475.17
5b	***Sequoiadendron giganteum***	***Abies alba***	***Picea glauca***	***Pinus lambertiana***	***Pinus taeda***	***Pseudotsuga menzesii***	***Ginkgo biloba***	***Gnetum montanum***
Number of genes	37,936	94,209	14,462	38,518	51,751	46,688	41,840	27,493
Average overall CDS size	1,084	629	1,421	1,102	1,131	1,180	1,186	1,290
Average size multiexonic introns	4,067	315	603	11,468	5,596	4,685	7,884	1,769
Maximum intron length (kb)	1,399.11	36.01	119.32	1,254.69	758.52	351.90	1,272.92	342.13

Table 6: Gene models proposed by BRAKER2, before and after filtering. Intermediate set was filtered by removing monoexonic models, models with greater than 50% of their length in a masked region, models annotated as retrodomains, and models lacking functional annotation with EnTAP. The high-confidence set includes the intermediate set, plus mono- and multi-exonic models derived from transcript evidence, removing any fully nested gene models.

	Initial model set	**Intermediate filtered set**	**High-confidence set**
Total Genes	1,460,545	32,360	37,936
Average CDS length	613.90	1099.08	1083.00
Average number of exons	2.78	4.22	4.5
Average intron length (bp)	2,362	2,233	4,066
Max intron length	385,133	159,979	1,399,110
Total monoexonics	941,659	-	5,163
Total multiexonics	518,886	32,360	32,773

Table 7. BUSCO completeness for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*)

TreeGenes code	**Abba**	**Gibi**	**Gnmo**	**Megl**	**Pama**	**Pial**	**Piba**
taxon	***Abies balsamea***	***Ginkgo biloba***	***Gnetum gnemon***	***Metasequoia glyptostroboides***	***Picea mariana***	***Pinus albicaulis***	***Pinus banksiana***
	Balsam fir	**Ginkgo**	**Gnemon/milinjo**	**Dawn redwood**	**Black spruce**	**White pine**	**Jack pine**
Unigene set							
Data source(s)	transcriptome	annotation, transcriptome	annotation	transcriptome	transcriptome	transcriptome	transcriptome
Number of unigenes	21,250	110,296	21,887	19,237	22,876	27,226	21,278
Average length of unigenes	396.46	269.39	351.69	343.17	376.93	338.76	381.58
BUSCOv4.0.2							
Complete	1419	1437	1301	1109	1429	1453	1342
Complete & single copy	1357	1292	1265	1068	1377	1398	1283
Complete & duplicated	62	145	36	41	52	55	59
Fragmented	52	89	82	206	66	48	93
Missing	143	88	231	299	119	113	179
Total searched	1614	1614	1614	1614	1614	1614	1614
% complete	87.92%	89.03%	80.61%	68.71%	88.54%	90.02%	83.15%

Table 7. BUSCO completeness for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*)

TreeGenes code	**Pice**	**Picn**	**Pila**	**Pima**	**Pimn**	**Pipt**	**Pist**
taxon	***Pinus cembra***	***Pinus canariensis***	***Pinus lambertiana***	***Pinus massoniana***	***Pinus monticola***	***Pinus patula***	***Pinus strobus***
	Swiss stone pine	**Canary island pine**	**Sugar pine**	**Chinese red pine**	**Western white pine**	**Mexican weeping pine**	**Eastern white pine**
Unigene set							
Data source(s)	transcriptome	transcriptome	annotation, transcriptome	transcriptome	transcriptome	transcriptome	transcriptome
Number of unigenes	17,994	22,631	42,256	33,891	17,447	46,563	21,697
Average length of unigenes	411.38	327.27	357.80	322.13	388.92	348.04	372.89
BUSCOv4.0.2							
Complete	1300	1183	1369	1415	1202	1526	1338
Complete & single copy	1250	1147	1276	1367	1163	1435	1296
Complete & duplicated	50	36	93	48	39	91	42
Fragmented	119	226	88	84	146	28	95
Missing	195	205	157	115	266	60	181
Total searched	1614	1614	1614	1614	1614	1614	1614
% complete	80.55%	73.30%	84.82%	87.67%	74.47%	94.55%	82.90%

Table 7. BUSCO completeness for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*)

TreeGenes code	**Pita**	**Pnte**	**Psme**	**Segi**	**Sese**	**Thoc**	**Amtr**
taxon	***Pinus taeda***	***Pinus tecunumanii***	***Pseudotsuga menziesii***	***Sequoiadendron giganteum***	***Sequoia sempervirens***	***Thuja occidentalis***	***Amborella trichopoda***
	Loblolly pine	**Tecun Uman Pine**	**Douglas-fir**	**Giant sequoia**	**Coast redwood**	**Eastern white cedar**	
Unigene set							
Data source(s)	annotation, transcriptome	transcriptome	annotation, transcriptome	annotation	transcriptome	transcriptome	annotation
Number of unigenes	45255	22287	70036	42325	21798	19208	24753
Average length of unigenes	392.03	450.75	289.17	328.80	303.28	338.63	318.60
BUSCOv4.0.2							
Complete	1090	1517	1152	1113	1064	1187	1303
Complete & single copy	1000	1453	1030	1057	1030	1149	1292
Complete & duplicated	90	64	122	56	34	38	11
Fragmented	161	22	253	232	221	172	49
Missing	363	75	209	269	329	255	23
Total searched	1614	1614	1614	1614	1614	1614	1614
% complete	67.53%	93.99%	71.38%	68.96%	65.92%	73.54%	80.73%

*Not a TreeGenes code; Amtr peptide data were downloaded from Ensembl (Howe et al., 2019).

Table 8. Orthogroup assignment summary for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*; Amtr).

	Abba	**Gibi**	**Gnmo**	**Megl**	**Pama**	**Pial**	**Piba**
	Abies balsamea	***Gingkgo biloba***	***Gnetum gnemon***	***Metasequoia glyptostroboides***	***Picea mariana***	***Pinus albicaulis***	***Pinus banksiana***
Number of genes	21250	24753	110296	21887	19237	22876	27226
Number of genes in orthogroups	20397	19981	76213	19648	18318	21197	24571
Number of unassigned genes	853	4772	34083	2239	919	1679	2655
Percentage of genes in orthogroups	96	80.7	69.1	89.8	95.2	92.7	90.2
Percentage of unassigned genes	4	19.3	30.9	10.2	4.8	7.3	9.8
Number of orthogroups containing species	12169	10832	27140	10875	12029	13090	14212
Percentage of orthogroups containing species	27.2	24.2	60.6	24.3	26.9	29.2	31.7
Number of species-specific orthogroups	29	757	6081	531	13	57	94
Number of genes in species-specific orthogroups	64	4029	20762	2482	28	150	216
Percentage of genes in species-specific orthogroups	0.3	16.3	18.8	11.3	0.1	0.7	0.8

Table 8. Orthogroup assignment summary for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*; Amtr).

	Pice	**Picn**	**Pila**	**Pima**	**Pimn**	**Pipt**	**Pist**
	Pinus cembra	***Pinus canariensis***	***Pinus lambertiana***	***Pinus massoniana***	***Pinus monticola***	***Pinus patula***	***Pinus strobus***
Number of genes	17994	22631	48172	33891	17447	46563	21697
Number of genes in orthogroups	17772	21501	44729	29596	16858	40872	21005
Number of unassigned genes	222	1130	3443	4295	589	5691	692
Percentage of genes in orthogroups	98.8	95	92.9	87.3	96.6	87.8	96.8
Percentage of unassigned genes	1.2	5	7.1	12.7	3.4	12.2	3.2
Number of orthogroups containing species	11602	12880	16398	17568	11767	19669	12951
Percentage of orthogroups containing species	25.9	28.8	36.6	39.2	26.3	43.9	28.9
Number of species-specific orthogroups	5	29	763	238	7	678	15
Number of genes in species-specific orthogroups	10	62	2756	529	25	1643	32
Percentage of genes in species-specific orthogroups	0.1	0.3	5.7	1.6	0.1	3.5	0.1

Table 8. Orthogroup assignment summary for 20 gymnosperm taxa and an angiosperm outgroup (*Amborella trichopoda*; Amtr).

	Pita	**Pnte**	**Psme**	**Segi**	**Sese**	**Thoc**	**Amtr**
	Pinus taeda	***Pinus tecunumanii***	***Pseudotsuga menziesii***	***Sequoiadendron giganteum***	***Sequoia sempervirens***	***Thuja occidentalis***	***Amborella trichopoda***
Number of genes	42848	22287	70036	42325	21798	19208	24753
Number of genes in orthogroups	39461	21901	59920	38934	20070	17835	19981
Number of unassigned genes	3387	386	10116	3391	1728	1373	4772
Percentage of genes in orthogroups	92.1	98.3	85.6	92	92.1	92.9	80.7
Percentage of unassigned genes	7.9	1.7	14.4	8	7.9	7.1	19.3
Number of orthogroups containing species	14166	13044	18538	15665	13230	11612	10832
Percentage of orthogroups containing species	31.6	29.1	41.4	35	29.5	25.9	24.2
Number of species-specific orthogroups	479	14	1616	607	47	54	757
Number of genes in species-specific orthogroups	1441	33	7261	3364	103	123	4029
Percentage of genes in species-specific orthogroups	3.4	0.1	10.4	7.9	0.5	0.6	16.3

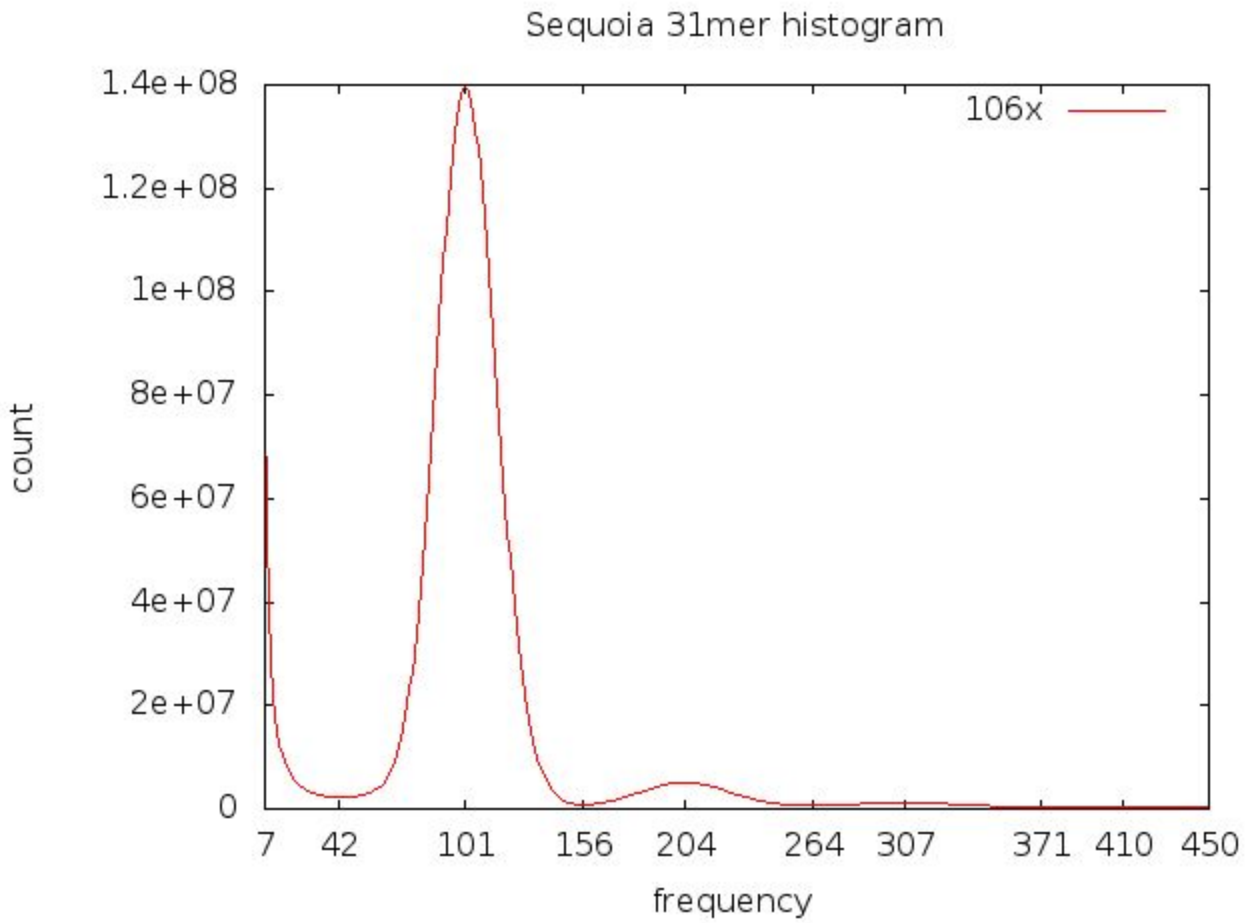


Figure 1. The histogram of 31-mer count in Illumina paired end reads. The red curve shows the number of 31-mers that are present in the reads X times, where X is the frequency plotted on horizontal axis. The main peak is at 101.

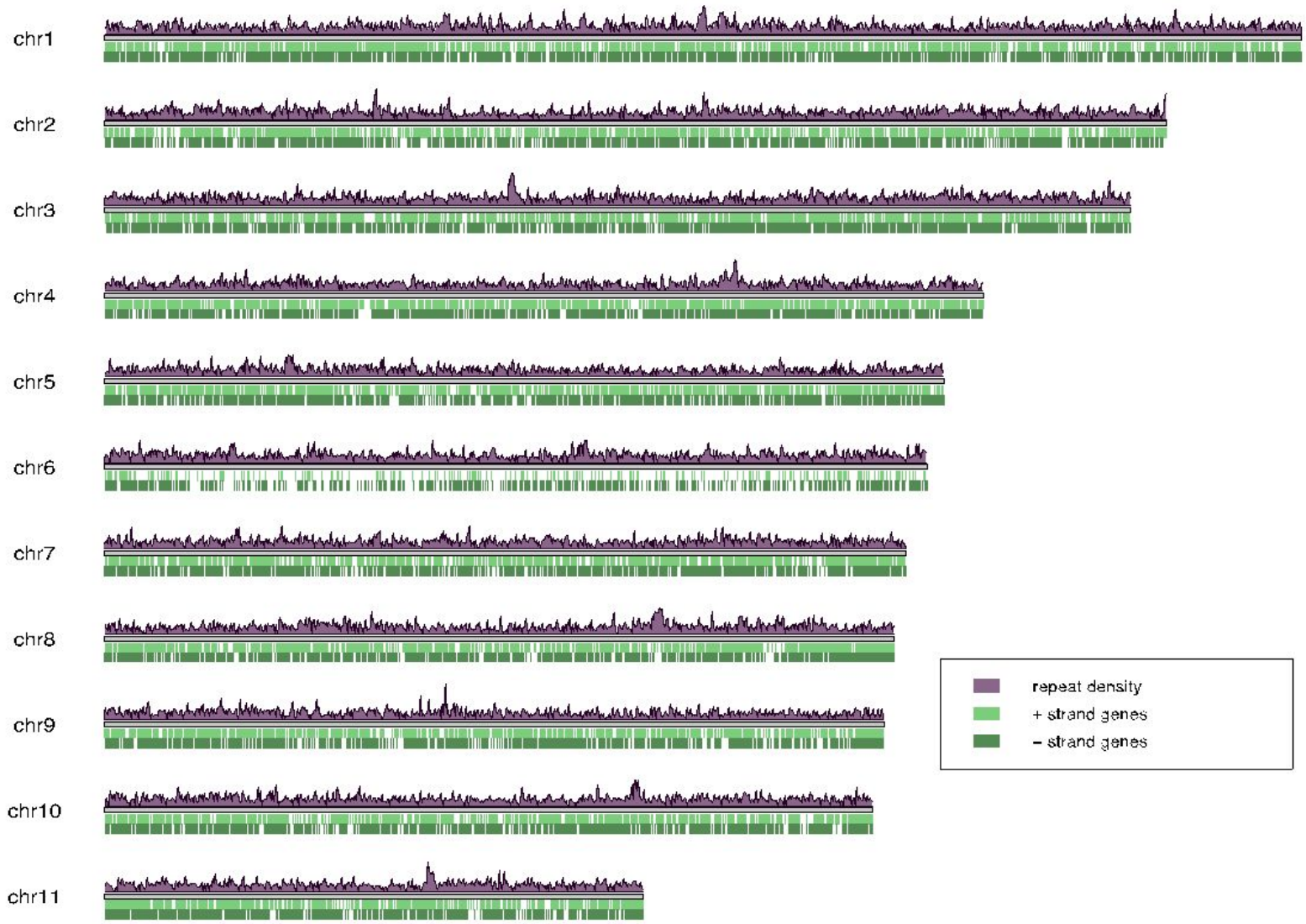


Figure 2. Repeat density and gene content of giant sequoia 2.0. Light green bars are + strand genes, dark green bars are - strand genes. Repeat density in purple, plotted in 1kb windows.

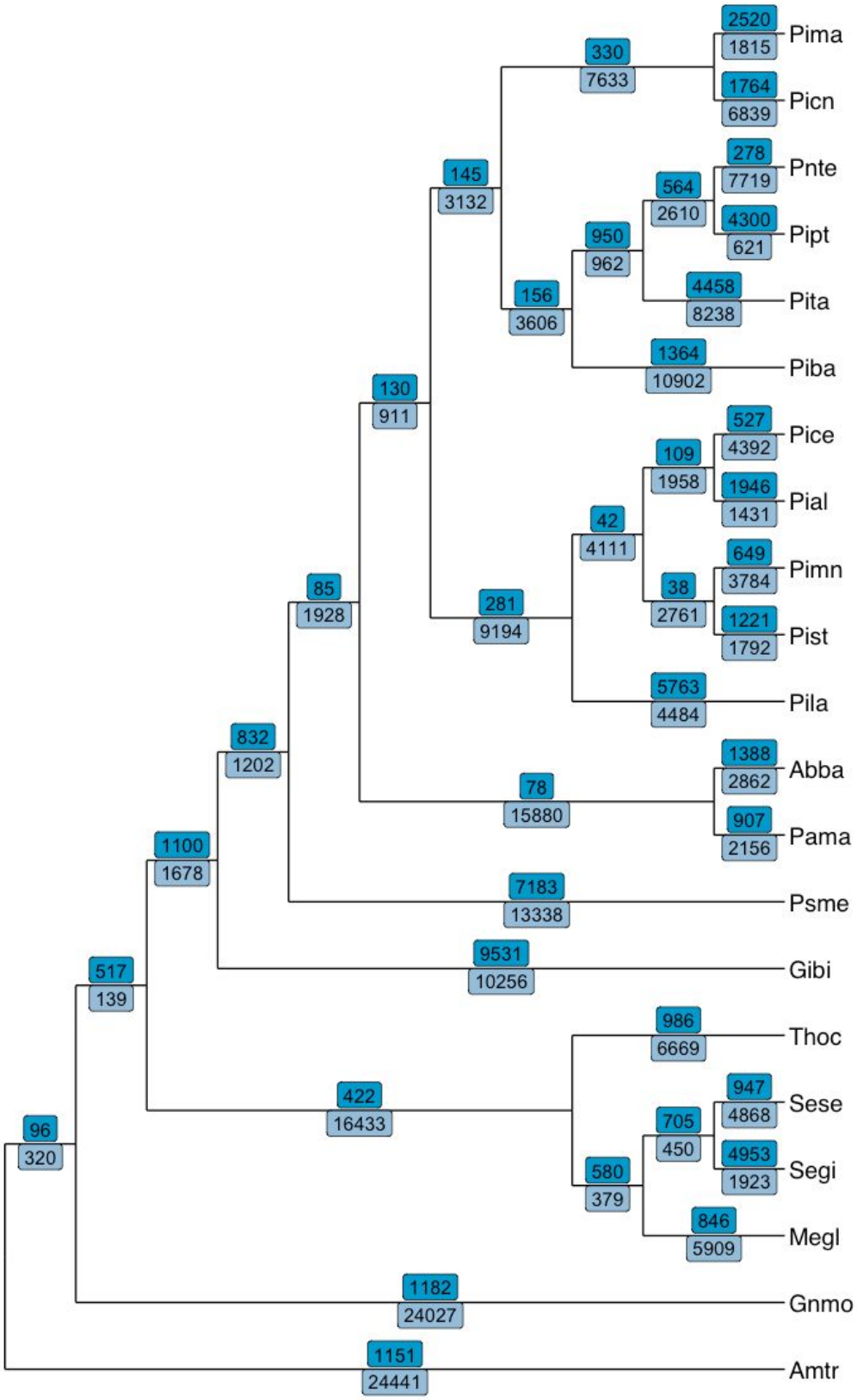


Figure 3: Gene family evolution along a gymnosperm cladogram. Numbers of expanded (bright blue, above branches) and contracted (light blue, below branches) orthogroups indicated in along each branch. Giant sequoia (Segi) experienced an overall expansion, with 4,953 orthogroups expanding and 1,923 contracting.

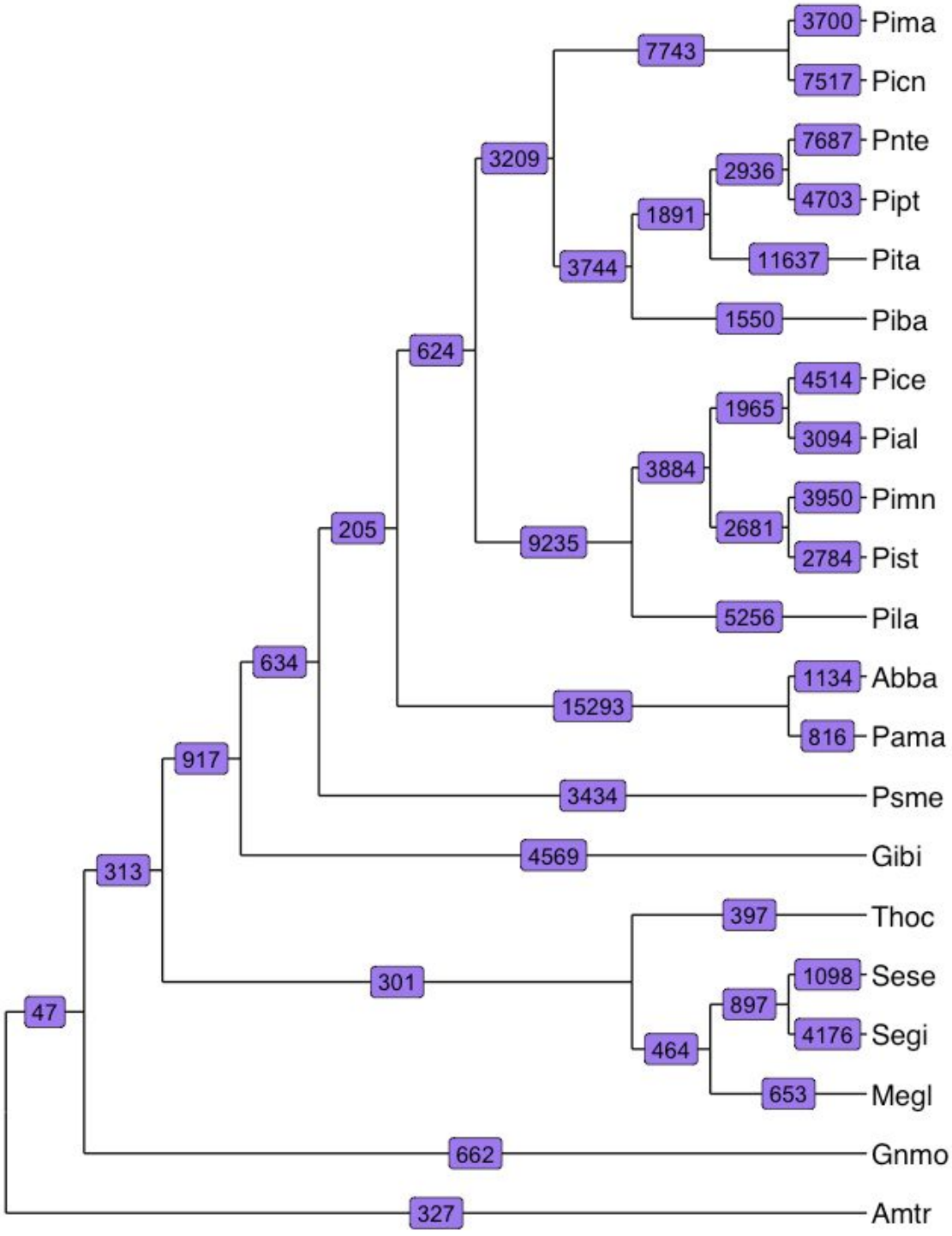


Figure 4: Rapid evolution along a gymnosperm cladogram. Numbers on each branch indicate the number of rapidly evolving gene families. Giant sequoia (Segi) has experienced rapid evolution in 4,176 gene families.

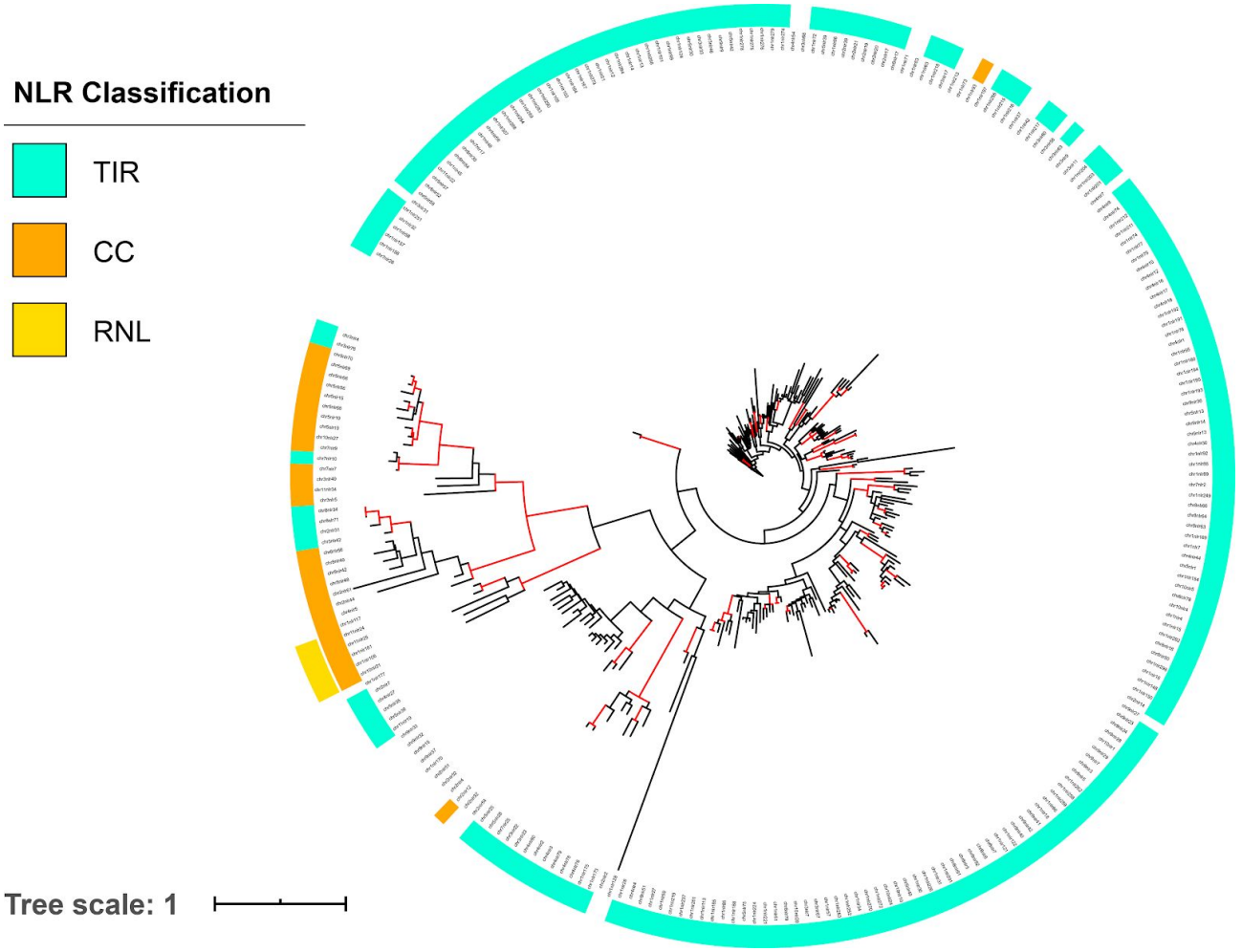


Figure 5: Maximum likelihood tree of NB-ARC domains of the 257 consensus NLR genes detected in the Segi assembly. Red branches indicate bootstrap support greater than 70%. The inner ring indicates predicted N-terminal TIR (blue) or CC (orange) domains. The outer ring indicates presence of an RPW8 motif present in the RNL sub-group of CC-NLRs. Tree is available at: http://itol.embl.de/shared/acr242

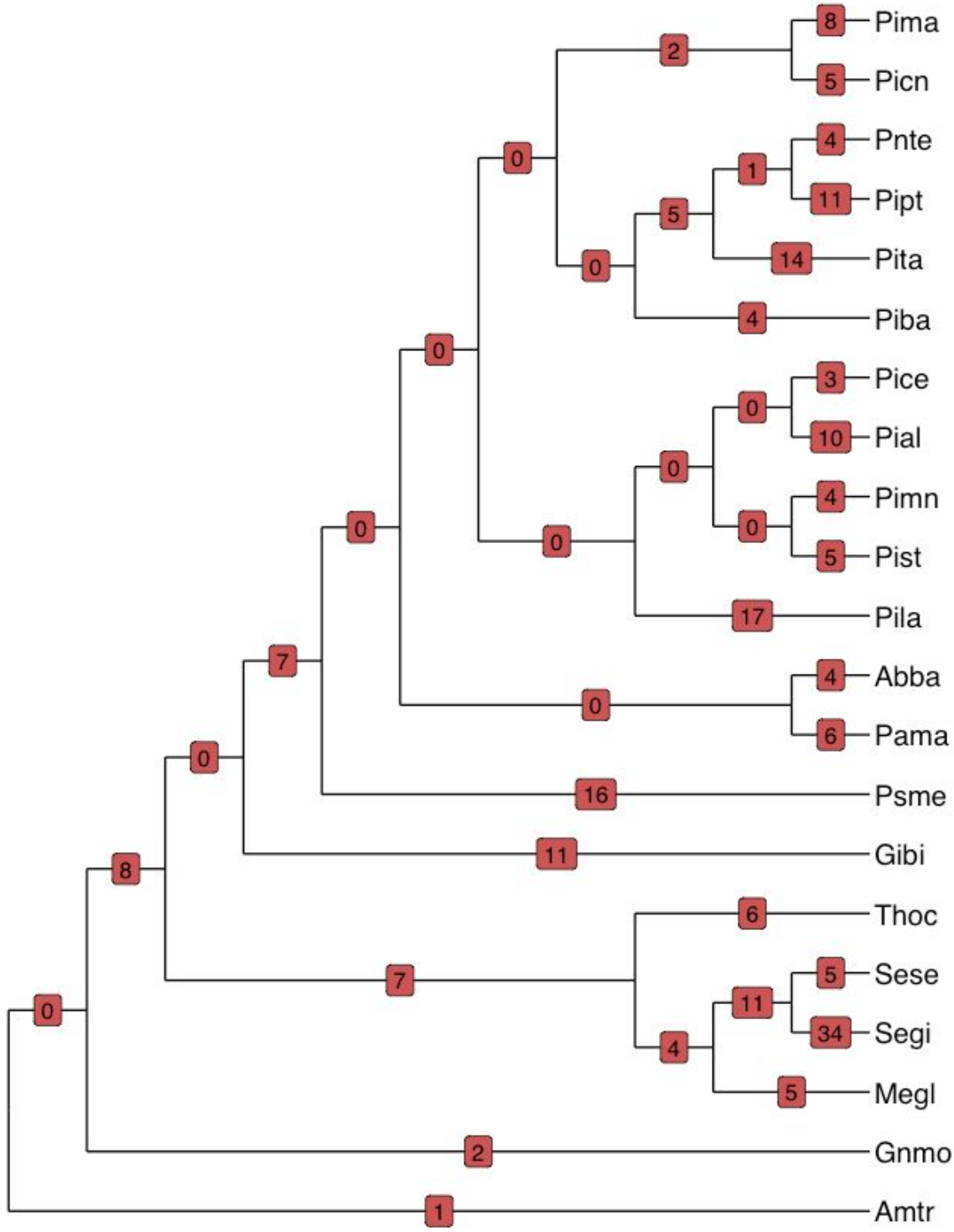


Figure 6: Rapid expansion in NLR-associated orthogroups along a gymnosperm cladogram. Numbers (red) on each branch indicate the number of rapidly expanding NLR orthogroups. Giant sequoia (Segi) has experienced rapid expansion in 34 NLR-associated orthogroups.

Table S1. Completeness of conifer genome assemblies assessed with BUSCOv3.0.2. Giant sequoia 2.0 is consistent with completeness of other conifer assemblies.

	Sequoiadendron giganteum	***Picea glauca***	***Picea abies***	***Pinus lambertiana***	***Pinus taeda***	***Pseudotsuga menziesii***
	Giant sequoia	**White spruce**	**Norway spruce**	**Sugar pine**	**Loblolly pine**	**Doug fir**
Complete BUSCOs (C)	611	443	505	396	636	484
Complete and single-copy BUSCOs (S)	575	316	434	349	508	412
Complete and duplicated BUSCOs (D)	36	127	71	47	128	72
Fragmented BUSCOs (F)	192	182	150	172	102	110
Missing BUSCOs (M)	811	815	785	872	702	846
Total BUSCO groups searched	1614	1440	1440	1440	1440	1440
Percentage found	37.86%	30.76%	35.07%	27.50%	44.17%	33.61%

Table S2. Classification and associated percentage of genome masked by repetitive elements in giant sequoia 2.0

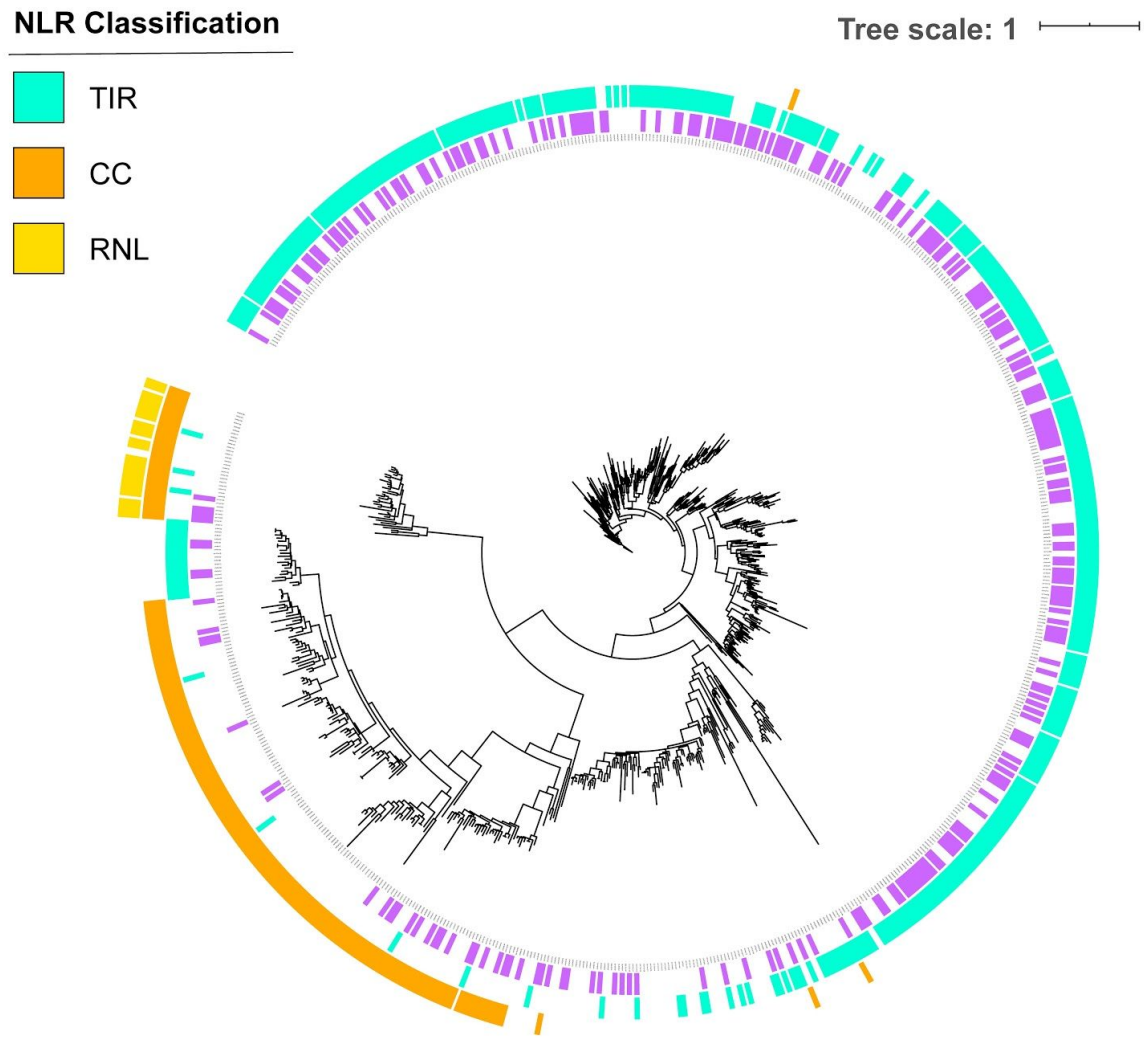
Repeat Class	Masked % of genome
DNA	
DNA/CMC-EnSpm	0.36161
DNA/MuLE-MuDR	1.55370
DNA/Sola	0.77113
DNA/TcMar-Fot1	0.05913
DNA/hAT-Tag1	0.47514
DNA/hAT-Tip100	0.13902
DNA total	3.35974
LINE	
LINE/L1	1.77165
LINE/L1-Tx1	0.40261
LINE/Penelope	0.02434
LINE/RTE-X	0.11875
LINE/Tad1?	0.00674
LINE total	2.32409
LTR	
LTR	0.21792
LTR/Copia	8.05066
LTR/ERVK	0.46223
LTR/Gypsy	19.62522
LTR total	28.35604
Low_complexity	0.16773
RC/Helitron	0.07451
Satellite	0.00005
Simple_repeat	2.03401
Unknown	42.34551

Table S3 Orthogroup clustering of 695,700 protein sequences from twenty gymnosperms plus an outgroup (*Amborella trichopoda*)

Number of species	21
Number of genes	695700
Number of genes in orthogroups	611441
Number of unassigned genes	84259
Percentage of genes in orthogroups	87.9
Percentage of unassigned genes	12.1
Number of orthogroups	44797
Number of species-specific orthogroups	12121
Number of genes in species-specific orthogroups	45127
Percentage of genes in species-specific orthogroups	6.5
Mean orthogroup size	13.6
Median orthogroup size	4
G50 (assigned genes)	30
G50 (all genes)	27
O50 (assigned genes)	4762
O50 (all genes)	6250
Number of orthogroups with all species present	5953
Number of single-copy orthogroups	206

Table S4. Annotation summary for 607 species-specific giant sequoia orthogroups

Total Sequences:	**607**
Similarity Search	
Total unique sequences with an alignment	218
Total unique sequences without an alignment	389
Gene Families	
Total unique sequences with family assignment	528
Total unique sequences without family assignment	79
Total unique sequences with at least one GO term	429
Total unique sequences with at least one pathway (KEGG) assignment	124
Totals	
Total unique sequences annotated (similarity search alignments only)	8
Total unique sequences annotated (gene family assignment only)	318
Total unique sequences annotated (gene family and/or similarity search)	536
Total unique sequences unannotated (gene family and/or similarity search)	71



Supplemental Figure 1: Maximum likelihood tree of NB-ARC domains of all NLR-Annotator detected NLR genes. The purple ring represents consensus NLR genes. N-terminal domains are indicated in the outer rings (TIR- light blue, CC- orange, RNL- yellow).