

Supplementary Tables

Supplementary Table 1 Sequencing reads for *Eucalyptus viminalis* ACT, VIC and TAS.

All PacBio HiFi reads, filtered ONT ultra-long reads (≥ 55 kb and $\geq Q20$) and filtered Hi-C reads (described in next table) were used in the genome assembly of *E. viminalis* ACT. Only filtered ONT reads (≥ 20 kb and $\geq Q20$) were used in the genome assembly of *E. viminalis* VIC and TAS. Therefore, these reads were filtered more stringently, but also benefited from updated sequencing chemistry and assembly tools.

	Evim ACT			Evim VIC		Evim TAS	
Metric	HiFi	ONT	ONT ≥ 55 kb $\geq Q7$	ONT	ONT ≥ 20 kb $\geq Q20$	ONT	ONT ≥ 20 kb $\geq Q20$
Reads	1,493,183	6,802,892	471,525	3,119,232	1,182,139	2,684,427	894,493
Yield (bp)	30,268,834,753	176,995,195,958	32,674,908,869	85,888,723,721	53,511,358,482	66,828,255,396	40,656,940,200
Mean read length (bp)	20,271.3	26,017.6	69,296.2	27,535.2	45,266.6	24,894.8	45,452.5
Median read length (bp)	19,515	23,524	64,736	28,391	42,292	19,822	42,314
N50 (bp)	20,332	36,617	66,993	46,246	47,264	46,378	47,725
Mean read quality	29.2	16.4	17.5	9.8	22.6	9.2	22.6
Median read quality	30.2	18.9	18.4	20.5	23.6	20.1	23.6
Five longest read lengths (and Q score)							
#1	55,114 (15.2)	463,629 (3.5)	564,886 (7.5)	1,875,904 (6.2)	375,909 (21.3)	1,410,201 (5.2)	307,800 (23.5)
#2	55,332 (24.0)	469,113 (3.9)	484,984 (7.3)	1,843,354 (6.2)	281,550 (22.9)	1,385,395 (3.5)	284,444 (21.6)
#3	55,411 (15.1)	484,984 (7.3)	293,940 (19.6)	1,554,198 (5.5)	269,294 (24.3)	1,218,598 (4.5)	259,566 (20.1)
#4	56,218 (17.9)	564,886 (7.5)	286,672 (19.9)	1,310,354 (6.4)	254,787 (24.0)	1,191,299 (2.9)	258,757 (20.8)
#5	57,165 (15.8)	806,429 (2.7)	282,977 (7.8)	1,271,470 (6.3)	250,349 (25.0)	1,123,223 (8.2)	251,260 (21.3)
Coverage (547 Mbp genome)	55x	324x	60x	157x	98x	122x	74x
Coverage per haplotype	28x	162x	30x	79x	49x	61x	37x

Supplementary Table 2. Hi-C sequencing results and chromosomal linkage statistics for the *E. viminalis* ACT reference genome. Statistics are shown for both haplotypes and were used for chromosome scaffolding and assembly validation.

	Haplotype 1	Haplotype 2
Hi-C Read Pairs	89,043,318	
Inter-chromosomal	10,570,983	10,599,530
Intra-chromosomal	19,716,057	19,602,535
Uninformative Read Pairs	58,756,278	58,841,253

Supplementary Table 3. Genome assembly statistics for *Eucalyptus viminalis* ACT, VIC and TAS. For each genome, the 11 chromosomes were haplotype resolved, at chromosome scale or higher (many being telomere-to-telomere). The *E. viminalis* ACT genome (first genome) was constructed with PacBio HiFi, ONT ultra-long and Hi-C. The *E. viminalis* VIC and TAS genomes were assembled with ONT ultra-long reads only, but benefited from newer sequencing chemistry and assembly algorithms, having only 1 gap at the chromosome two centromere. Karyotype is $2n = 22$.

Metric	Evim ACT		Evim VIC		Evim TAS	
	Hap1	Hap2	Hap 1	Hap 2	Hap 1	Hap 2
Sequences	294	287	392	25	298	28
Sequences >1 Mbp	11	11	11	11	11	11
N50 (bp)	51,735,356	50,558,970	43,272,629	52,040,736	40,964,566	52,728,202
N50 (bp) >1 Mbp	51,735,356	50,558,970	51,281,642	52,040,736	50,289,127	53,905,933
Size (bp)	546,531,677	551,267,619	589,735,305	531,995,131	559,924,252	536,652,899
Size (bp) >1 Mbp	530,050,200	530,973,922	538,353,018	529,912,749	526,300,622	533,344,571
Telomeres	2/22	3/22	22/22	22/22	22/22	21/22
T2T contigs	0/11	0/11	10/11	10/11	10/11	9/11
T2T scaffolds	0/11	0/11	11/11	11/11	11/11	10/11
Gaps	94	165	1	1	1	1

Supplementary Table 4. BUSCO completeness assessment of *E. viminalis* genome assemblies. Single-copy orthologs from the eudicotyledons odb12 dataset were identified using compleasm v0.2.7 to evaluate the completeness of the 11 haplotype-resolved chromosomal scaffolds for each genome.

	Evim ACT		Evim VIC		Evim TAS	
	Hap 1	Hap 2	Hap 1	Hap 2	Hap 1	Hap 2
BUSCO						
Complete	99.61% (2,794)	99.68% (2,796)	99.72% (2,797)	99.72% (2,797)	99.72% (2,797)	99.68% (2,796)
Single	98.11	97.79%	97.83%	98.04%	98.40%	97.93%

Supplementary Table 5. Assembly quality assessment using Quality Value (QV) scores for haplotype-resolved *E. viminalis* genomes. Assembly accuracy was assessed using yak v0.1 (r56) with default k=31 for each chromosome-scale haplotype (Cheng et al 2021 <https://www.nature.com/articles/s41592-020-01056-5>). Raw and coverage adjusted QV scores are shown as Phred-scaled values and accuracy percentages.

	Evim ACT (HiFi)		Evim VIC (ONT ≥20 kb ≥Q20)		Evim TAS (ONT ≥20 kb ≥Q20)	
	Hap 1	Hap 2	Hap 1	Hap 2	Hap 1	Hap 2
Raw QV	56.415	55.525	66.772	67.085	64.368	64.116
Raw QV %	99.999772	99.999720	99.999979	99.999980	99.999963	99.999962
Adjusted QV	57.591	55.845	64.562	64.620	60.366	60.395
Adjusted QV %	99.999826	99.999740	99.999965	99.999965	99.999910	99.999911

Supplementary Table 6. ONT direct RNA sequencing results for an *E. viminalis* ACT leaf sample. Reads were filtered to retain only those ≥200 bp in length and ≥Q10 quality score for the annotation of the genome and identification of isoforms.

Metric	Raw RNA reads	Filtered RNA reads
Mean Read Length (bp)	1,134.2	1,303.4
Mean Quality	12.2	19.2
Median Read Length (bp)	937.0	1,092.0
Median Quality	20.9	21.6
Reads	22,125,880	18,024,536
N50	1,605.0	1,618.0
STDEV read length	1,130.1	877.9
Yield (bp)	25,094,940,983	23,493,482,035
Five longest reads (and Q score) #1	241,297 (5.7)	27,417 (22.8)
#2	224,525 (3.9)	25,793 (22.0)
#3	210,414 (4.2)	21,167 (21.4)
#4	208,103 (6.7)	20,781 (22.2)
#5	205,564 (4.0)	20,457 (23.4)

Supplementary Table 7. Sequencing summary from Oxford Nanopore Technologies (ONT) long-read sequencing of environmental samples (n = 72). after quality (average Q score > 7) and read size (minimum read length > 1 kb) filtering.

	sample_name	coverage	num_reads	read_length_N50	total_bases_Gb	max_read_length	median_read_length
2	NSW0083	8.95098	962129	6.156	4.70225	56.823	4.745
3	NSW0105	11.4163	1486687	5.395	5.99737	61.321	3.132
4	NSW0108	5.89502	685649	5.833	3.09686	43.319	4.271
5	NSW0110	2.04307	225281	6.623	1.0733	44.32	3.978
6	NSW0111	7.71839	992345	5.721	4.05473	42.2	3.167
7	NSW0113	11.3181	950043	8.076	5.9458	66.902	5.839
8	NSW0119	7.69559	864114	5.994	4.04276	47.049	4.427
9	NSW0120	3.36433	429528	5.481	1.7674	79.467	3.651
10	NSW0133	1.00533	154295	4.417	0.528136	42.071	2.562
11	NSW0141	18.6357	1586697	7.729	9.78998	84.917	5.53
12	NSW0144	13.3328	1213005	7.767	7.00416	74.695	4.778
13	NSW0159	12.5647	884301	10.233	6.60067	84.868	6.54
14	NSW0163	13.6504	1025529	9.094	7.17101	73.836	6.453
15	NSW0170	17.7472	1428360	8.257	9.32323	92.544	5.734
16	NSW0175	9.84936	829086	8.335	5.1742	98.098	5.216
17	NSW0192	3.55784	437749	5.807	1.86906	47.266	3.498
18	NSW0194	4.04678	472992	6.177	2.12591	121.675	3.702
19	NSW0207	11.1162	1514158	5.189	5.8397	89.842	3.055
20	NSW0209	2.39105	301305	5.776	1.2561	105.191	3.288
21	NSW0250	16.8051	1240465	9.404	8.82829	87.516	6.374
22	NSW0293	29.9479	1556811	13.1	15.7326	156.083	9.485
23	NSW0296	13.7463	1044468	8.396	7.22143	73.649	6.264
24	NSW0299	9.98593	1434379	4.918	5.24595	65.534	2.84
25	NSW0306	24.4949	2717245	6.317	12.868	66.36	3.937
26	NSW0308	19.8459	2221206	6.244	10.4257	343.493	3.921
27	NSW0313	18.9491	1681010	7.596	9.95462	81.822	5.156
28	NSW0315	6.49355	544534	8.263	3.41129	76.15	5.524
29	NSW0323	9.19755	886616	6.575	4.83179	67.277	5.444
30	NSW0324	14.9675	1095822	9.919	7.86292	82.278	6.192
31	NSW0328	11.1906	775432	10.455	5.8788	105.051	6.5255
32	NSW0334	12.7057	907636	10.161	6.67474	90.242	6.347
33	NSW0335	10.751	806298	9.894	5.64788	71.769	5.852

34	NSW0337	16.0358	1313350	7.708	8.42416	63.244	5.994
35	NSW0341	13.883	1202451	7.381	7.29322	77.341	5.73
36	NSW0343	11.1506	863310	9.66	5.85778	74.817	5.524
37	NSW0347	9.58598	758783	9.411	5.03584	73.146	5.453
38	NSW0348	10.1471	781891	9.652	5.33063	86.062	5.603
39	NSW0350	15.6948	1651628	6.49	8.24502	61.998	4.421
40	NSW0354	14.2373	1517831	6.588	7.47936	65.054	4.12
41	NSW0356	13.5844	1428460	6.751	7.13637	59.6	3.999
42	NSW0357	13.7632	1045792	8.816	7.23029	58.483	6.504
43	NSW0359	16.8905	1608212	7.258	8.87317	79.355	4.755
44	NSW0360	0.177873	21904	5.78	0.0934427	39.709	3.305
45	NSW0366	21.2167	1967794	7	11.1459	102.709	5.291
46	NSW0438	23.6787	1369640	11.573	12.4392	105.884	8.143
47	NSW0482	0.643269	91807	4.935	0.337931	32.976	2.775
48	NSW0491	17.7734	2328606	5.465	9.33695	52.861	3.165
49	NSW0493	14.8659	1202607	8.905	7.80955	55.711	5.677
50	NSW0496	20.0196	2301955	6.008	10.517	84.553	3.886
51	NSW0507	0.891656	123623	5.077	0.468417	110.646	2.835
52	NSW0532	17.8178	1163923	10.001	9.36032	90.239	7.416
53	NSW0536	12.7913	1175455	7.649	6.71972	77.711	4.936
54	NSW0544	18.8856	1480507	8.65	9.92127	90.58	6.162
55	NSW0555	11.2373	1646519	4.768	5.90335	206.865	2.805
56	NSW0561	13.5694	1104498	8.972	7.12846	66.231	5.372
57	NSW0566	12.2311	1820740	4.64	6.42541	160.07	2.794
58	NSW0573	12.383	1622781	5.407	6.50519	118.511	3.243
59	NSW0575	8.35824	850173	6.382	4.39087	64.073	5.01
60	NSW0576	20.1635	1455137	10.067	10.5926	100.658	6.28
61	NSW0934	17.2554	1979918	6.175	9.06485	61.207	3.759
62	NSW0935	2.77763	382010	5.059	1.45918	56.067	2.916
63	NSW0936	1.81097	251023	5.055	0.951362	55.849	2.801
64	NSW0938	2.33443	313364	5.242	1.22636	68.637	2.966
65	NSW0939	4.3979	573694	5.349	2.31037	58.417	3.116
66	NSW0940	3.20183	425685	5.346	1.68203	105.466	3.278
67	NSW0954	6.24472	492787	8.714	3.28057	64.006	6.035
68	NSW0962	19.2845	1780998	7.04	10.1308	104.905	5.262
69	NSW0963	4.04471	491526	5.778	2.12483	45.066	3.639
70	NSW0966	6.39348	672144	6.409	3.35871	97.581	4.335

71	NSW0970	10.1207	857272	8.3	5.31674	94.322	5.231
72	NSW0972	11.1123	936771	8.229	5.83765	80.982	5.496

Supplementary Table 8. Haplotype-resolved assembly quality of environmental samples (n = 71). Genomes assembled with hifiasm (--ont). Table ordered by BUSCO scores, highest to lowest.

sample_name	BUSCO	population	coverage	haplotype	mean_length	median_length	contigs	n50_length	stdev_length	total_bases
NSW0293	98.79	C	29.9479	hap2	1,547,592	1,036,918	343	2,588,597	1,521,859	530,824,011
NSW0293	98.45	C	29.9479	hap1	1,103,047	658,789	503	2,037,153	1,278,829	554,832,757
NSW0438	95.18	B	23.6787	hap1	151,300	95,764	3,758	255,713	162,315	568,583,354
NSW0438	93.9	B	23.6787	hap2	184,573	131,570	2,727	280,315	175,737	503,330,782
NSW0306	92.73	C	24.4949	hap1	67,245	43,741	8,176	106,540	80,182	549,792,405
NSW0306	91.23	C	24.4949	hap2	75,405	50,902	6,516	113,726	84,158	491,339,593
NSW0576	93.64	A	20.1635	hap1	109,783	74,990	4,996	170,727	110,774	548,475,552
NSW0576	90.15	A	20.1635	hap2	121,546	87,360	4,018	176,608	112,517	488,372,190
NSW0544	88.74	A	18.8856	hap1	62,473	44,269	8,418	86,132	69,306	525,899,167
NSW0496	86.98	A	20.0196	hap1	47,287	32,496	11,132	69,946	47,864	526,402,658
NSW0496	85.04	A	20.0196	hap2	52,953	37,983	8,709	75,038	51,707	461,170,727
NSW0544	84.73	A	18.8856	hap2	70,038	52,252	6,428	93,761	68,081	450,202,316
NSW0313	85.43	C	18.9491	hap1	50,778	37,686	10,318	69,271	46,491	523,922,927
NSW0141	84.44	A	18.6357	hap1	51,439	38,021	9,645	70,661	45,653	496,128,445
NSW0313	82.93	C	18.9491	hap2	52,157	39,787	8,186	69,384	44,181	426,959,842
NSW0962	83.71	A	19.2845	hap1	45,806	33,925	11,199	62,089	40,252	512,979,448
NSW0532	83.79	A	17.8178	hap1	59,209	45,638	8,512	76,829	46,281	503,987,373
NSW0532	81.99	A	17.8178	hap2	69,239	54,565	6,472	90,321	51,601	448,114,270
NSW0250	85.34	A	16.8051	hap1	59,296	44,576	8,721	79,777	55,503	517,118,994
NSW0141	80.56	A	18.6357	hap2	57,661	43,917	7,491	77,501	48,296	431,935,882
NSW0308	81.17	C	19.8459	hap1	36,601	26,961	13,107	49,317	36,583	479,725,070
NSW0170	81.6	A	17.7472	hap1	47,744	36,535	10,120	62,879	38,601	483,167,736
NSW0962	79.63	A	19.2845	hap2	47,885	36,780	8,806	63,026	39,211	421,673,339
NSW0324	81	C	14.9675	hap1	48,921	38,543	9,700	61,626	38,011	474,530,096
NSW0366	79.32	C	21.2167	hap1	37,129	27,810	12,808	48,089	33,381	475,543,718
NSW0250	78.42	A	16.8051	hap2	63,539	49,071	6,428	83,853	50,076	408,425,847
NSW0170	77.73	A	17.7472	hap2	55,849	44,249	7,543	72,053	46,242	421,268,467
NSW0359	77.08	C	16.8905	hap1	33,615	26,849	13,125	41,914	24,960	441,196,281
NSW0324	75.15	C	14.9675	hap2	53,583	42,312	6,934	66,843	42,523	371,544,227

NSW0493	76.27	A	14.8659	hap1	42,726	33,208	10,838	52,725	40,837	463,068,729
NSW0308	73.99	C	19.8459	hap2	38,652	29,756	9,969	49,974	34,279	385,323,715
NSW0337	77.04	C	16.0358	hap1	37,298	29,626	12,406	45,914	27,221	462,714,827
NSW0934	76.35	A	17.2554	hap1	32,211	24,209	14,508	43,082	40,785	467,323,567
NSW0350	75.67	C	15.6948	hap1	31,941	24,022	14,059	40,295	33,279	449,051,886
NSW0366	74.46	C	21.2167	hap2	41,448	31,864	9,612	52,411	46,169	398,394,630
NSW0491	73.69	A	17.7734	hap1	26,088	19,805	16,265	33,807	26,558	424,318,531
NSW0337	72.39	C	16.0358	hap2	44,662	35,637	8,661	55,664	34,082	386,812,955
NSW0357	72.36	C	13.7632	hap1	36,389	29,986	11,831	43,592	24,032	430,515,197
NSW0296	71.03	C	13.7463	hap1	34,693	28,372	11,964	41,449	27,297	415,066,115
NSW0356	69.05	C	13.5844	hap1	26,038	21,124	14,579	31,617	19,647	379,613,800
NSW0561	71.02	A	13.5694	hap1	36,892	30,253	11,306	45,057	25,756	417,099,249
NSW0934	69.35	A	17.2554	hap2	33,799	26,285	10,835	42,864	38,797	366,214,720
NSW0359	68.44	C	16.8905	hap2	39,196	31,340	9,010	49,461	30,879	353,157,770
NSW0163	70.68	A	13.6504	hap1	37,948	31,294	11,167	45,785	25,790	423,762,578
NSW0341	70.55	C	13.883	hap1	31,540	25,543	13,042	38,004	23,224	411,339,821
NSW0491	68.15	A	17.7734	hap2	31,553	23,989	11,359	40,929	32,769	358,408,570
NSW0159	71.41	A	12.5647	hap1	40,826	33,108	9,976	49,117	28,723	407,276,499
NSW0334	69.39	C	12.7057	hap1	38,525	32,167	10,696	46,056	26,053	412,068,205
NSW0536	67.67	A	12.7913	hap1	29,309	24,168	13,056	34,831	21,233	382,652,192
NSW0493	67.15	A	14.8659	hap2	46,060	36,519	7,733	56,505	41,002	356,179,336
NSW0354	66.13	C	14.2373	hap1	24,837	20,157	14,853	29,936	18,419	368,908,822
NSW0144	65.52	A	13.3328	hap1	29,448	24,281	12,461	36,041	20,534	366,954,707
NSW0350	64.1	C	15.6948	hap2	33,495	26,306	9,678	41,664	29,408	324,166,737
NSW0113	64.75	B	11.3181	hap1	29,275	24,316	12,271	33,704	21,479	359,238,294
NSW0343	64.19	C	11.1506	hap1	32,968	27,677	10,813	39,119	21,501	356,477,162
NSW0328	63.5	C	11.1906	hap1	37,828	31,262	9,464	44,571	27,518	357,999,517
NSW0972	61.74	A	11.1123	hap1	29,178	24,511	11,805	34,175	19,772	344,446,817
NSW0335	62.12	C	10.751	hap1	33,237	28,349	10,180	38,965	20,677	338,351,538
NSW0348	59.76	C	10.1471	hap1	30,964	26,286	9,699	36,252	18,850	300,317,063
NSW0561	58.17	A	13.5694	hap2	42,154	34,781	7,095	51,612	28,216	299,079,802
NSW0573	57.61	A	12.383	hap1	17,521	14,628	17,567	21,024	12,790	307,798,328
NSW0970	58.86	A	10.1207	hap1	27,345	23,157	10,559	32,242	17,587	288,739,168
NSW0347	56.06	C	9.58598	hap1	29,475	25,165	9,350	34,616	18,034	275,591,477
NSW0105	55.46	B	11.4163	hap1	17,650	14,559	17,951	21,708	13,209	316,834,955
NSW0357	55.5	C	13.7632	hap2	42,089	34,188	7,177	51,001	28,214	302,069,105
NSW0163	54.73	A	13.6504	hap2	41,844	34,414	6,988	50,526	29,291	292,403,542
NSW0175	54.47	A	9.84936	hap1	27,837	23,107	9,981	32,607	18,523	277,839,838

NSW0341	53.87	C	13.883	hap2	33,571	27,632	7,944	40,210	22,829	266,684,305
NSW0207	53.13	A	11.1162	hap1	15,593	13,197	18,349	18,658	10,552	286,122,173
NSW0566	51.72	A	12.2311	hap1	14,295	11,874	19,866	17,219	10,254	283,989,607
NSW0296	51.46	C	13.7463	hap2	38,452	31,668	7,167	45,860	30,357	275,587,477
NSW0555	50.94	A	11.2373	hap1	14,123	11,668	17,706	17,025	10,983	250,060,142
NSW0159	51.33	A	12.5647	hap2	45,933	37,900	5,489	55,355	31,654	252,127,784
NSW0334	47.89	C	12.7057	hap2	41,908	35,064	5,784	49,933	28,214	242,393,300
NSW0144	45.58	A	13.3328	hap2	34,396	28,716	6,565	41,939	23,445	225,809,010
NSW0356	44.59	C	13.5844	hap2	29,764	24,635	7,763	36,712	22,953	231,054,062
NSW0536	44.11	A	12.7913	hap2	32,975	27,500	7,089	39,516	22,896	233,755,982
NSW0354	43.29	C	14.2373	hap2	29,040	24,054	7,711	35,144	21,541	223,925,553
NSW0083	43.21	B	8.95098	hap1	18,659	16,348	10,773	20,780	11,728	201,015,574
NSW0299	38	C	9.98593	hap1	13,100	11,088	14,452	15,771	9,511	189,326,335
NSW0343	38.61	C	11.1506	hap2	37,569	31,721	4,641	44,563	24,672	174,357,823
NSW0575	37.14	A	8.35824	hap1	19,149	17,056	9,225	21,242	10,629	176,644,650
NSW0328	36.71	C	11.1906	hap2	43,967	36,150	4,134	52,360	33,175	181,760,317
NSW0113	31.81	B	11.3181	hap2	34,306	27,857	5,136	40,054	27,180	176,196,528
NSW0119	31.55	B	7.69559	hap1	16,842	14,862	8,219	18,886	11,410	138,422,365
NSW0335	31.99	C	10.751	hap2	37,694	32,057	4,177	44,913	23,454	157,448,980
NSW0972	31.51	A	11.1123	hap2	32,158	26,939	4,885	37,448	22,556	157,093,964
NSW0348	28.85	C	10.1471	hap2	35,548	30,037	3,597	41,265	22,751	127,865,091
NSW0315	27.99	C	6.49355	hap1	24,122	20,990	4,659	27,553	14,370	112,385,863
NSW0970	26.61	A	10.1207	hap2	31,801	26,366	3,707	37,754	22,294	117,884,897
NSW0573	25.88	A	12.383	hap2	20,394	16,793	6,974	24,263	15,526	142,229,146
NSW0111	25.58	B	7.71839	hap1	15,157	12,913	8,899	18,135	10,910	134,884,111
NSW0105	24.63	B	11.4163	hap2	20,766	17,204	6,855	24,944	16,346	142,351,612
NSW0347	23.73	C	9.58598	hap2	33,867	28,881	3,075	39,472	21,440	104,140,489
NSW0954	23.82	A	6.24472	hap1	25,288	21,753	4,025	28,704	16,036	101,783,963
NSW0175	20.59	A	9.84936	hap2	32,866	27,394	3,308	38,857	23,069	108,720,548
NSW0207	20.08	A	11.1162	hap2	18,475	15,593	6,041	22,104	12,884	111,608,044
NSW0966	18.61	A	6.39348	hap1	17,200	14,685	4,228	19,468	11,857	72,720,101
NSW0566	18.78	A	12.2311	hap2	17,078	13,954	6,846	20,149	13,110	116,914,401
NSW0555	15.99	A	11.2373	hap2	17,208	13,819	4,818	20,549	15,110	82,907,674
NSW0108	15.18	B	5.89502	hap1	15,495	13,852	4,399	17,346	9,272	68,160,652
NSW0083	9.24	B	8.95098	hap2	21,249	18,281	2,389	23,485	17,719	50,763,044
NSW0299	7.74	C	9.98593	hap2	16,302	13,639	2,936	19,384	14,463	47,861,999
NSW0575	6.83	A	8.35824	hap2	21,558	18,333	1,731	24,342	15,440	37,316,211
NSW0939	6.11	A	4.3979	hap1	12,216	9,979	2,049	14,303	13,893	25,031,483

NSW0133	5.42	B	1.00533	hap1	12,999	11,088	1,856	15,295	17,427	24,125,211
NSW0133	5.42	B	1.00533	hap2	12,951	11,088	1,842	15,186	15,987	23,856,154
NSW0119	3.49	B	7.69559	hap2	20,002	16,268	1,065	22,419	24,296	21,302,456
NSW0194	3.23	A	4.04678	hap1	14,395	11,986	1,069	16,836	15,621	15,388,655
NSW0954	3.06	A	6.24472	hap2	27,621	23,009	541	30,764	23,414	14,943,080
NSW0315	2.75	C	6.49355	hap2	28,804	23,845	571	33,159	22,767	16,446,969
NSW0111	2.71	B	7.71839	hap2	19,511	15,968	1,338	22,716	16,889	26,105,869
NSW0963	2.24	A	4.04471	hap1	13,226	11,274	1,028	14,918	13,527	13,596,083
NSW0192	2.06	A	3.55784	hap1	13,592	11,371	783	15,911	15,257	10,642,654
NSW0966	1.42	A	6.39348	hap2	22,514	17,738	444	26,181	25,232	9,996,073
NSW0940	1.2	A	3.20183	hap1	12,505	9,736	437	14,141	16,856	5,464,778
NSW0120	0.99	B	3.36433	hap1	13,049	10,269	479	14,475	18,775	6,250,283
NSW0108	0.86	B	5.89502	hap2	18,742	15,794	440	20,743	18,608	8,246,245
NSW0194	0.9	A	4.04678	hap2	16,722	12,954	326	18,536	25,152	5,451,270
NSW0963	0.95	A	4.04471	hap2	16,164	12,943	319	17,557	21,887	5,156,270
NSW0939	0.56	A	4.3979	hap2	18,832	13,931	231	22,391	26,325	4,350,083
NSW0935	0.47	A	2.77763	hap1	11,526	7,646	366	13,891	22,458	4,218,391
NSW0209	0.39	A	2.39105	hap1	11,634	9,309	205	14,924	12,026	2,384,899
NSW0192	0.34	A	3.55784	hap2	18,521	13,397	211	20,835	26,583	3,907,861
NSW0120	0.26	B	3.36433	hap2	19,429	12,870	135	20,567	31,756	2,622,878
NSW0209	0.26	A	2.39105	hap2	12,415	9,818	136	15,372	13,614	1,688,383
NSW0935	0.26	A	2.77763	hap2	12,359	8,231	231	14,489	21,278	2,854,909
NSW0938	0.3	A	2.33443	hap1	12,667	8,809	235	16,530	16,705	2,976,792
NSW0110	0.21	B	2.04307	hap1	20,843	13,308	96	23,739	44,267	2,000,950
NSW0938	0.21	A	2.33443	hap2	14,441	9,365	145	18,588	19,016	2,093,878
NSW0940	0.17	A	3.20183	hap2	19,011	12,053	124	20,198	29,443	2,357,384
NSW0110	0.13	B	2.04307	hap2	25,362	13,411	59	35,391	55,638	1,496,370
NSW0936	0.13	A	1.81097	hap1	14,616	8,967	119	19,628	20,827	1,739,330
NSW0936	0.13	A	1.81097	hap2	14,289	8,855	116	19,124	20,894	1,657,519

Supplementary Table 9 Size and frequency of SVs discovered by Sniffles2

Variant type	Count	Median size	Mean size	Total bases (Mb)	Percentage (%)
Deletions	339,168	119	807.44	273.86	59.10
Insertions	229,942	231	641.01	147.39	40.07
Duplications	3,280	12,467.5	20,343.52	66.73	0.57
Inversions	1,499	3,709	16,788.48	25.17	0.26

Total	573,889	16,526.5	38,580.45	513.14	100.00
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Supplementary Table 10. Percent of different chromosomes of the reference genome covered by SVs discovered by Sniffles2

Chr	Percent of chromosome with SVs (%)
1	37.57
2	40.15
3	40.44
4	39.11
5	46.25
6	32.8
7	40.29
8	40.51
9	34.65
10	33.79
11	33.67
Overall	38.46

Supplementary Table 11. Number of SVs intersecting with different gene features

SV type	5 kb upstream	Genic	Exonic	Intronic	5 kb downstream	Intergenic
Deletion	19,705	15,039	3,546	10,789	19,612	291,341
Insertion	17,661	12,361	5,266	8,167	17,345	190,302
Duplication	617	623	568	548	596	2,353
Inversion	219	180	155	166	216	1,160
All	38,202	28,203	9,535	19,670	37,769	485,156
Percentage	6.66%	4.91%	1.66%	3.43%	6.58%	84.54%

Supplementary Table 12. SV-GWAS results for *CHILL1* and *CHILL8*

SV type	Size (bp)	Locus	Chr	Pos	GWAS -log ₁₀ (p-value)	Freq (%)	Effect (°C)	SE	% variation	Associated genes
Intercept (BioClim6)							-0.85	0.18		
Full model									84	
Insertion	485	<i>CHILL-1.1</i>	1	27,696,962	8.0	45	-1.95	0.15 ***	69	
Deletion	64	<i>CHILL-1.2</i>	1	27,698,414	9.0	43	-2.03	0.14 ***	73	
Deletion	58	<i>CHILL-1.3</i>	1	27,713,800	12.5	43	-2.06	0.13 ***	76	GPI-anchor protein, Chlorophyll a reductase
Deletion	402	<i>CHILL-1.4</i>	1	27,736,703	7.8	45	-1.96	0.15 ***	68	
Insertion	323	<i>CHILL-1.5</i>	1	27,799,070	9.2	43	-2.04	0.14 ***	74	
Insertion	1024	<i>CHILL-4.1</i>	4	25,787,765	8.2	47	-1.96	0.14 ***	71	
Deletion	123	<i>CHILL-6.1</i>	6	34,505,641	9.9	49	-1.92	0.13 ***	76	
Deletion	60	<i>CHILL-8.1</i>	8	38,494,586	7.1	10	1.25	0.26 ***	64	Phosphatase, Phosphate transporter
Deletion	52	<i>CHILL-8.2</i>	8	38,503,991	8.5	51	-1.85	0.14 ***	71	
Deletion	221	<i>CHILL-8.3</i>	8	38,507,136	9.0	51	-1.86	0.13 ***	72	

Supplementary Table 13. Characterisation of genes residing at the *CHILL8* locus.

Expression was detected by Oxford Nanopore Technologies direct RNA sequencing and reported as Transcripts Per Million (TPM).

Gene ID	Gene name	Protein length	Arabidopsis ortholog	Expression (TPM)
g34497.t1	probable inorganic phosphate transporter 1-3 [Eucalyptus grandis]	534	AT5G43350 phosphate transporter 1;1 (PHT1;1)	1,476.39 (reverse strand)
g34498.t1	serine/threonine-protein phosphatase PP1 isozyme 3 isoform X1 [Eucalyptus grandis]	324	AT2G39840 type one serine/threonine protein phosphatase 4 (TOPP4)	40.27 (forward strand)

Supplementary Table 14. Characterisation of genes residing at the *CHILL1* locus.

Expression was detected by Oxford Nanopore Technologies direct RNA sequencing and reported as Transcripts Per Million (TPM). Uncharacterised genes with no match or ortholog are labelled NA.

Gene ID	Gene name	Protein length	Arabidopsis ortholog	Expression (TPM)
g2294.t1	α -Terpineol synthase	711	Terpene Synthase 4 AT4G16740	0
g2295.t1	GPI-anchored protein	205	GPI-anchored protein At1G61900	0
g2296.t1	NA	169	NA	0
g2297.t1	7-hydroxymethyl chlorophyll a reductase	350	AT1G04620	13.42
g2298.t1	(+)- α -Terpineol synthase-like [Syzygium oleosum]	714	terpene synthase-like sequence-1,8-cineole AT3G25820	0
g2299.t1	GPI-anchored protein	212	GPI-anchored protein At1G61900	0

Supplementary Table 15. Results from linear models exploring relationships between BioClim6, geography, *CHILL1*, species and principal components (PCs) based on genome-wide and *CHILL1* SNPs.

Dependent variable	Factors in model	Variance explained by model (R ²) (%)	Factor level	P-value	Effect size	Units for effect size	Change required to achieve equivalent shift in BioClim6 from -2°C to 0°C
BioClim6	Latitude + elevation	72.5	Latitude	10 ⁻¹⁶	0.50	Latitude degrees (111km)	Translocate from latitude -36° to -32° (444km) if at 1200m
			Elevation	10 ⁻¹⁶	-0.35	100m elevation	Translocate from altitude of 1200m to 600m if at latitude of -36°
BioClim6	<i>CHILL1</i>	15.8	<i>CHILL1/CHILL1</i>	-	0 ^a	-	From <i>CHILL1/CHILL1</i> to <i>chill1/chill1</i>
			<i>CHILL1/chill1</i>	10 ⁻⁶	0.83	One allele	
			<i>chill1/chill1</i>	10 ⁻¹⁶	1.59	Two alleles	
BioClim6	Species	0.8	<i>E. dalrympleana</i>	-	0 ^a	-	-
			<i>E. rubida</i>	0.37	-0.17	-	-
			<i>E. viminalis</i>	0.36	0.164	-	-
BioClim6	Latitude + elevation + <i>CHILL1</i>	74.7	Latitude	10 ⁻¹⁶	0.59	-	-
			Elevation	10 ⁻¹⁶	-0.34	-	-
			<i>CHILL1/CHILL1</i>	-	0 ^a	-	-
			<i>CHILL1/chill1</i>	0.02	-0.24	-	-
			<i>chill1/chill1</i>	10 ⁻⁹	-0.82	-	-
BioClim6	Latitude +	73.8	Latitude	10 ⁻¹⁶	0.49	-	-

	elevation + species		Elevation	10 ⁻¹⁶	-0.38	-	-
			<i>E. dalrympleana</i>	-	0 ^a	-	-
			<i>E. rubida</i>	0.007	-0.29	-	-
			<i>E. viminalis</i>	10 ⁻⁷	-0.44	-	-
BioClim6	Latitude + elevation + species + CHILL1	75.4	Latitude	10 ⁻¹⁶	0.57	-	-
			Elevation	10 ⁻¹⁶	-0.37	-	-
			CHILL1/CHILL1	-	0 ^a	-	-
			CHILL1/chill1	0.007	-0.18	-	-
			chill1/chill1	10 ⁻⁷	-0.74	-	-
			<i>E. dalrympleana</i>	-	0 ^a	-	-
			<i>E. rubida</i>	0.006	-0.29	-	-
			<i>E. viminalis</i>	0.001	-0.32	-	-
BioClim6	Genome-wide PC1 & PC2	0.9	PC1	0.10	0.002	-	-
			PC2	0.250	0.002	-	-
BioClim6	Genome-wide PC3 & PC4	54.00	PC3	10 ⁻¹⁶	0.031	-	-
			PC4	0.07	-0.004	-	-
BioClim6	CHILL1 + PC1 & PC2	15.0	PC1	10 ⁻¹⁶	0.022	-	-
			PC2	0.79	0.003	-	-

BioClim6 <i>CHILL1</i> + PC3 & PC4	0.04		PC3	0.68	0.005	-	-
			PC4	0.99	0.0001	-	-
<i>CHILL1</i> BioClim6		^b 14.4	BioClim6	10 ⁻¹¹	0.58		Decrease in frequency of <i>CHILL1</i> allele from 0.88 to 0.69.
BioClim6 + species		24.0	BioClim6	10 ⁻¹¹	0.60		Decrease in frequency of <i>CHILL1</i> allele from 0.79 to 0.53 if <i>E. viminalis</i> .
			<i>E. dalrympleana</i>	-	0		-
			<i>E. rubida</i>	0.005	-1.19		
			<i>E. viminalis</i>	0.002	0.86		
<i>CHILL1</i> BioClim6 + Genome-wide PC1 + Genome-wide PC2		22.1	BioClim6	10 ⁻¹¹	0.60		-
			PC1	10 ⁻⁷	0.011		-
			PC2	0.60	0.0026		-
<i>CHILL1</i> Latitude + elevation		37.3	Latitude	10 ⁻¹⁷	0.68		-
			Elevation	0.08	-0.13		-
<i>CHILL1</i> BioClim6 + latitude + elevation		40.1	BioClim6	0.0007	-0.65		-
			Latitude	10 ⁻¹⁵	1.020		-

			Elevation	0.0006	-0.35	-
<i>CHILL1</i>	BioClim6 + Genome-wide PC1 + Genome-wide PC2 + Genome-wide PC3 + Genome-wide PC4	46.2	BioClim6	0.3	-0.16	
			PC1	0.001	-0.020	-
			PC2	0.50	0.006	-
			PC3	10 ⁻¹⁰	0.103	-
			PC4	10 ⁻⁷	-0.074	-

- Reference factor level against which estimates for other factor levels are compared. “-” denotes irrelevant or uninterpretable.
- McFadden’s R^2 was used for models where the dependent variable was binomial.
- Estimates of effects are on the logit scale but translated to the response scale in the table’s last column.

Supplementary Table 16. Frequencies of *CHILL1* genotypes and alleles by species and geographic population

Species	No. of genotypes	^a Population		
		South	Central	North
<i>E. viminalis</i>				
<i>CHILL1/CHILL1</i>	89	0.83	0.08	0.03
<i>CHILL1/chill1</i>	43	0.15	0.51	0.24
<i>chill1/chill1</i>	44	0.02	0.38	0.74
Total	176	102	26	38
<i>E. dalrympleana</i>				
<i>CHILL1/CHILL1</i>	102	0.89	0.92	0.09
<i>CHILL1/chill1</i>	15	0.11	0.06	0.22
<i>chill1/chill1</i>	21	0.03	0.04	0.70
Total	138	66	48	24
<i>E. rubida</i>				
<i>CHILL1/CHILL1</i>	103	0.83	0.90	0.00
<i>CHILL1/chill1</i>	16	0.15	0.11	0.00
<i>chill1/chill1</i>	1	0.01	0.00	0.00
Total	120	82	38	0
All species				
<i>CHILL1/CHILL1</i>	294	0.84	0.66	0.05
<i>CHILL1/chill1</i>	74	0.14	0.21	0.23
<i>chill1/chill1</i>	66	0.02	0.13	0.73
Total	434	250	122	62

a. South, below -35° latitude; North, greater than -30°; Central, between -35° and -30°