GENOME NOTE

The genome sequence of Limestone woundwort, *Stachys alpina* L. (Lamiales: Lamiaceae)

[version 1; peer review: 1 approved]

Maarten J. M. Christenhusz[1,2], José Ignacio Márquez-Corro[1], Ilia J. Leitch[1], Andrew G. Shaw[3], Michael F. Fay[1],
Royal Botanic Gardens Kew Genome Acquisition Lab,
Plant Genome Sizing Collective, Darwin Tree of Life Barcoding collective,
Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team,
Wellcome Sanger Institute Scientific Operations: Sequencing Operations,
Wellcome Sanger Institute Tree of Life Core Informatics team,
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

[1]Royal Botanic Gardens Kew, Richmond, Surrey, England, UK
[2]Botanical Society of Britain and Ireland, St Albans, Hertfordshire, England, UK
[3]The Rare British Plants Nursery, Builth Wells, Wales, UK

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Abstract

We present a genome assembly of *Stachys alpina* (Limestone woundwort; Streptophyta; Magnoliopsida; Lamiales; Lamiaceae). The assembly consists of two haplotypes with total lengths of 744.30 megabases and 741.28 megabases. Most of the haplotype 1 assembly (99.34%) was scaffolded into 15 chromosomal pseudomolecules. Haplotype 2 was assembled to scaffold level. The mitochondrial sequences have lengths of 77.79, 90.96, 82.64, 47.76 and 58.93 kilobases and the plastid genome assembly has a length of 150.22 kilobases. This assembly was generated as part of the Darwin Tree of Life project, which produces reference genomes for eukaryotic species found in Britain and Ireland.

Keywords

Stachys alpina, Limestone woundwort, genome sequence, chromosomal, Lamiales

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1. **Automated Benchmarking**, Wellcome Sanger Institute, Hinxton, UK

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the Tree of Life gateway.

Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

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Species taxonomy

Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Tracheophyta; Euphyllophyta; Spermatophyta; Magnoliopsida; Mesangiospermae; eudicotyledons; Gunneridae; Pentapetalae; asterids; lamiids; Lamiales; Lamiaceae; Lamioideae; Stachydeae; *Stachys*; *Stachys alpina* L. (NCBI:txid694383)

Background

Limestone woundwort (*Stachys alpina* L.) is a short-lived perennial plant with a rosette of petioled leaves at the base and stems reaching about 1.5 m with opposite, petioled leaves and whorls of pinkish-purple flowers in the leaf axils. The species is native to western, central and southern Europe and western Asia (POWO, 2026). It is a woodland edge plant, often occurring together with its relative *Stachys sylvatica*. The plant responds to soil disturbance and canopy opening, germinating from seeds in the soil seed bank. It is generally associated with moist, moderately nutrient-rich to nutrient-rich calcareous soils and does not persist in deep shade (Rich, 2022).

There has been considerable discussion about its native status in Britain. It was cultivated in England by 1597 and was first recorded in the wild in 1897 near Wotton-under-Edge, Gloucestershire (Rich, 2022). A lack of isozyme variation and the late discovery of the species in the wild supported the view that it was an introduction to the UK flora rather than a native species (Kay & John, 1995; Pearman, 2007). However, more recently Jones (2011) and Rich (2022) have presented evidence that it may well be native rather than introduced. The five or so surviving sub-populations in Denbighshire are thought to be declining, although repeated conservation plantings make the trend difficult to assess (Walker, 2023).

The reported sporophytic chromosome number is $2n = 30$ (e.g. Henniges *et al.*, 2022), with numerous counts listed in the Chromosome Counts Database (Rice *et al.*, 2015), including one report made from material collected in West Gloucestershire (see the BSBI Cytology Database). Consistent with this count, most of the haplotype 1 assembly is scaffolded into 15 chromosomal pseudomolecules, corresponding to a haploid chromosome number of $n = 15$.

This study provides a resource for comparative genomics and may help clarify the origin of the British populations of this species.

As part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland, we sequenced the genome of *S. alpina* using a specimen cultivated at The Rare British Plants Nursery from seed collected at a woodland edge near Wotton-under-Edge, Gloucestershire, England, UK (Figure 1).

Figure 1. ***S. alpina*** **(daStaAlpi1) from which samples were taken for genome sequencing.**

Methods

Sample acquisition, flow cytometry and DNA barcoding

A specimen of *S. alpina* (specimen ID KDTOL10945, ToLID daStaAlpi1; Figure 1) was used for genome sequencing. It was collected from The Rare British Plants Nursery, Builth Wells, Powys, Wales, UK (latitude 52.1419, longitude −3.456) on 2023-06-29. The specimen was collected by Maarten Christenhusz, Jose Ignacio Marquez-Corro, Ilia Leitch and Andrew Shaw and identified by Andrew Shaw. The daStaAlpi1 specimen was also used for Hi-C and RNA sequencing. The herbarium voucher for the sequenced plant (collector number MC9616) is deposited in the Herbarium, Royal Botanic Gardens, Kew (K), as specimen K001527240.

The genome size was estimated by flow cytometry following the 'one-step' method outlined in Pellicer *et al.* (2021) and using propidium iodide as the fluorochrome. The General Purpose Buffer (GPB) supplemented with 3% PVP was used for isolation of nuclei (Loureiro *et al.*, 2007), and the internal calibration standard was *Petroselinum crispum* 'Champion Moss Curled' with an assumed 1C-value of 2,200 Mb (Obermayer *et al.*, 2002).

The initial identification was verified by an additional DNA barcoding process according to the framework developed by Twyford *et al.* (2024). Part of the plant specimen was preserved in silica gel desiccant (Chase & Hills, 1991). DNA extracted from the dried plant was amplified by PCR for standard barcode markers, with the amplicons sequenced and compared to public sequence databases including GenBank and the Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007). Following whole genome sequence generation, the relevant DNA barcode region was also used alongside the initial barcoding data for sample tracking at the WSI (Twyford *et al.*, 2024). The standard operating procedures for Darwin Tree of Life barcoding are available on protocols.io.

Nucleic acid extraction

High molecular weight (HMW) DNA extraction protocols developed by the Tree of Life Core Laboratory at the Wellcome Sanger Institute (WSI) are available on protocols.io (Howard *et al.*, 2025). Tissue from the daStaAlpi1 sample was weighed and triaged to select the appropriate extraction workflow. HMW DNA was prepared for two PacBio HiFi libraries: DTOL15059345 and DTOL15186200. The tissue input, HMW DNA extraction, downstream fragmentation and clean-up, and post-shearing quality-control measurements for each PacBio library are summarised in Table 1.

The concentration of the sheared and purified DNA was assessed with a NanoDrop spectrophotometer and a Qubit Fluorometer using the Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the Femto Pulse system.

Table 1. DNA extraction and post-extraction processing for each PacBio library used for *S. alpina*.

PacBio library ID	Tissue preparation	HMW DNA extraction	Fragmentation and clean-up	Post-shearing QC
DTOL15059345	88.0 mg leaf; Bead Beating	Automated Plant Organic Extraction	Fragmentation: Diagenode Megaruptor3 for LI PacBio; clean-up: Automated SPRI	Qubit 21.2 ng/μL; fragment size 18.0 kb
DTOL15186200	59.0 mg leaf; Bead Beating	Plant MagAttract 48xrn v4	Fragmentation: Diagenode Megaruptor3 for LI PacBio; clean-up: Automated SPRI	Qubit 9.01 ng/μL; fragment size 14.9 kb; GQN 7.4

RNA was extracted from leaf tissue of daStaAlpi1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol. The RNA concentration was assessed with a NanoDrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. RNA integrity was assessed using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

PacBio HiFi library preparation and sequencing

Library preparation and sequencing took place in the WSI Scientific Operations core. Libraries DTOL15059345 and DTOL15186200 were prepared with the SMRTbell Prep Kit 3.0 (Pacific Biosciences) according to the manufacturer's instructions. The kit includes reagents for end repair/A-tailing, adapter ligation, post-ligation SMRTbell bead clean-up, and nuclease treatment. Size selection and clean-up were performed using diluted AMPure PB beads (Pacific Biosciences). DNA concentration was quantified with a Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and the Qubit 1X dsDNA HS assay kit. Final library fragment size was assessed with the Agilent Femto Pulse Automated Pulsed Field CE Instrument (Agilent Technologies) using the gDNA 55 kb BAC analysis kit.

The libraries were sequenced on a Revio instrument (Pacific Biosciences, California, USA). The prepared libraries selected for multiplexing were pooled based on genome size, library molarity, and the intended plex level. Primers were annealed and polymerases were bound to generate circularised complexes, following the manufacturer's instructions. Complexes were purified using SMRTbell beads, diluted to the Revio loading concentration, and spiked with a Revio sequencing internal control. The pooled libraries were sequenced on a Revio 25M Plex SMRT cell in a 2-plex run. SMRT Link software (Pacific Biosciences), a web-based workflow manager, was used to configure and monitor the runs and to carry out primary and secondary data analysis.

Hi-C

Sample preparation and crosslinking

Hi-C data were generated from the daStaAlpi1 leaf sample using the Arima-HiC v2 kit (Arima Genomics). Tissue was finely ground using the Covaris cryoPREP Dry Pulverizer (Covaris), and then subjected to nuclei isolation. Nuclei were isolated using a modified protocol based on the Qiagen QProteome Cell Compartment Kit (Qiagen), in which only the Lysis and CE2 buffers were used, with QIAshredder spin columns. After isolation, nuclei were fixed using formaldehyde to a final concentration of 2% to crosslink the DNA. The crosslinked DNA was then digested and biotinylated according to the manufacturer's instructions. A clean-up step was performed with SPRIselect beads before library preparation. DNA concentration was quantified using the Qubit Fluorometer v4.0 (Thermo Fisher Scientific) and the Qubit HS Assay Kit, following the manufacturer's instructions.

Hi-C library preparation and sequencing

Biotinylated DNA constructs were fragmented using a Covaris E220 sonicator and size-selected to 400–600 bp using SPRISelect beads. DNA was enriched with Arima-HiC v2 kit Enrichment beads. End repair, A-tailing, and adapter ligation were carried out with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), following a modified protocol where library preparation occurs while DNA remains bound to the Enrichment beads. Libraries were amplified with KAPA HiFi HotStart mix and a custom Unique Dual Index (UDI) barcode set (Integrated DNA Technologies). Depending on sample concentration and biotinylation percentage determined at the crosslinking stage, libraries were amplified with 10–16 PCR cycles. Post-PCR clean-up was performed with SPRISelect beads. Libraries were quantified using the AccuClear Ultra High Sensitivity dsDNA Standards Assay Kit (Biotium) and a FLUOstar Omega plate reader (BMG Labtech).

Prior to sequencing, libraries were normalised to 10 ng/μL. Normalised libraries were quantified again to create equimolar and/or weighted 2.8 nM pools. Pool concentrations were checked using the Agilent 4200 TapeStation (Agilent) with High Sensitivity D500 reagents before sequencing. Libraries were sequenced on the Illumina NovaSeq X, generating paired-end 150-bp reads.

RNA library preparation and sequencing

Libraries were prepared with the NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs), following the manufacturer's instructions. Poly(A) mRNA was isolated from 100 ng of total RNA using oligo(dT) beads, converted to cDNA, and uniquely indexed; 14 PCR cycles were performed. Libraries were prepared with an intended average fragment size of 100–300 bp. Libraries were quantified, normalised, and pooled equimolarly to a final concentration of 2.8 nM before dilution to 150 pM for loading. Sequencing was carried out on the Illumina NovaSeq X, generating paired-end reads.

Genome assembly

Prior to assembly of the PacBio HiFi reads, a database of *k*-mer counts ($k = 31$) was generated from the filtered reads using FastK. The *k*-mer frequency distribution was analysed with GenomeScope (version 2.1.0) (Ranallo-Benavidez *et al.*, 2020), providing fitted point estimates of genome size, heterozygosity and repeat content.

The HiFi reads were assembled using Hifiasm in Hi-C phasing mode (Cheng *et al.*, 2021, 2022), producing two haplotypes. Hi-C reads (Rao *et al.*, 2014) were mapped to contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). Contigs were further scaffolded with Hi-

C data in YaHS (Zhou *et al.*, 2023), using the --break option for handling potential misassemblies. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MerquryFK (Rhie *et al.*, 2020). The organelle genomes were assembled using OATK (Zhou *et al.*, 2025).

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline. The flat files and maps for curation were generated with TreeVal. Manual curation was conducted primarily in PretextView and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). The two haplotype assemblies were combined for curation. Manual corrections included 34 breaks and 63 joins, which reduced the scaffold count by 14.5%. The curation process is described at sanger-tol/curation-resources. A Hi-C contact map of the haplotype 1 assembly was generated with PretextSnapshot.

Assembly quality assessment

The MerquryFK tool (Rhie *et al.*, 2020) was run in a Singularity container (Kurtzer *et al.*, 2017) to evaluate *k*-mer completeness and assembly quality for both haplotypes using the *k*-mer database ($k = 31$) computed prior to genome assembly. The analysis outputs included assembly QV scores and completeness statistics.

The haplotype 1 assembly was assessed using BlobToolKit (Challis *et al.*, 2020). PacBio reads were mapped to the assembly to derive sequence coverage, and BUSCO (Manni *et al.*, 2021) was used to assess gene-set completeness. DIAMOND BLASTp searches of extracted BUSCO proteins and DIAMOND BLASTx searches of assembly sequences against UniProt Reference Proteomes (Buchfink *et al.*, 2021), followed by BLASTn searches of sequences without DIAMOND BLASTx hits against the NCBI nt database (Altschul *et al.*, 1990), supported taxonomic assignment. Coverage, sequence composition, taxonomic assignment and BUSCO results were integrated into a BlobDir for interactive inspection and visualisation. The snail and blob plots were rendered locally from this hosted BlobDir using BlobTk v0.8.3. The scaffold-length radial scale function for the snail plot was set to linear (Challis & Blaxter, 2026). The blob plot used circular markers with area proportional to scaffold length (Challis *et al.*, 2020).

Genome sequence report

Sequence data

The genome of a specimen of *S. alpina* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating 23.15 Gb (gigabases) from 2.38 million reads. We assembled the genome from these reads. GenomeScope analysis using a $p = 2$ model gave a *k*-mer-based haploid genome size estimate of 775.31 Mb, with heterozygosity of 0.09% and repeat content of 60.25%; the full-model fit was 94.12% (Figure 2). Using flow cytometry, the genome size (1C-value) of the sample was estimated to be 0.91 pg, equivalent to 890.00 Mb. These estimates guided expectations for the assembly. The sequencing data provided approximately 28× coverage of the haplotype 1 assembly. Hi-C sequencing produced 111.20 Gb from 368.23 million read pairs, which were used to scaffold the haplotype 1 assembly. RNA sequencing data were also generated and are available in public sequence repositories. Table 2 summarises the specimen and sequencing details.

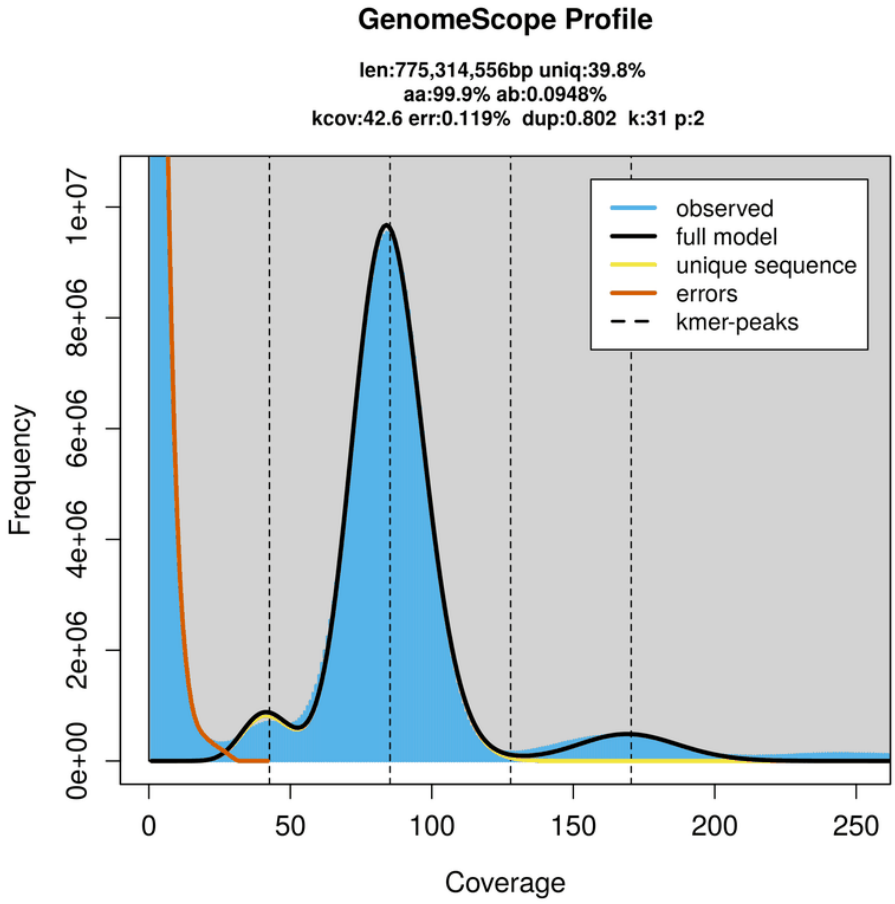

Figure 2. GenomeScope model fitted to the *k*-mer frequency distribution. The plot shows the observed and modelled *k*-mer spectra used to estimate genome size, heterozygosity and repeat content from unassembled sequencing reads.

Table 2. Specimen and sequencing data for *S. alpina* (BioProject PRJEB85036)

Platform	PacBio HiFi	Hi-C	RNA-seq
ToLID	daStaAlpi1	daStaAlpi1	daStaAlpi1
Specimen ID	KDTOL10945	KDTOL10945	KDTOL10945
BioSample (source individual)	SAMEA114564285	SAMEA114564285	SAMEA114564285
BioSample (tissue)	SAMEA114564376	SAMEA114564376	SAMEA114564376
Tissue	leaf	leaf	leaf
Instrument	Revio (2 runs)	Illumina NovaSeq X	Illumina NovaSeq X
Library ID & run accession(s)	DTOL15059345: ERR14209146; DTOL15186200: ERR14209147	ERR14224626	ERR15551497
Read count total	2.38 million reads	368.23 million read pairs	48.45 million read pairs
Base count total	23.15 Gb	111.20 Gb	14.63 Gb

Assembly statistics

The genome was assembled into two haplotypes using Hi-C phasing. The haplotype 1 assembly was curated to chromosome level, while haplotype 2 was assembled to scaffold level. The haplotype 1 assembly, which is proposed as the reference assembly for this species, has a total length of 744.30 Mb in 124 scaffolds (excluding organelle sequences), with 200 gaps, and a scaffold N50 of 49.75 Mb (Table 3). The haplotype 1 assembly has a scaffold auN of 49.7 Mb.

Table 3. Genome assembly statistics for *S. alpina*

Genome assembly	Haplotype 1	Haplotype 2
Assembly name	daStaAlpi1.hap1.1	daStaAlpi1.hap2.1
Assembly accession	GCA_965217345.1	GCA_965217315.1
Assembly level	chromosome	scaffold
Span (Mb)	744.30	741.28
Number of chromosomes	15	-
Number of contigs	324	265
Contig N50 (Mb)	6.79	7.74
Number of scaffolds (excluding organelle sequences)	124	101
Scaffold N50 (Mb)	49.75	50.05
Scaffold auN (Mb)	49.7	49.6
Longest scaffold length (Mb)	59.87	58.14
Organelles	Mitochondrial genomes: 77.79; 90.96; 82.64; 47.76; 58.93 kb; Plastid genome: 150.22 kb	-

Most of the haplotype 1 assembly sequence (99.34%) was assigned to 15 chromosome-level scaffolds. These chromosome-level scaffolds, confirmed by Hi-C data, are named according to size (Figure 3; Table 4).

Multiple mitochondrial genomes (5) and the plastid genome (length 150.22 kb, OZ244446.1) were also assembled. These sequences are included as contigs in the multifasta file of the haplotype 1 genome submission and as standalone records.

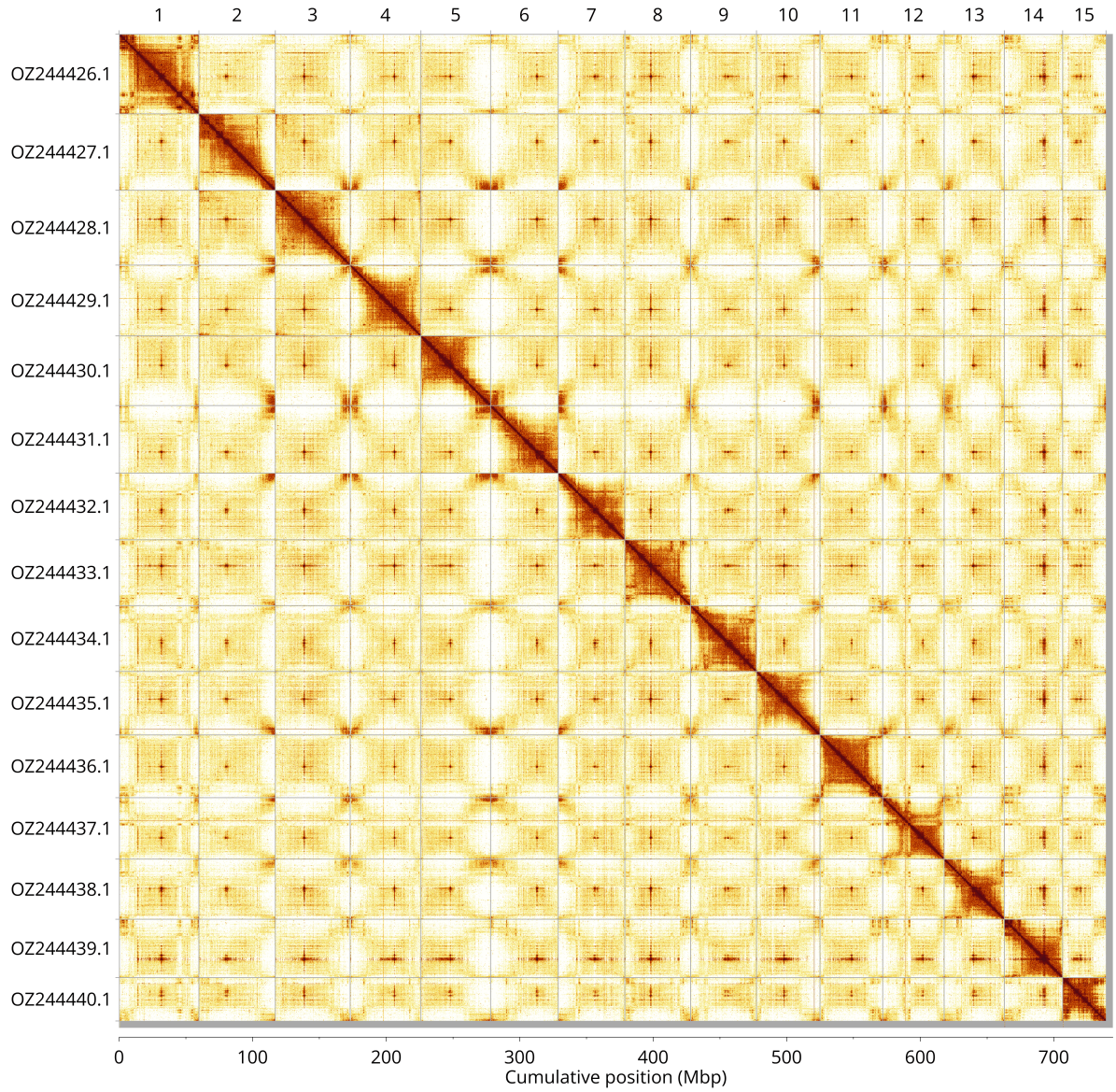

Figure 3. Hi-C contact map of the *S. alpina* haplotype 1 assembly. Assembled chromosomes are shown in order of size and labelled along the axes, with a megabase scale shown below. The plot was generated using PretextSnapshot.

Table 4. Chromosomal pseudomolecules in the haplotype 1 genome assembly of *S. alpina* daStaAlpi1

INSDC accession	Molecule	Length (Mb)	GC%
OZ244426.1	1	59.87	37.50
OZ244427.1	2	57.20	38
OZ244428.1	3	56.15	38
OZ244429.1	4	52.89	38
OZ244430.1	5	52.15	37.50
OZ244431.1	6	50.73	37
OZ244432.1	7	49.75	37.50

INSDC accession	Molecule	Length (Mb)	GC%
OZ244433.1	8	49.38	38
OZ244434.1	9	49.32	38
OZ244435.1	10	47.56	37.50
OZ244436.1	11	47	38
OZ244437.1	12	45.90	37
OZ244438.1	13	45.19	37.50
OZ244439.1	14	43.59	37.50
OZ244440.1	15	32.73	37.50

Assembly quality metrics

For the haplotype 1 assembly, the estimated QV is 68.4, and for the haplotype 2 assembly, 69.4. When the two haplotypes are combined, the assembly achieves an estimated QV of 68.9. The *k*-mer completeness is 97.03% for the haplotype 1 assembly, 97.14% for the haplotype 2 assembly, and 98.78% for the combined haplotypes (Figure 4).

BUSCO analysis using the eudicots_odb10 reference set (n = 2,326) identified 96.9% of the expected gene set (single = 92.0%, duplicated = 4.8%) in the haplotype 1 assembly. For the haplotype 2 assembly, BUSCO v.5.7.1 analysis identified 96.8% of the expected gene set (single = 92.0%, duplicated = 4.7%).

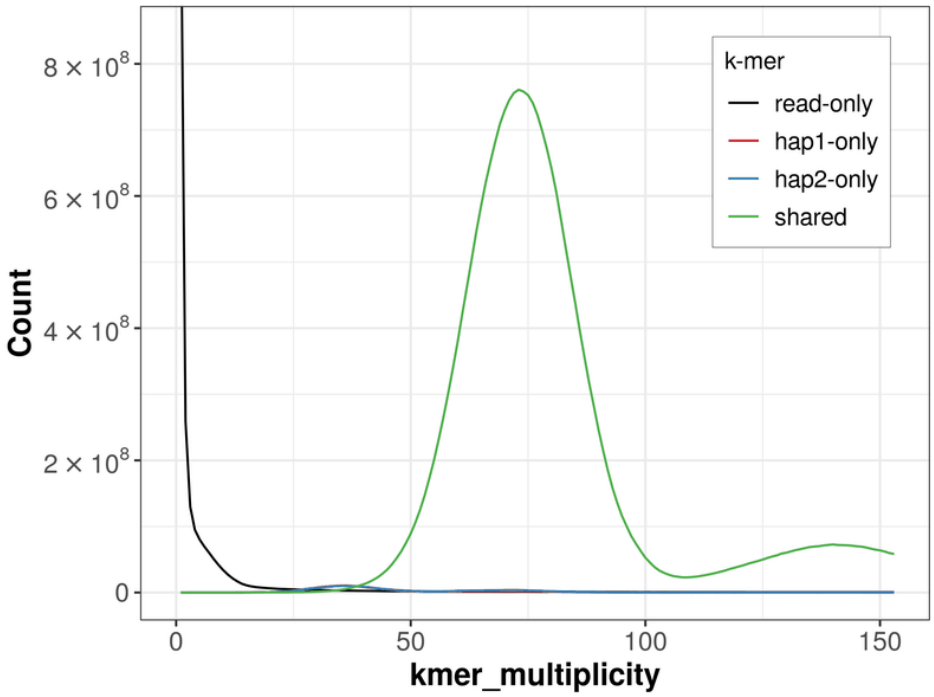

Figure 4. Evaluation of *k*-mer completeness using MerquryFK. This plot illustrates the recovery of *k*-mers from the original read data in the haplotype 1 assembly, the haplotype 2 assembly and the combined haplotypes. The horizontal axis represents *k*-mer multiplicity, and the vertical axis shows the number of *k*-mers. The black curve represents *k*-mers that appear in the reads but are not assembled, while the remaining curves correspond to *k*-mers recovered in the assemblies shown.

The snail plot in Figure 5 provides a summary of assembly contiguity, cumulative scaffold count, sequence composition and BUSCO completeness for the haplotype 1 assembly (Challis & Blaxter, 2026). The relative sizes of the longest scaffold, scaffold N50 and N90, together with the dark-grey scaffold-length distribution, show how much of the assembly is contained in long scaffolds. The outer composition track shows variation in GC, AT and N content, while the central track shows the cumulative scaffold count and the BUSCO inset summarises gene-set completeness.

The blob plot in Figure 6 displays scaffolds from the haplotype 1 assembly by GC proportion and base coverage. Marker area is proportional to scaffold length, and colour indicates taxonomic assignment (Challis *et al.*, 2020).

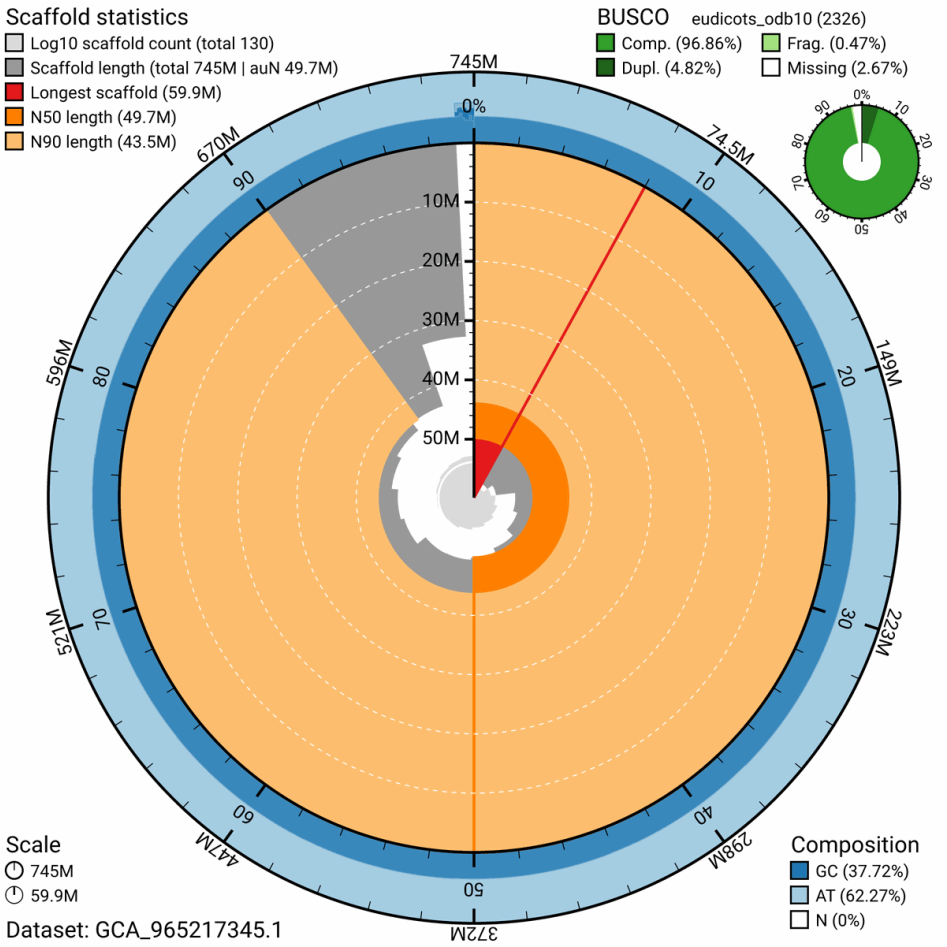

Figure 5. Assembly metrics for daStaAlpi1.hap1.1, the haplotype 1 assembly. The BlobToolKit snail plot provides an overview of assembly metrics and BUSCO gene completeness. The circumference represents the length of the haplotype 1 assembly, and the main plot is divided into 1,000 bins around the circumference. The outermost blue tracks display the distribution of GC, AT, and N percentages across the bins. Scaffolds are arranged clockwise from longest to shortest and are depicted in dark grey. Scaffold length is shown on a linear radial scale, scaled to the longest scaffold. Red shading indicates the longest scaffold, and the deeper orange and pale orange shading represent the N50 and N90 lengths. The summary statistics include scaffold auN, the area under the scaffold Nx curve. The light grey spiral at the centre shows the cumulative scaffold count on a logarithmic scale. A summary of complete, fragmented, duplicated, and missing BUSCO genes in the eudicots_odb10 set is presented at the top right. The assembly can be explored interactively in the BlobToolKit viewer.

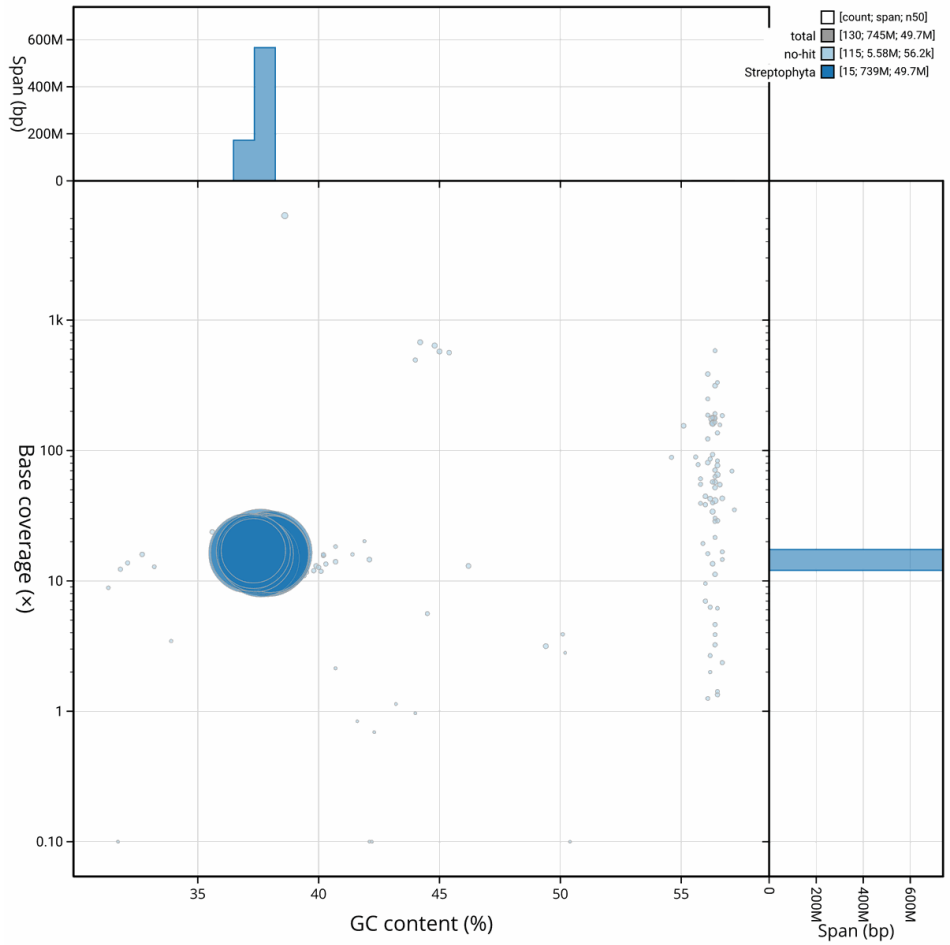

Figure 6. BlobToolKit blob plot for daStaAlpi1.hap1.1, the haplotype 1 assembly. The plot shows base coverage (vertical axis) and GC content (horizontal axis). Circles represent scaffolds; marker area is proportional to scaffold length, and colour indicates taxonomic assignment. The histograms along the axes display the total length of sequences distributed across different levels of coverage and GC content. The assembly can be explored interactively in the BlobToolKit viewer.

Table 5 lists the assembly metric benchmarks adapted from Rhie *et al.*. (2021) and the Earth BioGenome Project (EBP) Report on Assembly Standards. The three-part EBP metric calculated for the haplotype 1 assembly is **6.C.Q68**.

Table 5. Earth BioGenome Project summary metrics for the *S. alpina* assembly

Measure	Value	*Benchmark*
EBP summary (haplotype 1)	6.C.Q68	*6.C.Q40*
Contig N50 length	6.79 Mb	*> 1 Mb*
Scaffold N50 length	49.75 Mb	*= chromosome N50*
Consensus quality (QV)	Haplotype 1: 68.4; haplotype 2: 69.4; combined: 68.9	*# 40*
k-mer completeness	Haplotype 1: 97.03%; Haplotype 2: 97.14%; combined: 98.78%	*> 90%*
BUSCO	Haplotype 1: C:96.9% [S:92.0%, D:4.8%], F:0.5%, M:2.7%, n:2,326;	*S > 90%; D < 5%*

Measure	Value	*Benchmark*
	Haplotype 2: C:96.8% [S:92.0%, D:4.7%], F:0.5%, M:2.8%, n:2,326	
Percentage of assembly assigned to chromosomes	99.34%	*# 90%*

Notes: The EBP summary uses log10(Contig N50); chromosome-level (C) or log10(Scaffold N50); Q (Merqury QV). BUSCO v5.7.1 using the eudicots_odb10 lineage dataset: C=complete; S=single-copy; D=duplicated; F=fragmented; M=missing; n=orthologues.

Data availability

European Nucleotide Archive: Stachys alpina. Accession number PRJEB85036; https://identifiers.org/ena.embl/PRJEB85036. The genome sequence is released openly for reuse. The *S. alpina* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665) and the Sanger Institute Tree of Life Programme (PRJEB43745). All raw sequence data and the haplotype assemblies have been deposited in INSDC databases. The genome will be annotated using available RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 2 and Table 3.

Pipelines used for genome assembly at the WSI Tree of Life are available at https://pipelines.tol.sanger.ac.uk/pipelines. Table 6 lists software versions used in this study.

Table 6. Software versions and sources

Software	Version	Source
BLAST	2.14.0	https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
BlobToolKit	4.4.5	https://github.com/genomehubs/blobtoolkit
BlobTk (hosted BlobDir)	0.5.1	https://github.com/genomehubs/blobtk
BlobTk (snail and blob plot renderer)	0.8.3	https://github.com/genomehubs/blobtk
BUSCO	5.7.1	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
DIAMOND	2.1.8	https://github.com/bbuchfink/diamond
fasta_windows	0.2.4	https://github.com/tolkit/fasta_windows
FastK	1.2	https://github.com/thegenemyers/FASTK
GenomeScope	2.1.0	https://github.com/tbenavi1/genomescope2.0
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.19.8-r603	https://github.com/chhylp123/hifiasm
HiGlass	1.13.4	https://github.com/higlass/higlass
MerquryFK	1.1.0-c1	https://github.com/thegenemyers/MERQURY.FK
Minimap2	2.28-r1209	https://github.com/lh3/minimap2
Oatk	0.9	https://github.com/c-zhou/oatk
MultiQC	1.14; 1.17 and 1.18	https://github.com/MultiQC/MultiQC
Nextflow	24.10.4	https://github.com/nextflow-io/nextflow
PretextSnapshot	0.0.6	https://github.com/sanger-tol/PretextSnapshot

Software	Version	Source
PretextView	1.0.3	https://github.com/sanger-tol/PretextView
samtools	1.21	https://github.com/samtools/samtools
sanger-tol/ascc	0.1.0	https://github.com/sanger-tol/ascc
sanger-tol/blobtoolkit	v0.7.1	https://github.com/sanger-tol/blobtoolkit
sanger-tol/curationpretext	1.4.2	https://github.com/sanger-tol/curationpretext
Seqtk	1.4-r122	https://github.com/lh3/seqtk
Singularity	3.9.0	https://github.com/sylabs/singularity
TreeVal	1.2.1	https://github.com/sanger-tol/treeval
YaHS	1.2.2	https://github.com/c-zhou/yahs

Author information

- Members of the Royal Botanic Gardens Kew Genome Acquisition Lab
- Members of the Plant Genome Sizing Collective
- Members of the Darwin Tree of Life Barcoding collective
- Members of the Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory team
- Members of Wellcome Sanger Institute Scientific Operations – Sequencing Operations
- Members of the Wellcome Sanger Institute Tree of Life Core Informatics team
- Members of the Tree of Life Core Informatics collective
- Members of the Darwin Tree of Life Consortium

Wellcome Sanger Institute – Legal and Governance

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the **'Darwin Tree of Life Project Sampling Code of Practice'**, which can be found in full on the Darwin Tree of Life website. By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project. Further, the Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible. The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material
- Legality of collection, transfer and use (national and international)

Each transfer of samples is further undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Darwin Tree of Life Partner, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances, other Darwin Tree of Life collaborators.

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Open Peer Review

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✔ **Automated Benchmarking**
Wellcome Sanger Institute, Hinxton, UK

As this article is a Genome Note, Automated Benchmarking has been used to determine whether the reported assembly passes four defined checks established by the Earth BioGenome Project: the raw and assembled data are publicly available, the Earth BioGenome Project three-part metric is met, the k-mer completeness exceeds 90%, and the BUSCO score passes the stated threshold.

For Stachys alpina (daStaAlpi1), all four checks passed.

Data available: pass. ENA study PRJEB85036, raw-read accessions ERR14209146, ERR14209147, ERR14224626, and ERR15551497, and assembly accessions GCA_965217345.1 and GCA_965217315.1 are public.

Earth BioGenome Project three-part metric: pass. Haplotype 1 is reported as 6.C.Q68, meeting the 6.C.Q40 reference standard.

k-mer completeness: pass. The combined haplotype assemblies have 98.78% k-mer completeness, exceeding the Earth BioGenome Project target of 90%.

BUSCO: pass. Haplotype 1, which is proposed as the reference assembly for this species, has 96.9% complete BUSCOs (92.0% single-copy and 4.8% duplicated). BUSCO software version: 5.7.1; lineage dataset: eudicots_odb10. It meets the plant-specific target of greater than 90% complete BUSCOs.

This decision uses values reported in the Genome Note and public accession records.

Competing Interests: This Automated Benchmarking peer-review report was generated automatically and relies on analyses performed by the Tree of Life programme, which also

produced the Genome Note under review.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.