DATA NOTE

The genome sequence of the Red Twin-spot Carpet, *Xanthorhoe spadicearia* (Denis & Schiffermüller, 1775) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Xanthorhoe spadicearia* (the Red Twin-spot Carpet; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 276.7 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 17.03 kilobases in length. Gene annotation of this assembly on Ensembl identified 16,396 protein coding genes.

Keywords

Xanthorhoe spadicearia, Red Twin-spot Carpet, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

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

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Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Larentiinae; *Xanthorhoe*; *Xanthorhoe spadicearia* (Denis & Schiffermüller, 1775) (NCBI:txid934904).

Background

The Red Twin-spot Carpet (*Xanthorhoe spadicearia*) is a geometrid moth that occurs in a wide variety of habitats across Britain. In Ireland it is more scarce, and records are more widely scattered (GBIF Secretariat, 2023). Its caterpillars feed on a wide variety of herbaceous plants (Henwood *et al.*, 2020). There are two generations each year, and adult moths from both generations are now recorded earlier in the year than they were in the 1970s (Randle *et al.*, 2019; Waring *et al.*, 2017).

The genome of the Red Twin-spot Carpet, *Xanthorhoe spadicearia*, was sequenced 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. Here we present a chromosomally complete genome sequence for *Xanthorhoe spadicearia*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one female *Xanthorhoe spadicearia* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 62-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 3 missing joins or mis-joins and removed one haplotypic duplication.

Figure 1. Photograph of the *Xanthorhoe spadicearia* (ilXanSpad1) specimen used for genome sequencing.

The final assembly has a total length of 276.7 Mb in 32 sequence scaffolds with a scaffold N50 of 10.3 Mb (Table 1). Most (99.99%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. A summary of the assembly statistics is shown in Figure 2, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). There is half-coverage of the Z chromosome, and the species appears to be a ZO female. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited. The mitochondrial genome was also assembled and can be found as a contig within the multifasta file of the genome submission.

The estimated Quality Value (QV) of the final assembly is 71.1 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.4% (single = 98.1%, duplicated = 0.3%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, spectral estimates, sequencing runs, contaminants and pre-curation assembly statistics can be found at https://links.tol.sanger.ac.uk/species/934904.

Genome annotation report

The *Xanthorhoe spadicearia* genome assembly (GCA_947086425.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Xanthorhoe_spadicearia_GCA_947086425.1/Info/Index). The resulting annotation includes 16,623 transcribed mRNAs from 16,396 protein-coding genes.

Methods

Sample acquisition and nucleic acid extraction

A female *Xanthorhoe spadicearia* (specimen ID Ox001823, ToLID ilXanSpad1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-07-24 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford).

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) includes a sequence of core procedures: sample preparation; sample homogenisation; DNA extraction; DNA fragmentation; and fragmented DNA clean-up. The sample was prepared for DNA extraction at the WSI Tree of Life laboratory: the ilXanSpad1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing (Jay *et al.*, 2023). Tissue from the head and thorax was homogenised using a PowerMasher

Table 1. Genome data for *Xanthorhoe spadicearia*, ilXanSpad1.1.

Project accession data		
Assembly identifier	ilXanSpad1.1	
Species	*Xanthorhoe spadicearia*	
Specimen	ilXanSpad1	
NCBI taxonomy ID	934904	
BioProject	PRJEB55721	
BioSample ID	SAMEA10979081	
Isolate information	ilXanSpad1, female: head and thorax (DNA and Hi-C sequencing), abdomen (RNA sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	71.1	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.4%[S:98.1%,D:0.3%], F:0.5%,M:1.1%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.99%	*≥ 95%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10168715	
Hi-C Illumina	ERR10149545	
PolyA RNA-Seq Illumina	ERR11641098	
Genome assembly		
Assembly accession	GCA_947086425.1	
Accession of alternate haplotype	GCA_947086435.1	
Span (Mb)	276.7	
Number of contigs	51	
Contig N50 length (Mb)	7.6	
Number of scaffolds	32	
Scaffold N50 length (Mb)	10.3	
Longest scaffold (Mb)	14.5	
Genome annotation		
Number of protein-coding genes	16,396	
Number of gene transcripts	16,623	

* Assembly metric benchmarks are adapted from column VGP-2020 of "Table 1: Proposed standards and metrics for defining genome assembly quality" from (Rhie *et al.*, 2021).

** BUSCO scores based on the lepidoptera_odb10 BUSCO set using v5.3.2. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/ilXanSpad1.1/dataset/CAMTYT01.1/busco.

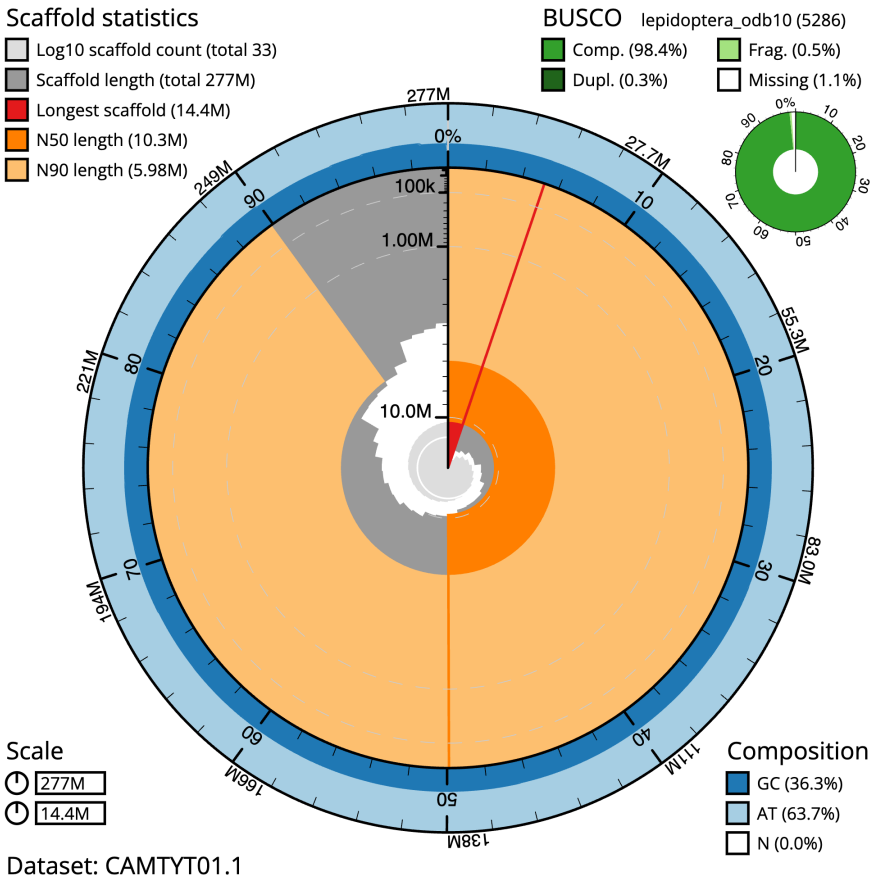

Figure 2. Genome assembly of *Xanthorhoe spadicearia*, ilXanSpad1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 276,691,867 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (14,446,717 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (10,252,953 and 5,980,574 bp), respectively. The pale grey spiral shows the cumulative scaffold count on a log scale with white scale lines showing successive orders of magnitude. The blue and pale-blue area around the outside of the plot shows the distribution of GC, AT and N percentages in the same bins as the inner plot. A summary of complete, fragmented, duplicated and missing BUSCO genes in the lepidoptera_odb10 set is shown in the top right, An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilXanSpad1.1/dataset/CAMTYT01.1/snail.

II tissue disruptor (Denton *et al.*, 2023). HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 31 (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of AMPure PB beads to sample to eliminate shorter fragments and concentrate the DNA. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from abdomen tissue of ilXanSpad1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ *mir*Vana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Protocols developed in the Tree of Life laboratory are available on protocols.io (https://dx.doi.org/10.17504/protocols.io.8epv5xxy6g1b/v1).

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations core

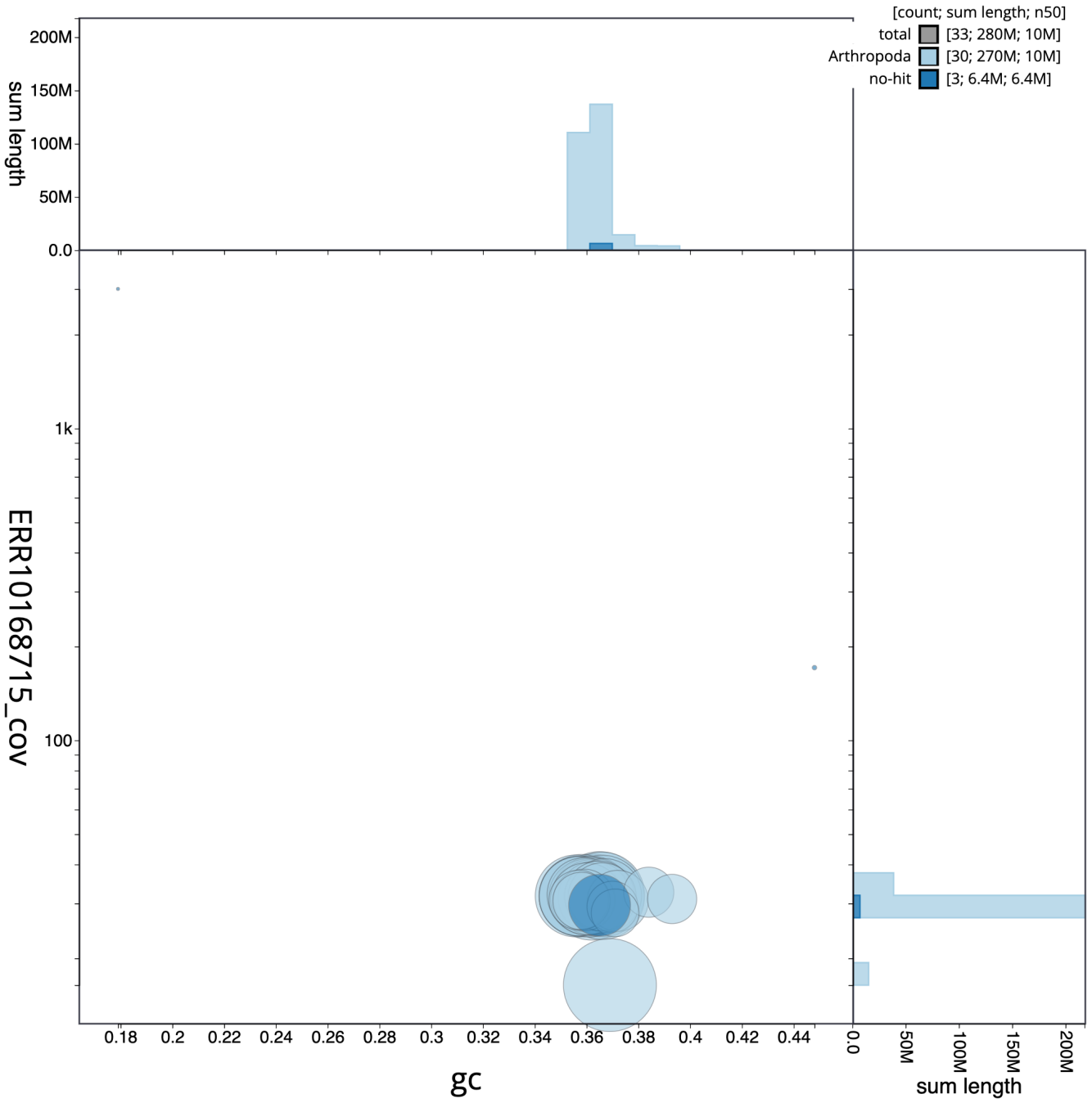

Figure 3. Genome assembly of *Xanthorhoe spadicearia*, ilXanSpad1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilXanSpad1.1/dataset/CAMTYT01.1/blob.

at the WSI on Pacific Biosciences SEQUEL II (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from remaining head and thorax tissue of ilXanSpad1 using the Arima2 kit and sequenced on the Illumina NovaSeq 6000 instrument.

Genome assembly, curation and evaluation

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021) and haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using YaHS

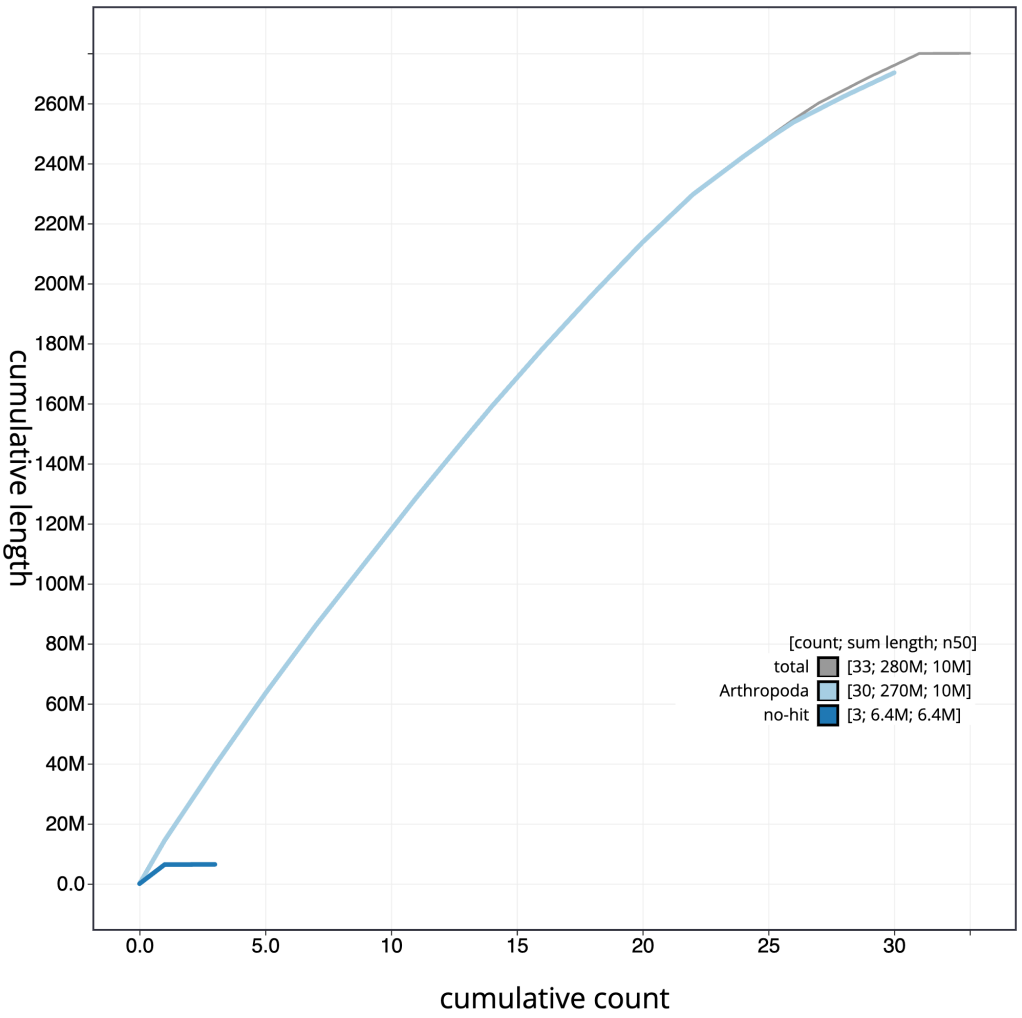

Figure 4. Genome assembly of *Xanthorhoe spadicearia*, ilXanSpad1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilXanSpad1.1/dataset/CAMTYT01.1/cumulative.

(Zhou *et al.*, 2023). The assembly was checked for contamination and corrected as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) or MITOS (Bernt *et al.*, 2013) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

A Hi-C map for the final assembly was produced using bwa-mem2 (Vasimuddin *et al.*, 2019) in the Cooler file format (Abdennur & Mirny, 2020). To assess the assembly metrics, the *k*-mer completeness and QV consensus quality values were calculated in Merqury (Rhie *et al.*, 2020). This work was done using Nextflow (Di Tommaso *et al.*, 2017) DSL2 pipelines "sanger-tol/readmapping" (Surana *et al.*, 2023a) and "sanger-tol/genomenote" (Surana *et al.*, 2023b). The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were calculated.

Table 3 contains a list of relevant software tool versions and sources.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Xanthorhoe spadicearia* assembly (GCA_947086425.1) in Ensembl Rapid Release.

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**

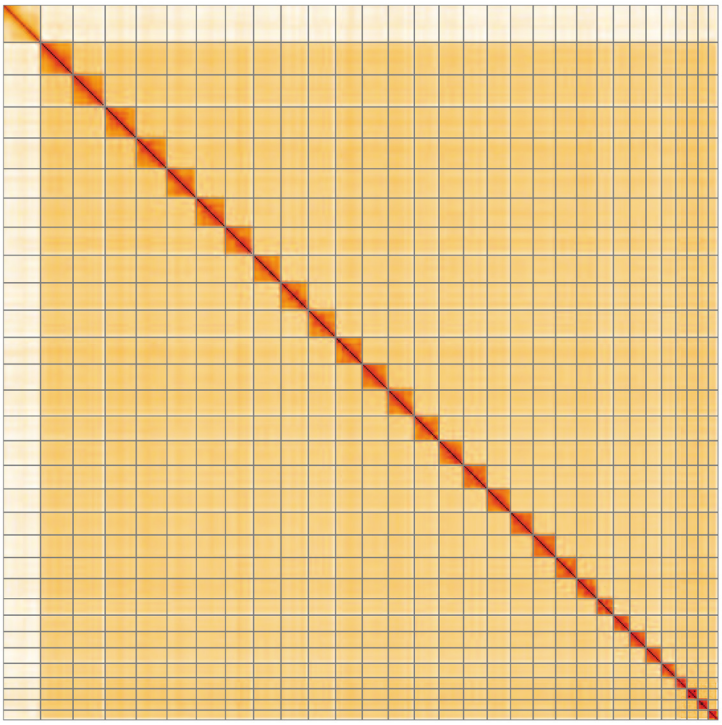

Figure 5. Genome assembly of *Xanthorhoe spadicearia*, ilXanSpad1.1: Hi-C contact map of the ilXanSpad1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=ZFpT9HYSTwajfMQ9VHRHkg.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Xanthorhoe spadicearia*, ilXanSpad1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX352195.1	1	12.64	36.5
OX352196.1	2	12.38	36.0
OX352197.1	3	11.99	36.5
OX352198.1	4	11.93	36.5
OX352199.1	5	11.37	36.0
OX352200.1	6	11.26	35.5
OX352201.1	7	10.84	36.0
OX352202.1	8	10.63	35.5
OX352203.1	9	10.61	35.5
OX352204.1	10	10.55	35.5
OX352205.1	11	10.25	36.0
OX352206.1	12	10.11	36.5
OX352207.1	13	10.02	36.0
OX352208.1	14	9.59	36.0
OX352209.1	15	9.47	36.0
OX352210.1	16	9.14	36.0
OX352211.1	17	9.07	36.5
OX352212.1	18	8.78	36.5
OX352213.1	19	8.71	36.0
OX352214.1	20	8.16	36.5
OX352215.1	21	7.76	36.5
OX352216.1	22	6.39	36.5
OX352217.1	23	6.35	36.0
OX352218.1	24	6.24	37.0
OX352219.1	25	5.98	35.5
OX352220.1	26	5.52	36.0
OX352221.1	27	4.37	37.0
OX352222.1	28	4.21	38.5
OX352223.1	29	4.07	39.5
OX352224.1	30	3.83	37.0
OX352194.1	Z	14.45	37.0
OX352225.1	MT	0.02	18.5

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.0.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
sanger-tol/genomenote	v1.0	https://github.com/sanger-tol/genomenote
sanger-tol/readmapping	1.1.0	https://github.com/sanger-tol/readmapping/tree/1.1.0
YaHS	yahs-1.1.91eebc2	https://github.com/c-zhou/yahs

Practice', which can be found in full on the Darwin Tree of Life website here. 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.

Data availability

European Nucleotide Archive: *Xanthorhoe spadicearia* (red twin-spot carpet). Accession number PRJEB55721; https://identifiers.org/ena.embl/PRJEB55721 (Wellcome Sanger Institute, 2022).

The genome sequence is released openly for reuse. The *Xanthorhoe spadicearia* genome sequencing initiative is part of the Darwin Tree of Life (DToL) project. All raw sequence data and the assembly have been deposited in INSDC databases. Raw data and assembly accession identifiers are reported in Table 1.

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Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013541.

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