DATA NOTE

The genome sequence of the Marbled Piercer, *Cydia splendana* (Hübner, 1799) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual female *Cydia splendana* (the Marbled Piercer; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence is 630.6 megabases in span. Most of the assembly is scaffolded into 28 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 16.67 kilobases in length. Gene annotation of this assembly on Ensembl identified 16,691 protein coding genes.

Keywords

Cydia splendana, Marbled Piercer, 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; Apoditrysia; Tortricoidea; Tortricidae; Olethreutinae; Grapholitini; *Cydia; Cydia splendana* (Hübner, 1799) (NCBI:txid1100963).

Background

The Marbled Piercer *Cydia splendana* (Hübner, 1799) is a moth of the Tortricidae family. The species is found across Europe, east to Russia, northern Iran, and Asia minor, and has also been recorded in the Azores and from Madeira (Bradley *et al.*, 1979; GBIF Secretariat, 2022). The species is widespread across Britain and Ireland, though appears to be scarcer in Ireland (Bradley *et al.*, 1979; Elliott *et al.*, 2018). Adult moths of this species show limited variation in forewing markings, typically exhibiting a marbled grey ground-colour, however a dark brown form also occurs (Bradley *et al.*, 1979).

The larva feeds from August to mid-October on acorns (*Quercus*), chestnuts (*Castanea*), and rarely walnuts (*Juglans*). Once full-fed the larva constructs a cocoon in the soil and overwinters within. Pupation occurs in June, and adults occur between June and August, flying from dusk and coming to light (Bradley *et al.*, 1979; Elliott *et al.*, 2018). The female lays 150 to 200 eggs on leaves near the fruit of the foodplant, which hatch in 10 to 12 days. The species can be common along margins and rides in woodland with oak and chestnut trees (Bradley *et al.*, 1979).

The genome of *Cydia splendana* 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 *Cydia splendana*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one female *Cydia splendana* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, –1.34). A total of 36-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 66-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 106 missing joins or mis-joins and removed one haplotypic duplication, reducing the scaffold number by 34.5% and increasing the scaffold N50 by 14.77%.

The final assembly has a total length of 630.6 Mb in 112 sequence scaffolds with a scaffold N50 of 22.1 Mb (Table 1). Most (94.81%) of the assembly sequence was assigned to 28 chromosomal-level scaffolds, representing 27 autosomes and the Z sex chromosome. Half-coverage of the Z chromosome reads indicated that this is a female specimen.

Figure 1. Photograph of the *Cydia splendana* (ilCydSple1) specimen used for genome sequencing.

Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 2–Figure 5; Table 2). 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 57.2 with *k*-mer completeness of 99.99%, and the assembly has a BUSCO v5.3.2 completeness of 97.7% (single = 97.2%, duplicated = 0.6%), 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/1100963.

Genome annotation report

The *Cydia splendana* genome assembly (GCA_910591565.2) was annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Cydia_splendana_GCA_910591565.1/Info/Index). The resulting annotation includes 35,924 transcribed mRNAs from 16,691 protein-coding and 6,602 non-coding genes.

Methods

Sample acquisition and nucleic acid extraction

Two *Cydia splendana* specimens (ilCydSple1, ilCydSple2) were collected in Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2020-07-20. The specimens were taken from woodland habitat using a light trap. The specimens were collected and identified by Douglas Boyes (University of Oxford) and were snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilCydSple1 sample was weighed and dissected on dry ice. Whole organism tissue was disrupted

Table 1. Genome data for *Cydia splendana*, ilCydSple1.2.

Project accession data		
Assembly identifier	ilCydSple1.2	
Species	*Cydia splendana*	
Specimen	ilCydSple1	
NCBI taxonomy ID	1100963	
BioProject	PRJEB45179	
BioSample ID	SAMEA7701547	
Isolate information	ilCydSple1, whole organism (DNA sequencing) ilCydSple2, whole organism (Hi-C scaffolding and RNA sequencing)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	57.2	*≥ 50*
k-mer completeness	99.99%	*≥ 95%*
BUSCO**	C:97.7%[S:97.2%,D:0.6%], F:0.6%,M:1.7%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	94.81%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR6454730	
10X Genomics Illumina	ERR6054876–ERR6054879	
Hi-C Illumina	ERR6054875	
PolyA RNA-Seq Illumina	ERR10123646	
Genome assembly		
Assembly accession	GCA_910591565.2	
Accession of alternate haplotype	GCA_910591525.1	
Span (Mb)	630.6	
Number of contigs	262	
Contig N50 length (Mb)	6.5	
Number of scaffolds	112	
Scaffold N50 length (Mb)	22.1	
Longest scaffold (Mb)	49.8	
Genome annotation		
Number of protein-coding genes	16,691	
Number of non-coding genes	6,602	
Number of gene transcripts	35,924	

* 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/ilCydSple1.2/dataset/CAJUYE02/busco.

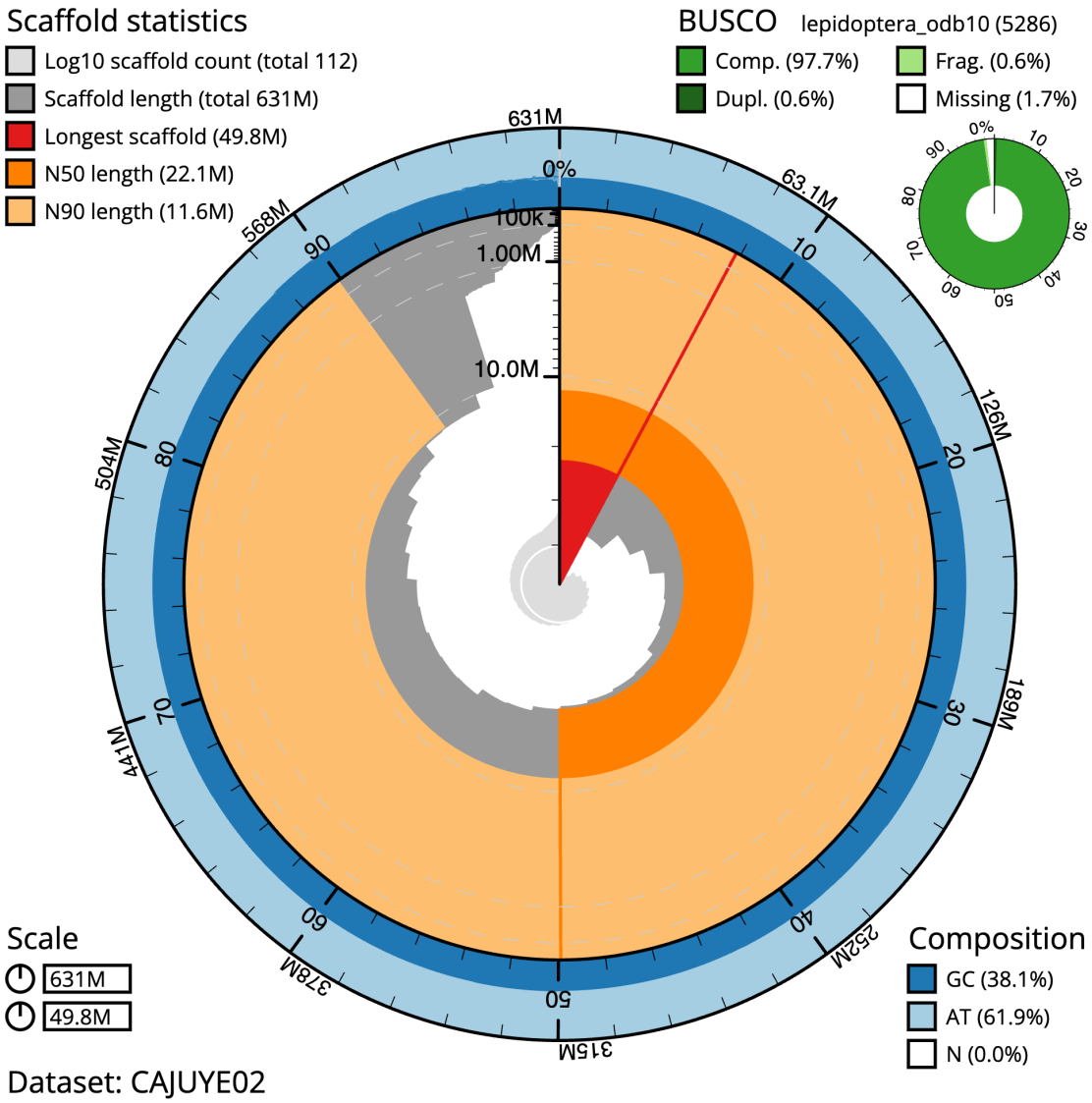

Figure 2. Genome assembly of *Cydia splendana*, ilCydSple1.2: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 630,618,321 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (49,817,474 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (22,055,301 and 11,648,489 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/ilCydSple1.2/dataset/CAJUYE02/snail.

using a Nippi Powermasher fitted with a BioMasher pestle. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from a 20 ng aliquot of extracted DNA using the 0.8X AMpure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the DNA sample. 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 whole tissue of ilCydSple2 in the Tree of Life Laboratory at the WSI using TRIzol, according to the manufacturer's instructions. RNA was eluted in 50 μl RNAse-free water and its concentration assessed using a

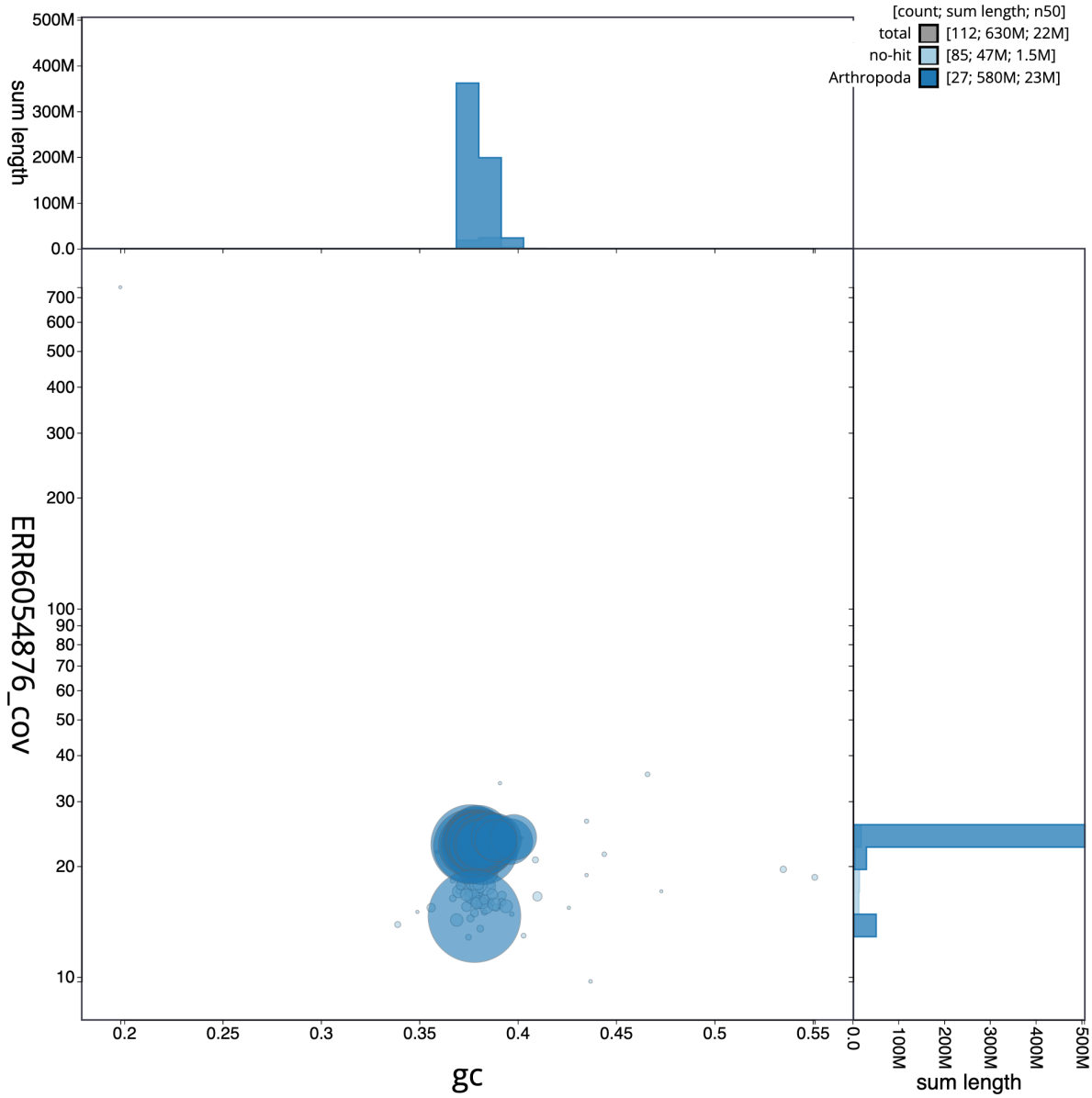

Figure 3. Genome assembly of *Cydia splendana*, ilCydSple1.2: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilCydSple1.2/dataset/CAJUYE02/blob.

Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud 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 at the WSI on Pacific Biosciences SEQUEL II (HiFi), Illumina NovaSeq 6000 (RNA-Seq and 10X) instruments. Hi-C data were also generated from whole organism tissue of ilCydSple2 using the Arimav2 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). One round of polishing

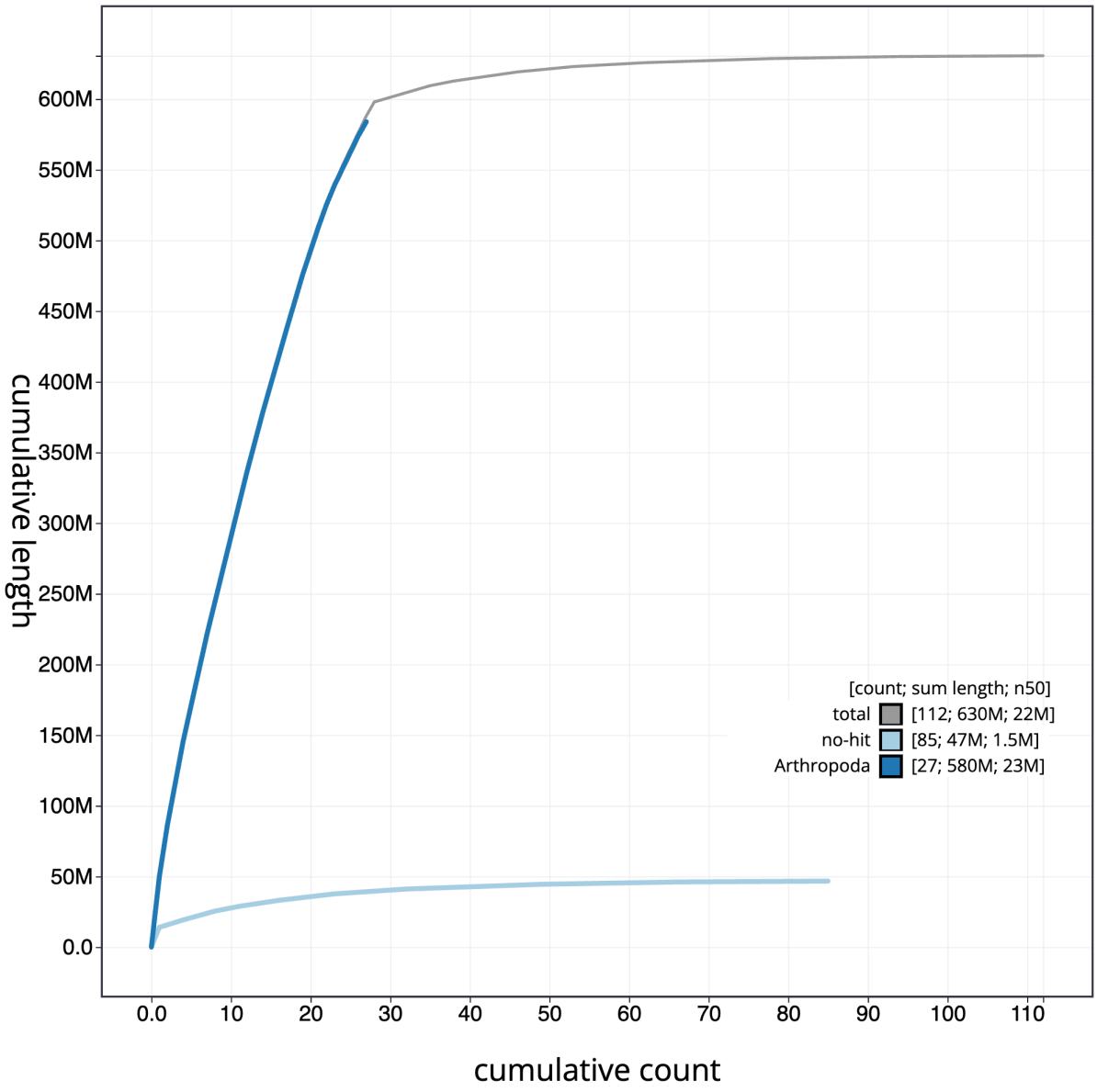

Figure 4. Genome assembly of *Cydia splendana*, ilCydSple1.2: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilCydSple1.2/dataset/CAJUYE02/cumulative.

was performed by aligning 10X Genomics read data to the assembly with Long Ranger ALIGN, calling variants with FreeBayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using SALSA2 (Ghurye *et al.*, 2019). The assembly was checked for contamination and corrected using the gEVAL system (Chow *et al.*, 2016) as described previously (Howe *et al.*, 2021). Manual curation was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2022), 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

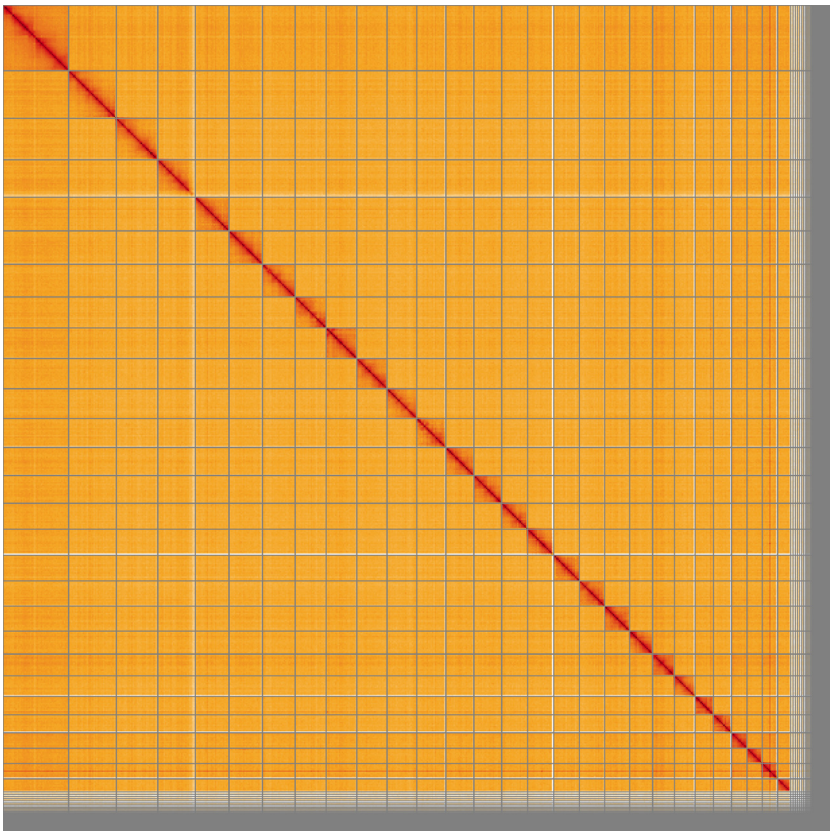

Figure 5. Genome assembly of *Cydia splendana*, ilCydSple1.2: Hi-C contact map of the ilCydSple1.2 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=fJgVs2sBTwi1LlDUxjUcjg.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Cydia splendana*, ilCydSple1.

INSDC accession	Chromosome	Length (Mb)	GC%
OU342872.1	1	36.11	37.5
OU342873.1	2	31.49	37.5
OU342874.1	3	28.51	38.0
OU342875.1	4	25.63	38.0
OU342876.1	5	25.31	38.0
OU342877.1	6	24.83	38.0
OU342878.1	7	23.52	37.5
OU342879.1	8	23.32	38.0
OU342880.1	9	22.98	38.0
OU342881.1	10	22.56	37.5
OU342882.1	11	22.06	38.5
OU342883.1	12	21.27	38.0
OU342884.1	13	21.09	38.0
OU342885.1	14	19.83	38.0
OU342886.1	15	19.69	38.0
OU342887.1	16	19.55	38.0
OU342888.1	17	19.2	38.0
OU342889.1	18	18.99	38.5
OU342890.1	19	17.39	38.5
OU342891.1	20	16.6	39.0
OU342892.1	21	15.66	38.0
OU342893.1	22	14.0	38.5
OU342894.1	23	13.6	38.0
OU342895.1	24	11.77	39.5
OU342896.1	25	11.65	39.0
OU342897.1	26	11.63	40.0
OU342898.1	27	9.89	39.0
OU342871.1	Z	49.82	38.0
OU342899.2	MT	0.02	20.0

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 Ensembl gene annotation system (Aken *et al.*, 2016) was used to generate annotation for the *Cydia splendana* assembly (GCA_910591565.2). Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

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 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.

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
FreeBayes	1.3.1-17-gaa2ace8	https://github.com/freebayes/freebayes
gEVAL	N/A	https://geval.org.uk/
Hifiasm	0.15.1-r329	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Long Ranger ALIGN	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
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
SALSA	2.2	https://github.com/salsa-rs/salsa
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

Data availability

European Nucleotide Archive: *Cydia splendana* (marbled piercer). Accession number PRJEB45179; https://identifiers.org/ena.embl/PRJEB45179. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Cydia splendana* 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.

Author information

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.4789928.

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Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/zenodo.4783585.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.4790455.

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

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.4783558.

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