DATA NOTE

The genome sequence of the Plain Wave moth, *Idaea straminata* (Borkhausen, 1794) [version 1; peer review: awaiting peer review]

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Abstract

We present a genome assembly from an individual male *Idaea straminata* (the Plain Wave; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence spans 411.30 megabases. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 17.15 kilobases in length. Gene annotation of this assembly on Ensembl identified 17,493 protein-coding genes.

Keywords

Idaea straminata, Plain Wave moth, 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; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Sterrhinae; *Idaea; Idaea straminata* (Borkhausen, 1794) (NCBI:txid104449).

Background

Idaea straminata, the Plain Wave, is a moth of the family Geometridae (Figure 1). The genus Idaea takes its name from Mount Ida in Crete, a sacred site in Greek mythology, while the common name "Wave" refers to the characteristic wing markings (Marren, 2019). This species requires careful identification, as it closely resembles *Idaea aversata*, the Riband Wave.

I. straminata is found throughout Europe including West Russia and Balkans, with scattered records into Asia (GBIF Secretariat, 2024). It is widely distributed in the UK but is not numerous (Kimber, 2024).

It is a univoltine species, with the larval stage from August to May, overwintering in larval form (Waring *et al.*, 2017). Like many of its close relatives, the larvae feed on dandelion (*Taraxacum)* and knotgrass (*Polygonum*) and also sallow (*Salix spp.*). Pupation occurs in plant debris, and adults are active from late June to early August (Waring *et al.*, 2017).

The genome of the plain wave, *Idaea straminata*, was sequenced as part of the Darwin Tree of Life Project, which aims to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *Idaea straminata*, based on a male specimen from Glen Strathfarrar in the Scottish Highlands.

Genome sequence report

The genome of an adult *Idaea straminata* was sequenced using Pacific Biosciences single-molecule HiFi long reads, generating a total of 27.36 Gb (gigabases) from 2.28 million reads, providing approximately 64-fold coverage. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data, which produced 104.64 Gb from 692.98 million reads, yielding an approximate coverage of 254-fold. Specimen and sequencing information is summarised in Table 1.

Figure 1. Photograph of *Idaea straminata* by Patrick Clement (not the specimen used for genome sequencing).

Manual assembly curation corrected two missing joins or mis-joins. The final assembly has a total length of 411.30 Mb in 34 sequence scaffolds with a scaffold N50 of 14.4 Mb (Table 2). The total count of gaps in the scaffolds is 33. The snail plot in Figure 2 provides a summary of the assembly statistics, 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. Most (99.96%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 3). Chromosome Z was assigned by synteny to the genome of *Idaea aversata* (GCA_907269075.1) (Boyes *et al.*, 2023). 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 67.4 with *k*-mer completeness of 100.0%, and the assembly has a BUSCO v5.3.2 completeness of 98.5% (single = 98.2%, duplicated = 0.3%), using the lepidoptera_odb10 reference set (n = 5,286).

Metadata for specimens, BOLD barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/104449.

Genome annotation report

The *Idaea straminata* genome assembly (GCA_951213275.1) was annotated at the European Bioinformatics Institute (EBI) on Ensembl Rapid Release. The resulting annotation includes 17,659 transcribed mRNAs from 17,493 protein-coding genes (Table 2; https://rapid.ensembl.org/Idaea_straminata_GCA_951213275.1/Info/Index). The average transcript length is 6,516.01. There are 5.73 exons per transcript.

Methods

Sample acquisition

An *Idaea straminata* (specimen ID SAN00002585, ToLID ilIdaStra1) was collected from Glen Strathfarrar, Scotland, UK (latitude 57.41, longitude –4.73) on 2022-06-27, using a moth trap. The specimen was collected and identified by Marc Botham (UK Centre for Ecology & Hydrology), and then preserved by flash freezing.

Table 1. **Specimen and sequencing data for *Idaea straminata*.**

Project information			
Study title	*Idaea straminata* (plain wave)		
Umbrella BioProject	PRJEB61363		
Species	*Idaea straminata*		
BioSample	SAMEA112198465		
NCBI taxonomy ID	104449		
Specimen information			
Technology	**ToLID**	**BioSample accession**	**Organism part**
PacBio long read sequencing	ilIdaStra1	SAMEA112198493	thorax
Hi-C sequencing	ilIdaStra1	SAMEA112198493	thorax
RNA sequencing	ilIdaStra1	SAMEA112198494	abdomen
Sequencing information			
Platform	**Run accession**	**Read count**	**Base count (Gb)**
Hi-C Illumina NovaSeq 6000	ERR11242565	6.93e+08	104.64
PacBio Sequel IIe	ERR11242141	2.28e+06	27.36
RNA Illumina NovaSeq 6000	ERR12245560	7.31e+07	11.03

Nucleic acid extraction

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) Tree of Life Core Laboratory includes a sequence of core procedures: sample preparation and homogenisation, DNA extraction, fragmentation and purification. Detailed protocols are available on protocols.io (Denton *et al.*, 2023b). The ilIdaStra1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023), and tissue from the thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a).

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 (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation, using AMPure PB beads to eliminate shorter fragments and concentrate the DNA (Strickland *et al.*, 2023). The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the 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 ilIdaStra1 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.

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 at the WSI on Pacific Biosciences Sequel IIe (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments.

Hi-C data were generated from frozen thorax tissue of ilIdaStra1, using the Arima-HiC v2 kit. The tissue was fixed with a TC buffer containing formaldehyde, resulting in crosslinked DNA. The crosslinked DNA was digested with a restriction enzyme master mix. The resulting 5'-overhangs were filled in and labelled with a biotinylated nucleotide. The biotinylated DNA was then fragmented, enriched, barcoded, and amplified using the NEBNext Ultra II DNA Library Prep Kit. Hi-C sequencing was performed on an Illumina NovaSeq 6000 instrument, using paired-end sequencing with a read length of 150 bp.

Genome assembly, curation and evaluation

Assembly

The HiFi reads were first assembled using Hifiasm (Cheng *et al.*, 2021) with the --primary option. Haplotypic duplications

Table 2. Genome assembly data for *Idaea straminata*, ilIdaStra1.1.

Genome assembly		
Assembly name	ilIdaStra1.1	
Assembly accession	GCA_951213275.1	
Accession of alternate haplotype	*GCA_951215995.1*	
Span (Mb)	411.30	
Number of contigs	68	
Contig N50 length (Mb)	9.8	
Number of scaffolds	34	
Scaffold N50 length (Mb)	14.4	
Longest scaffold (Mb)	20.47	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	67.4	*≥ 50*
k-mer completeness	100.0%	*≥ 95%*
BUSCO**	C:98.5%[S:98.2%,D:0.3%], F:0.3%,M:1.1%,n:5286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.96%	*≥ 95%*
Sex chromosomes	Z	*localised homologous pairs*
Organelles	Mitochondrial genome: 17.15 kb	*complete single alleles*
Genome annotation of assembly GCA_951213275.1 at Ensembl		
Number of protein-coding genes	17,493	
Number of gene transcripts	17,659	

* 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 version 5.3.2. 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/ilIdaStra1_1/dataset/ilIdaStra1_1/busco.

were identified and removed using purge_dups (Guan *et al.*, 2020). The Hi-C reads were mapped to the primary contigs using bwa-mem2 (Vasimuddin *et al.*, 2019). The contigs were further scaffolded using the provided Hi-C data (Rao *et al.*, 2014) in YaHS (Zhou *et al.*, 2023) using the --break option. The scaffolded assemblies were evaluated using Gfastats (Formenti *et al.*, 2022), BUSCO (Manni *et al.*, 2021) and MERQURY.FK (Rhie *et al.*, 2020).

The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) and uses these annotations to select the final mitochondrial contig and to ensure the general quality of the sequence.

Assembly curation

The assembly was decontaminated using the Assembly Screen for Cobionts and Contaminants (ASCC) pipeline (article in preparation). Manual curation was primarily conducted using PretextView (Harry, 2022), with additional insights provided by JBrowse2 (Diesh *et al.*, 2023) and HiGlass (Kerpedjiev *et al.*, 2018). Scaffolds were visually inspected and corrected as described by Howe *et al.* (2021). Any identified contamination, missed joins, and mis-joins were corrected, and duplicate sequences were tagged and removed. Sex chromosomes were identified by synteny analysis. The curation process is documented at https://gitlab.com/wtsi-grit/rapid-curation (article in preparation).

Evaluation of the final assembly

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 the "sanger-tol/readmapping" (Surana *et al.*, 2023a) and

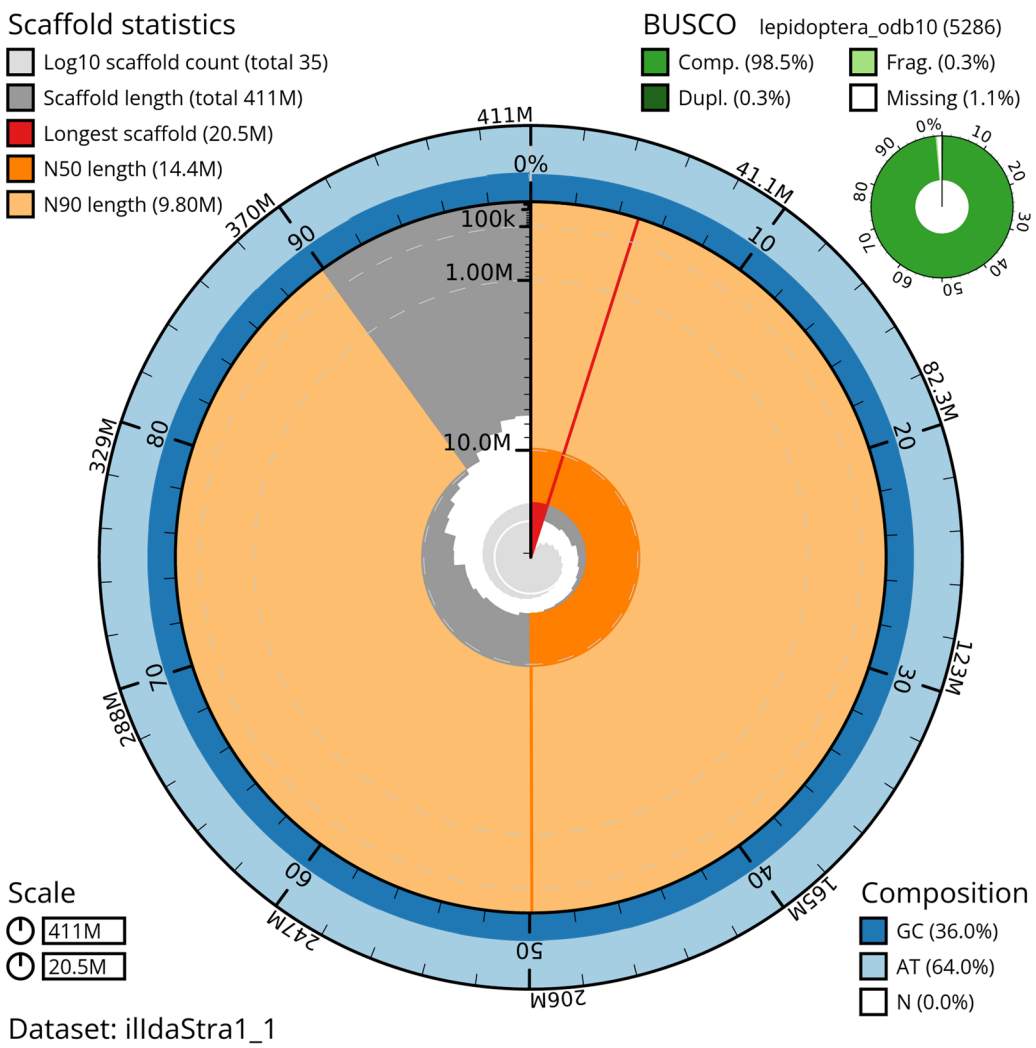

Figure 2. Genome assembly of *Idaea straminata*, ilIdaStra1.1: metrics. The BlobToolKit snail plot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 411,300,018 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (20,470,204 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (14,441,715 and 9,796,490 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/ilIdaStra1_1/dataset/ilIdaStra1_1/snail.

"sanger-tol/genomenote" (Surana *et al.*, 2023b) pipelines. The genome readmapping pipelines were developed using the nf-core tooling (Ewels *et al.*, 2020), use MultiQC (Ewels *et al.*, 2016), and make extensive use of the Conda package manager, the Bioconda initiative (Grüning *et al.*, 2018), the Biocontainers infrastructure (da Veiga Leprevost *et al.*, 2017), and the Docker (Merkel, 2014) and Singularity (Kurtzer *et al.*, 2017) containerisation solutions. The genome was also analysed within the BlobToolKit environment (Challis *et al.*, 2020) and BUSCO scores (Manni *et al.*, 2021) were calculated.

Table 4 contains a list of relevant software tool versions and sources.

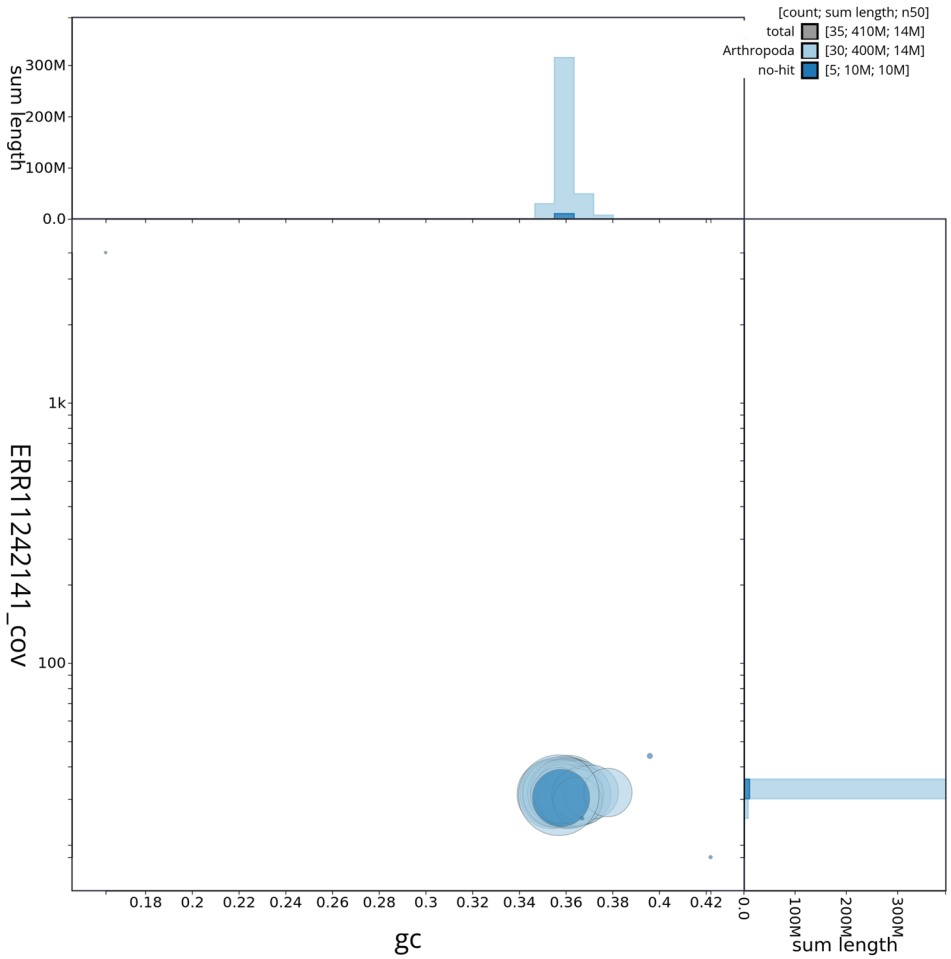

Figure 3. Genome assembly of *Idaea straminata*, ilIdaStra1.1: Blob plot of base coverage against GC proportion for sequences in the assembly. Scaffolds are coloured by phylum. Circles are sized in proportion to sequence length. Histograms show the distribution of sequence length sum along each axis. An interactive version of this figure is available at_https://blobtoolkit.genomehubs.org/view/ilIdaStra1_1/dataset/ilIdaStra1_1/blob.

Genome annotation

The BRAKER2 pipeline (Brůna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Idaea straminata* assembly (GCA_951213275.1) in Ensembl Rapid Release at the EBI.

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)

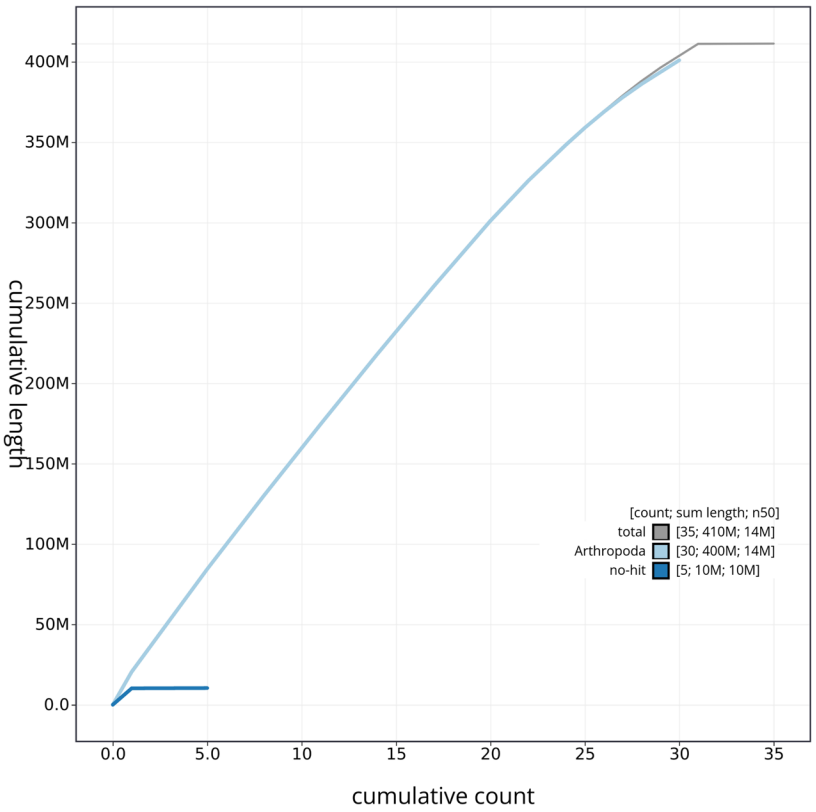

Figure 4. Genome assembly of *Idaea straminata* ilIdaStra1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all sequences. Coloured lines show cumulative lengths of sequences assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilIdaStra1_1/dataset/ilIdaStra1_1/cumulative.

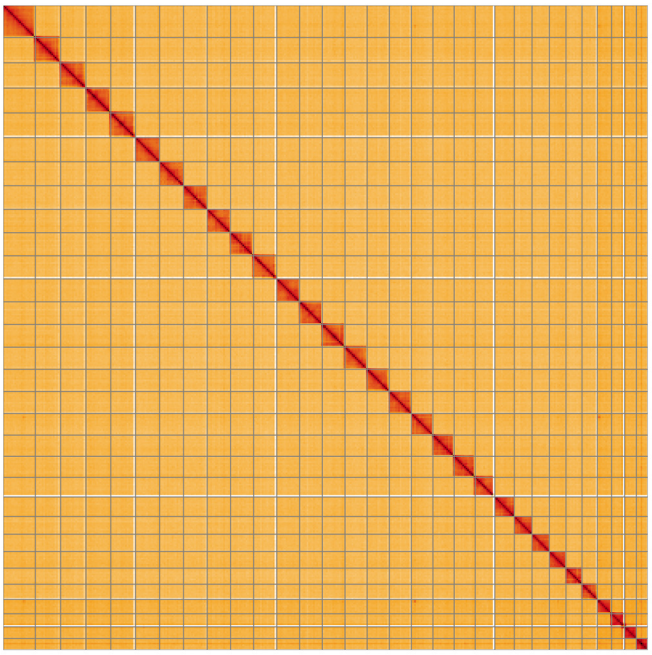

Figure 5. Genome assembly of *Idaea straminata* ilIdaStra1.1: Hi-C contact map of the ilIdaStra1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=CDOtlfGsRD2OBEkG5YWFng.

Table 3. Chromosomal pseudomolecules in the genome assembly of *Idaea straminata*, ilIdaStra1.

INSDC accession	Name	Length (Mb)	GC%
OX577846.1	1	16.14	36.0
OX577847.1	2	16.12	36.0
OX577848.1	3	15.85	36.0
OX577849.1	4	15.79	36.0
OX577850.1	5	15.35	35.5
OX577851.1	6	15.26	35.5
OX577852.1	7	15.08	35.5
OX577853.1	8	14.91	36.0
OX577854.1	9	14.75	35.5
OX577855.1	10	14.73	35.5
OX577856.1	11	14.63	36.0
OX577857.1	12	14.48	35.5
OX577858.1	13	14.44	36.0
OX577859.1	14	14.13	35.5
OX577860.1	15	14.1	36.0
OX577861.1	16	14.09	36.0
OX577862.1	17	13.77	36.0
OX577863.1	18	13.5	36.0
OX577864.1	19	13.45	36.5
OX577865.1	20	12.48	36.0
OX577866.1	21	12.39	36.5
OX577867.1	22	11.33	36.0
OX577868.1	23	11.1	36.0
OX577869.1	24	10.6	36.5
OX577870.1	25	10.21	36.0
OX577871.1	26	9.8	35.5
OX577872.1	27	9.11	37.0
OX577873.1	28	8.27	36.5
OX577874.1	29	7.5	36.5
OX577875.1	30	7.33	38.0
OX577845.1	Z	20.47	35.5
OX577876.1	MT	0.02	17.0

Table 4. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
bwa-mem2	2.2.1	https://github.com/bwa-mem2/bwa-mem2
Cooler	0.8.11	https://github.com/open2c/cooler
Gfastats	1.3.6	https://github.com/vgl-hub/gfastats
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury.FK	d00d98157618f4e8d1a91 90026b19b471055b22e	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	3	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.5	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
Singularity	3.9.0	https://github.com/sylabs/singularity
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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: *Idaea straminata* (plain wave). Accession number PRJEB61363; https://identifiers.org/ena.embl/PRJEB61363 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Idaea straminata* 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 and Table 2.

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Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: https://doi.org/10.5281/zenodo.12160324.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.12205391.

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

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