




DATA NOTE

The genome sequence of the common grass-veneer, *Agriphila tristella* (Denis & Schiffermüller, 1775) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual male *Agriphila tristella* (the common grass-veneer; Arthropoda; Insecta; Lepidoptera; Crambidae). The genome sequence is 802 megabases in span. Most of the assembly (99.83%) is scaffolded into 23 chromosomal pseudomolecules with the Z sex chromosome assembled. The mitochondrial genome was also assembled and is 15.3 kilobases in length.

Keywords

Agriphila tristella, common grass-veneer, genome sequence, chromosomal, Lepidoptera





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Open Peer Review

Approval Status  

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| 16 Dec 2022 | view | view |

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

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Author roles: **Boyes D:** Investigation, Resources; **Parkerson L:** Writing – Original Draft Preparation;

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Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Pyraloidea; Crambidae; Crambinae; *Agriphila*; *Agriphila tristella* (Denis and Schiffermüller, 1775) (NCBI: txid1594226).

Background

The common grass-veneer *Agriphila tristella* (Denis & Schiffermüller, 1775) is a micro-moth of the Crambinae subfamily. It can usually be recognised by its yellow median streak on the forewing which branches into four ‘fingers’ towards the apex of the wing. However, the species can be quite variable and difficult to separate from *Agriphila selasella*. In these cases, the prominent facial cone and differences in the genitalia can be used to identify *A. tristella* reliably (Lewis, 2012). The species is common in grassland and rough meadows throughout the British Isles, where the eggs are laid on various grasses.

A. tristella larvae can be found from September to June, feeding in a vertical silken gallery along the lower part of a grass stem. Pupae can then be found in June and July within oval frass-covered silken cocoons in loose soil amongst the grass roots. The adults typically fly between late June to mid-September, with a peak in August. During this time, they can be readily disturbed by day or attracted to light at night (Langmaid *et al.*, 2018).

The genome of the common grass-veneer was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all the named eukaryotic species in the Atlantic Archipelago of Britain and Ireland.

Genome sequence report

The genome was sequenced from a single male *A. tristella* (Figure 1), collected in Wytham Woods, Oxford, Berkshire, UK. A total of 29-fold coverage in Pacific Biosciences



Figure 1. Image of the *A. tristella* specimen taken prior to preservation and processing.

single-molecule HiFi long reads and 56-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 86 missing/misjoins and removed 40 haplotypic duplications, reducing the assembly size by 3.91% and the scaffold number by 39.22%, and increasing the scaffold N50 by 32.77%.

The final assembly has a total length of 802 Mb in 62 sequence scaffolds with a scaffold N50 of 51.7 Mb (Table 1). Most of the assembly sequence (99.83%) was assigned to 23 chromosomal-level scaffolds, representing 22 autosomes (numbered by sequence length) and the Z sex chromosome (Figure 2–Figure 5; Table 2). Heterozygous inversion was

Table 1. Genome data for *A. tristella*, ilAgrTris1.1.

| Project accession data | |
|----------------------------------|---|
| Assembly identifier | ilAgrTris1.1 |
| Species | <i>Agriphila tristella</i> |
| Specimen | ilAgrTris1 (genome assembly, Hi-C) |
| NCBI taxonomy ID | 1594226 |
| BioProject | PRJEB48050 |
| BioSample ID | SAMEA8603174 |
| Isolate information | Male. Thorax (ilAgrTris1, genome assembly); head (ilAgrTris1, Hi-C) |
| Raw data accessions | |
| PacificBiosciences SEQUEL II | ERR7123973-ERR7123974 |
| 10X Genomics Illumina | ERR7113557-ERR7113560 |
| Hi-C Illumina | ERR7113556 |
| Genome assembly | |
| Assembly accession | GCA_928269145.1 |
| Accession of alternate haplotype | GCA_928269205.1 |
| Span (Mb) | 801.8 |
| Number of contigs | 149 |
| Contig N50 length (Mb) | 15.3 |
| Number of scaffolds | 62 |
| Scaffold N50 length (Mb) | 51.7 |
| Longest scaffold (Mb) | 62.13 |
| BUSCO* genome score | C:98.0%[S:97.4%,D:0.6%],F:0.6%,M:1.4%,n:5,286 |

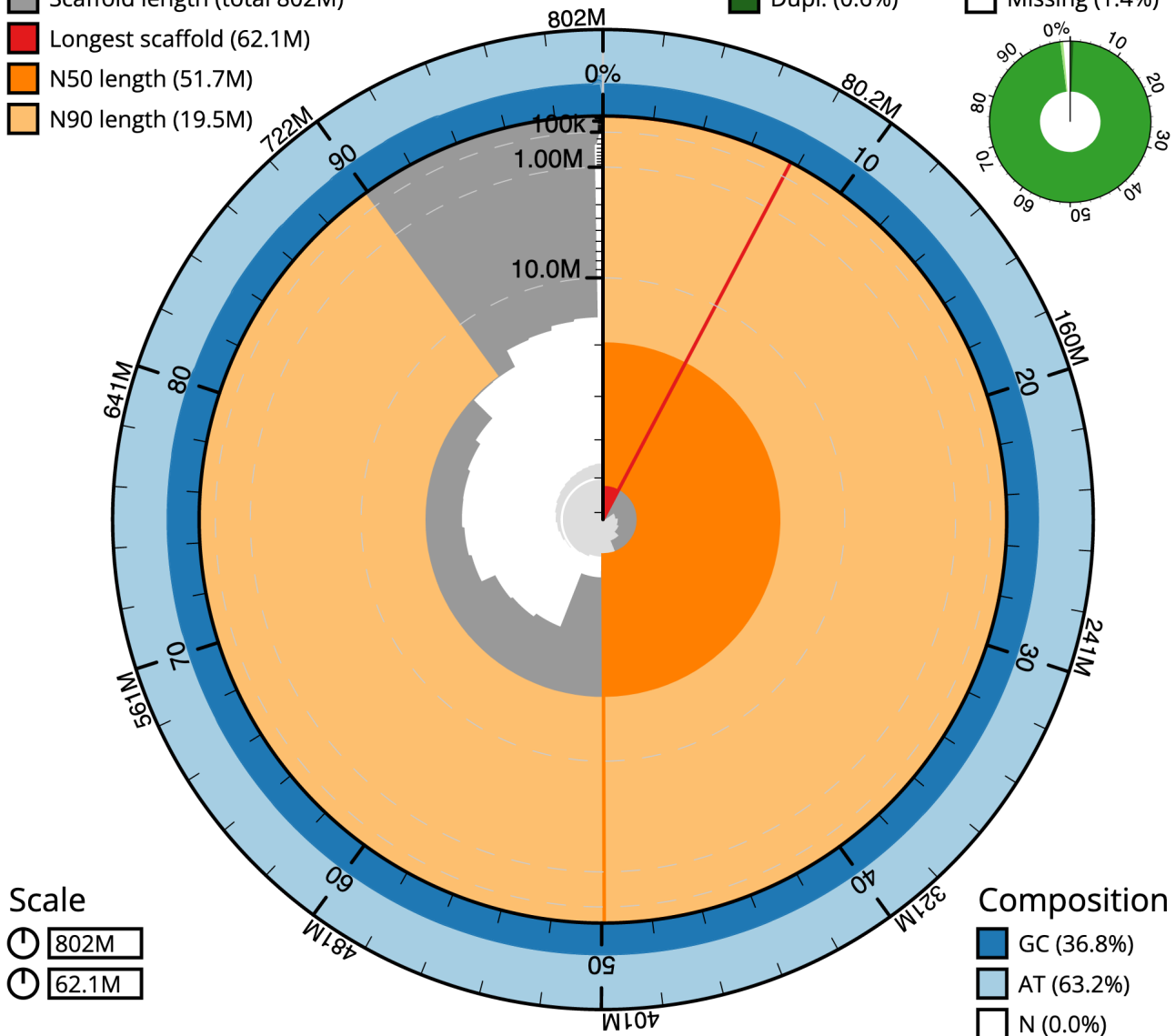
*BUSCO scores based on the lepidoptera_odb10 BUSCO set using v5.3.2. C = complete [S single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at <https://blobtoolkit.genomehubs.org/view/ilAgrTris1.1/dataset/CAKMRQ01/busco>.

Scaffold statistics

- Log10 scaffold count (total 63)
- Scaffold length (total 802M)
- Longest scaffold (62.1M)
- N50 length (51.7M)
- N90 length (19.5M)

BUSCO lepidoptera_odb10 (5286)

- Comp. (98.0%)
- Frag. (0.6%)
- Dupl. (0.6%)
- Missing (1.4%)



Dataset: CAKMRQ01

Figure 2. Genome assembly of *A. tristella*, ilAgrTris1.1: 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 801,775,791 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (62,134,667 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (51,702,748 and 19,521,324 bp respectively). The pale grey spiral shows the cumulative chromosome 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/ilAgrTris1.1/dataset/CAKMRQ01/snail>.

observed on chromosome 1 (19.75–29.97 Mb). A large size differential between haplotypes on several chromosomes was observed, with additional sequence not aligning to

comparators. Since difficulty was experienced in reconciling the chromosome 13 longer haplotype with the Hi-C map, 3.3 Mb of the chromosome was left in an alternate assembly.

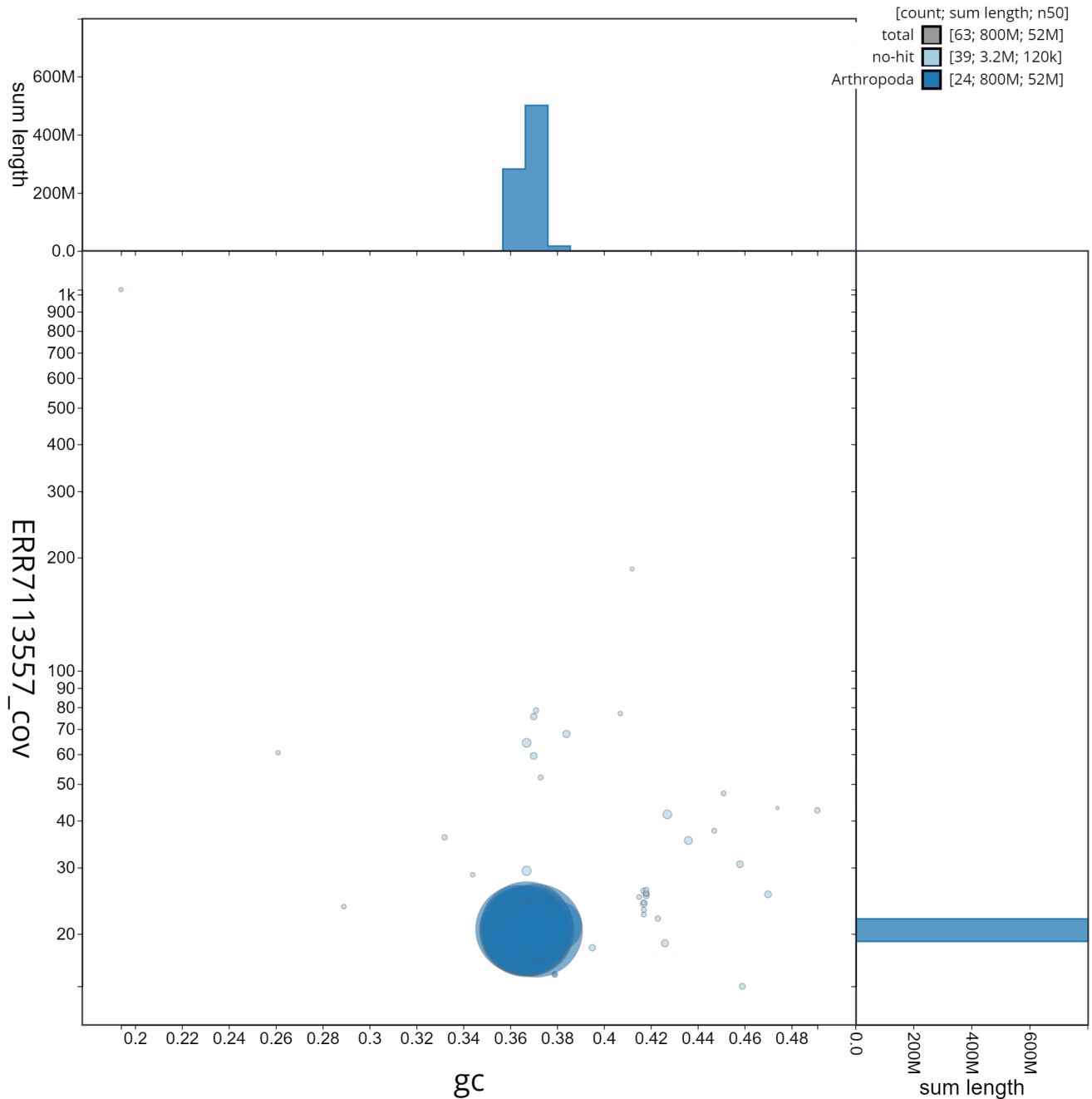


Figure 3. Genome assembly of *A. tristella*, ilAgrTris1.1: GC coverage. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilAgrTris1.1/dataset/CAKMRQ01/blob>.

The assembly has a BUSCO v5.3.2 (Manni *et al.*, 2021) completeness of 98.0% (single 97.4%, duplicated 0.6%) using the lepidoptera_odb10 reference set ($n = 5,286$). While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Methods

Sample acquisition and nucleic acid extraction

A single male *A. tristella* specimen (ilAgrTris1) was collected in Wytham Woods, Oxford, Berkshire, UK (latitude 51.772, longitude -1.338) by Douglas Boyes (University of Oxford),

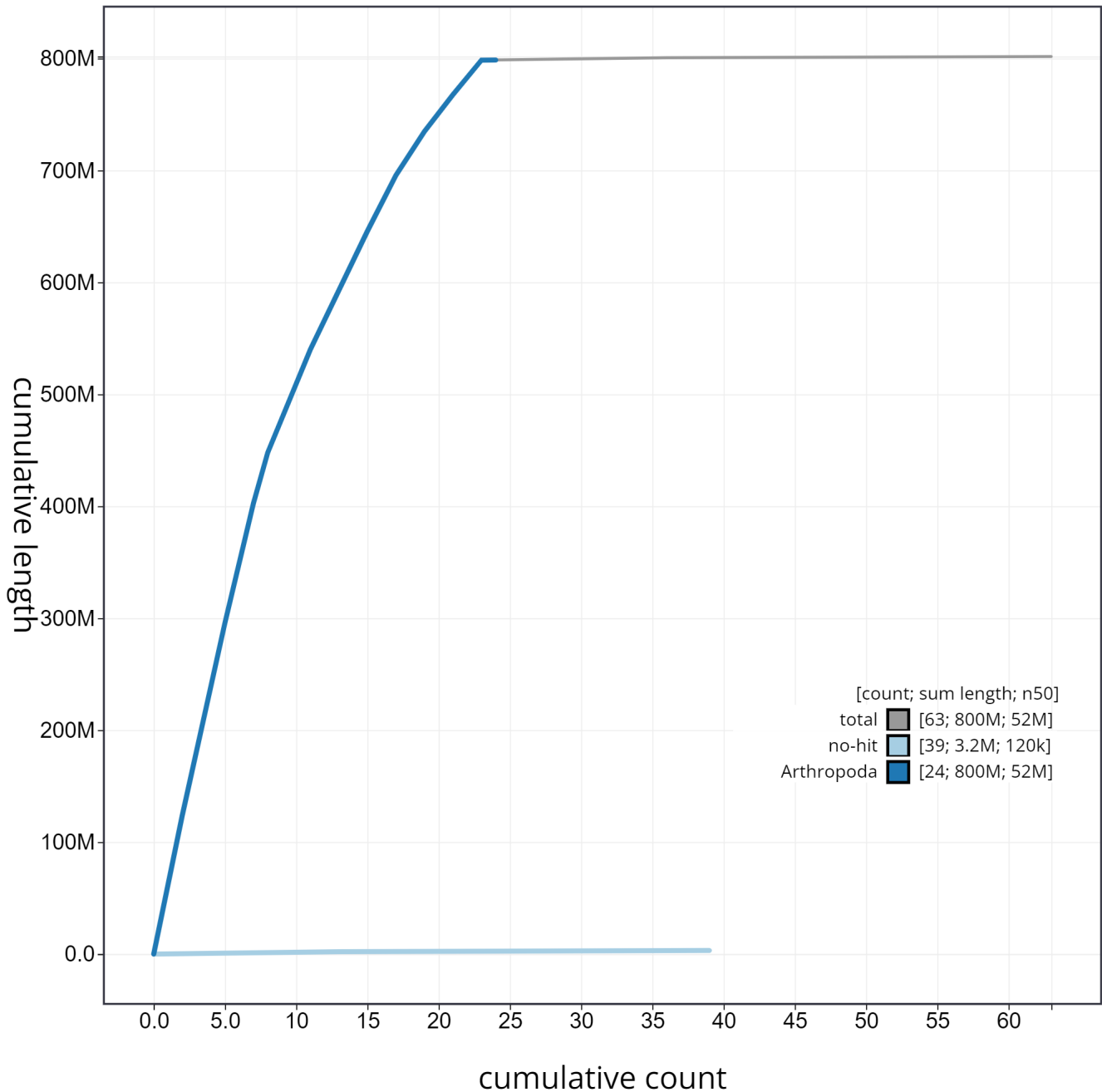


Figure 4. Genome assembly of *A. tristella*, ilAgrTris1.1: cumulative sequence. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilAgrTris1.1/dataset/CAKMRQ01/cumulative>.

using a light trap. The specimen was identified by Douglas Boyes and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ilAgrTris1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Thorax tissue was disrupted using a Nippi Powermasher

fitted with a BioMasher pestle. Fragment size analysis of 0.01–0.5 ng of DNA was then performed using an Agilent FemtoPulse. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low-molecular weight DNA was removed from a 200 ng aliquot of extracted DNA using 0.8X AMPure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng

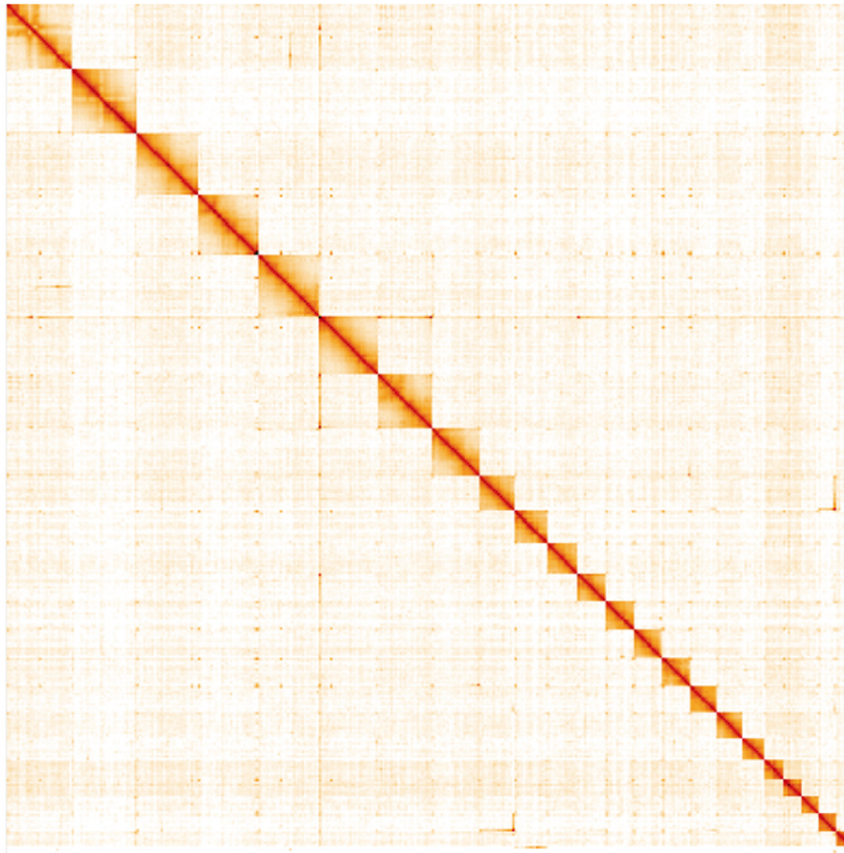


Figure 5. Genome assembly of *A. tristella*, ilAgrTris1.1: Hi-C contact map. Hi-C contact map of the ilAgrTris1.1 assembly, visualised in HiGlass. Chromosomes are arranged in size order from left to right and top to bottom. The interactive Hi-C map can be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=e5E_CDuZQM6vlEwHYyzUjA.

Table 2. Chromosomal pseudomolecules in the genome assembly of *A. tristella*, ilAgrTris1.1.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|------|
| OV743429.1 | 1 | 62.13 | 37.1 |
| OV743431.1 | 2 | 58.03 | 36.8 |
| OV743432.1 | 3 | 57.33 | 36.6 |
| OV743433.1 | 4 | 56.74 | 36.7 |
| OV743434.1 | 5 | 55.11 | 36.4 |
| OV743435.1 | 6 | 51.7 | 36.6 |
| OV743436.1 | 7 | 45.28 | 36.6 |
| OV743437.1 | 8 | 30.63 | 37 |
| OV743438.1 | 9 | 31.47 | 36.8 |
| OV743439.1 | 10 | 30.18 | 36.7 |
| OV743440.1 | 11 | 27.08 | 36.3 |
| OV743441.1 | 12 | 26.54 | 36.8 |

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|------|
| OV743442.1 | 13 | 26.08 | 36.9 |
| OV743443.1 | 14 | 26 | 37.3 |
| OV743444.1 | 15 | 25.48 | 36.4 |
| OV743445.1 | 16 | 24.34 | 37.2 |
| OV743446.1 | 17 | 19.6 | 36.5 |
| OV743447.1 | 18 | 19.52 | 37.4 |
| OV743448.1 | 19 | 16.52 | 38 |
| OV743449.1 | 20 | 16.29 | 36.7 |
| OV743450.1 | 21 | 15.62 | 36.9 |
| OV743451.1 | 22 | 15.29 | 37.1 |
| OV743430.1 | Z | 61.58 | 36.7 |
| OV743452.1 | MT | 0.02 | 19.6 |
| - | Unplaced | 3.21 | 40.4 |

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. 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.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were generated in the Tree of Life laboratory from head tissue of *iAgrTris1* using the Arima v2 kit and sequenced on a NovaSeq 6000 instrument.

Genome assembly

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021); haplotypic duplication was identified and removed with purge_dups (Guan *et al.*, 2020). One round of polishing was performed by aligning 10X Genomics read data to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014), using YaHS (Zhou *et al.*, 2022). The assembly was checked for contamination as described previously (Howe *et al.*, 2021). Manual curation was performed using HiGlass (Kerpedjiev *et al.*, 2018) and PretextView (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021), which performs annotation using MitoFinder (Allio *et al.*, 2020). The genome was analysed and BUSCO scores were generated within the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of all software tool versions used, where appropriate.

Ethics/compliance issues

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. 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. 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 used.

| Software tool | Version | Source |
|------------------|-------------------|---|
| Hifiasm | 0.15.3 | (Cheng <i>et al.</i> , 2021) |
| purge_dups | 1.2.3 | (Guan <i>et al.</i> , 2020) |
| YaHS | 1.0 | (Zhou <i>et al.</i> , 2022) |
| longranger align | 2.2.2 | https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines |
| freebayes | 1.3.1-17-gaa2ace8 | (Garrison & Marth, 2012) |
| MitoHiFi | 2.0 | (Uliano-Silva <i>et al.</i> , 2021) |
| HiGlass | 1.11.6 | (Kerpedjiev <i>et al.</i> , 2018) |
| PretextView | 0.2.x | https://github.com/wtsi-hpag/PretextView |
| BlobToolKit | 3.2.6 | (Challis <i>et al.</i> , 2020) |

Data availability

European Nucleotide Archive: *Agriphila tristella* (common grass-venerer). Accession number PRJEB48050; <https://identifiers.org/ena.embl/PRJEB48050> (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *A. tristella* 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. The genome will be annotated and presented through the Ensembl pipeline at the European Bioinformatics Institute. 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>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.4893703>.

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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[Publisher Full Text](#)

Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 25 May 2024

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Annabel Whibley 

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² Bragato Research Institute, Blenheim, New Zealand

Review of the genome assembly of *Agriphila tristella* by Douglas Boyes and colleagues at the University of Oxford and Wellcome Trust Sanger Centre. The authors have assembled another extremely high-quality genome and provided comprehensive and clearly structured information to support this effort. Yet again, the well-informed natural history detail that is included provides very helpful framing. Methods, analyses and reporting are all exemplary.

I note two omissions in this report compared to an earlier report that I reviewed: (1) Genome annotation- is there a reason why this assembly hasn't been run through the Ensembl rapid annotation pathway? (2) A link to the metadata, such as kmer spectra- which can be a really useful resource.

The level of heterozygosity in the genome individual seems quite marked and, from the haplotype size differences recorded, it would appear that structural variation may be an important feature of genome biology in this species. Whilst to explore this in detail is probably beyond the scope of these reports, it would have been nice to have these "quirks" also presented as tables/figures. As the reports of the primary assemblies become increasingly standardized (and these short reports are models in the field), as a collective I would love to see the DToL and its partners explore ways to effectively disseminate learnings from unexpected and challenging findings.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.**Reviewer Expertise:** Genomics**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.**

Reviewer Report 29 November 2023

<https://doi.org/10.21956/wellcomeopenres.20589.r70179>

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**Kuppusamy Sivasankaran** 

Loyola College, Chennai, India

I would like to appreciate the authors for assembling the *Agriphila tristella* (Denis & Schiffermuller, 1775) of whole genome sequence. The authors sequenced almost 800 million base pairs in the genome assembly using 51 scaffolds. The authors have properly submitted the sequence in the public database. This robust data will be useful for the phylogenomic researchers.

Suggestions:

In the abstract the first sentence can be rewritten as "The genome of *Agriphila tristella* (Insecta: Lepidoptera: Crambidae) was sequenced and assembled".

The last sentence of the abstract can be changed as "The mitochondrial genome was also annotated and is 15.3 kilobases in size".

Under the subheading "Background" Third paragraph of the first line can be modified as "The genome of *A. tristella* was sequenced....."

Under the subheading "Genome sequence report" first paragraph sixth line" with chromosome conformation" It can be changed as "with chromosome confirmation"

Query:

Why the authors haven't included the details about the annotation of Protein-coding genes, non-coding sequences, number of gene transcripts in the tables.

The manuscript is well prepared, and it can be accepted for indexing.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phylogenetic analysis of lepidopteran moths using complete mitochondrial genome sequence

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.
