

DATA NOTE

# The genome sequence of the swallow prominent, *Pheosia tremula* (Clerck, 1759) [version 1; peer review: 2 approved]

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**V1** First published: 13 Dec 2021, 6:335  
<https://doi.org/10.12688/wellcomeopenres.17484.1>  
Latest published: 13 Dec 2021, 6:335  
<https://doi.org/10.12688/wellcomeopenres.17484.1>

## Abstract

We present a genome assembly from an individual male *Pheosia tremula* (the swallow prominent; Arthropoda; Insecta; Lepidoptera; Notodontidae). The genome sequence is 290 megabases in span. The majority of the assembly, 99.94%, is scaffolded into 31 chromosomal pseudomolecules, with the Z sex chromosome assembled.

## Keywords

*Pheosia tremula*, swallow prominent, genome sequence, chromosomal, Lepidoptera



This article is included in the [Tree of Life gateway](#).

## Open Peer Review

Approval Status  

	1	2
<b>version 1</b>		
13 Dec 2021	<a href="#">view</a>	<a href="#">view</a>

1. **Simon H. Martin** , University of Edinburgh, Edinburgh, UK

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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; **Holland PWH:** Supervision, Writing – Original Draft Preparation, Writing – Review & Editing;

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by Wellcome through core funding to the Wellcome Sanger Institute (206194) and the Darwin Tree of Life Discretionary Award (218328).

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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**How to cite this article:** Boyes D, Holland PWH, University of Oxford and Wytham Woods Genome Acquisition Lab *et al.* **The genome sequence of the swallow prominent, *Pheosia tremula* (Clerck, 1759) [version 1; peer review: 2 approved]** Wellcome Open Research 2021, 6:335 <https://doi.org/10.12688/wellcomeopenres.17484.1>

**First published:** 13 Dec 2021, 6:335 <https://doi.org/10.12688/wellcomeopenres.17484.1>

## Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Notodontidae; Notodontinae; Pheosia; *Pheosia tremula* (Clerck, 1759) (NCBI:txid988019).

## Background

*Pheosia tremula* (swallow prominent) is a strikingly patterned moth considered (Allan, 1947) as “aesthetically the perfect insect”; its larvae feed on poplar (*Populus* sp.) and willow (*Salix* sp.). *Pheosia tremula* can be found throughout northern and central Europe and Russia. Within the UK the moth is relatively common in England and Wales but more local in Scotland. The flight period in the UK peaks in May to June, and again in August, and the moth can be found in woodland, plantations, riversides, gardens and parks. Like other moths in the family Notodontidae, *P. tremula* has a single auditory receptor cell associated with each tympanic membrane on the second thoracic segment; *P. tremula* has therefore been used to investigate the electrophysiological basis of auditory reception in simple “one-celled ears” (Fullard *et al.*, 1998). The genome of *P. tremula* was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all of the named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *P. tremula*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

## Genome sequence report

The genome was sequenced from a single male *P. tremula* collected from Wytham Woods (Figure 1), Oxfordshire, UK (latitude 51.768, longitude -1.337). A total of 73-fold coverage in Pacific Biosciences single-molecule long reads and 120-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation



**Figure 1. Image of the ilPheTrem1 specimen taken prior to preservation and processing.** Specimen shown next to FluidX storage tube, 43.9 mm in length.

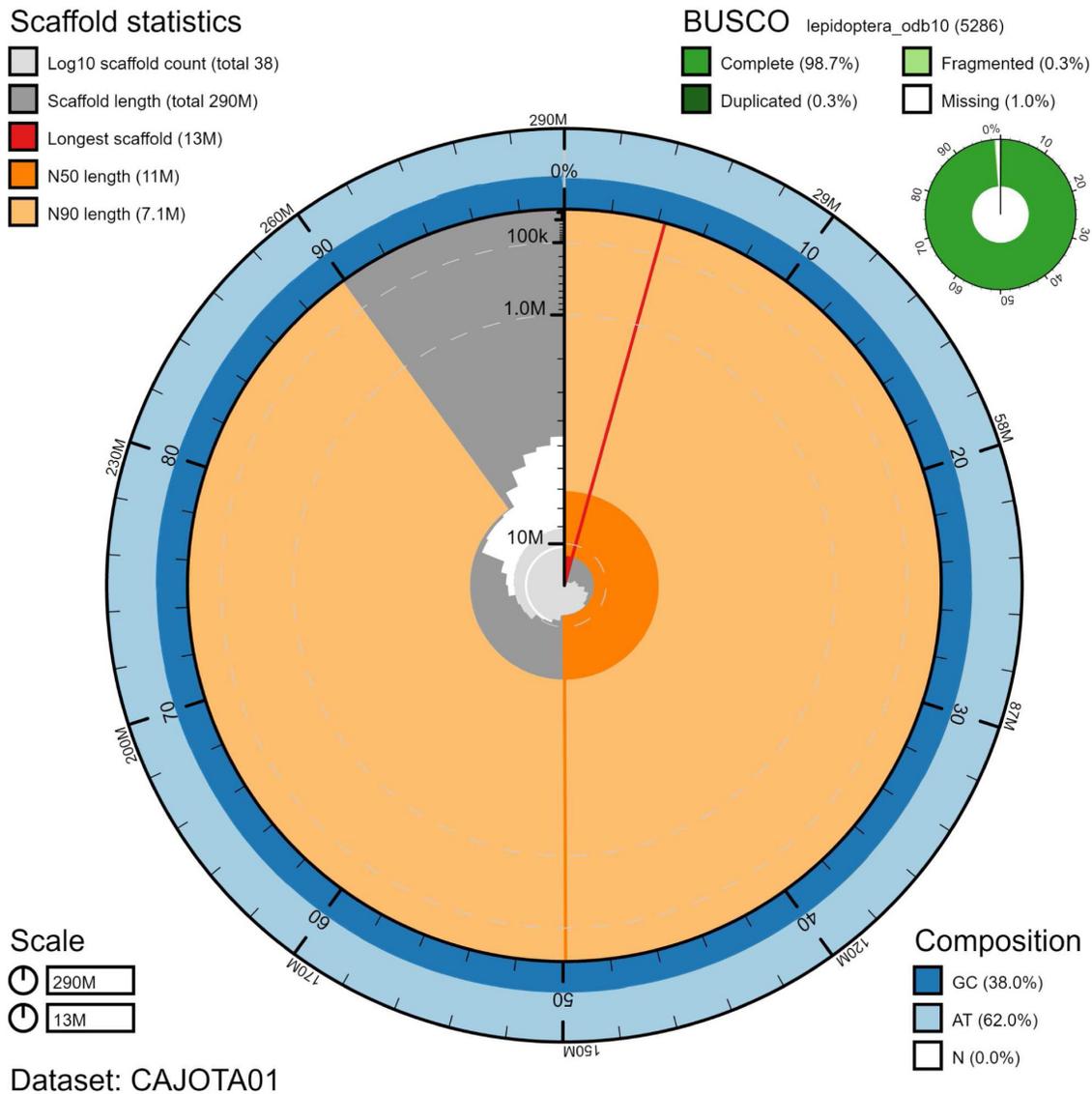
Hi-C data. Manual assembly curation corrected 70 missing/misjoins and removed 9 haplotypic duplications, reducing the assembly length by 0.03% and the scaffold number by 48.61%, and increasing the scaffold N50 by 3.55%.

The final assembly has a total length of 290 Mb in 38 sequence scaffolds with a scaffold N50 of 11 Mb (Table 1). Of the assembly sequence, 99.9% was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes (numbered by sequence length), and the Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.1.2 (Manni *et al.*, 2021) completeness of 98.7% (single 98.4%, duplicated 0.3%) using the lepidoptera\_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

**Table 1. Genome data for *Pheosia tremula*, ilPheTrem1.1.**

Project accession data	
Assembly identifier	ilPheTrem1.1
Species	<i>Pheosia tremula</i>
Specimen	ilPheTrem1
NCBI taxonomy ID	NCBI:txid988019
BioProject	PRJEB43537
BioSample ID	SAMEA7520523
Isolate information	Male, thorax/abdomen
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6412031, ERR6412363
10X Genomics Illumina	ERR6054530-ERR6054533
Hi-C Illumina	ERR6054529
Genome assembly	
Assembly accession	GCA_905333125.1
Accession of alternate haplotype	GCA_905333115.1
Span (Mb)	290
Number of contigs	106
Contig N50 length (Mb)	7.7
Number of scaffolds	37
Scaffold N50 length (Mb)	10.6
Longest scaffold (Mb)	12.6
BUSCO* genome score	C:98.7%[S:98.4%,D:0.3%], F:0.3%,M:1.0%,n:5286

\*BUSCO scores based on the lepidoptera\_odb10 BUSCO set using v5.1.2. 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/ilPheTrem1.1/dataset/CAJOTA01/busco>.



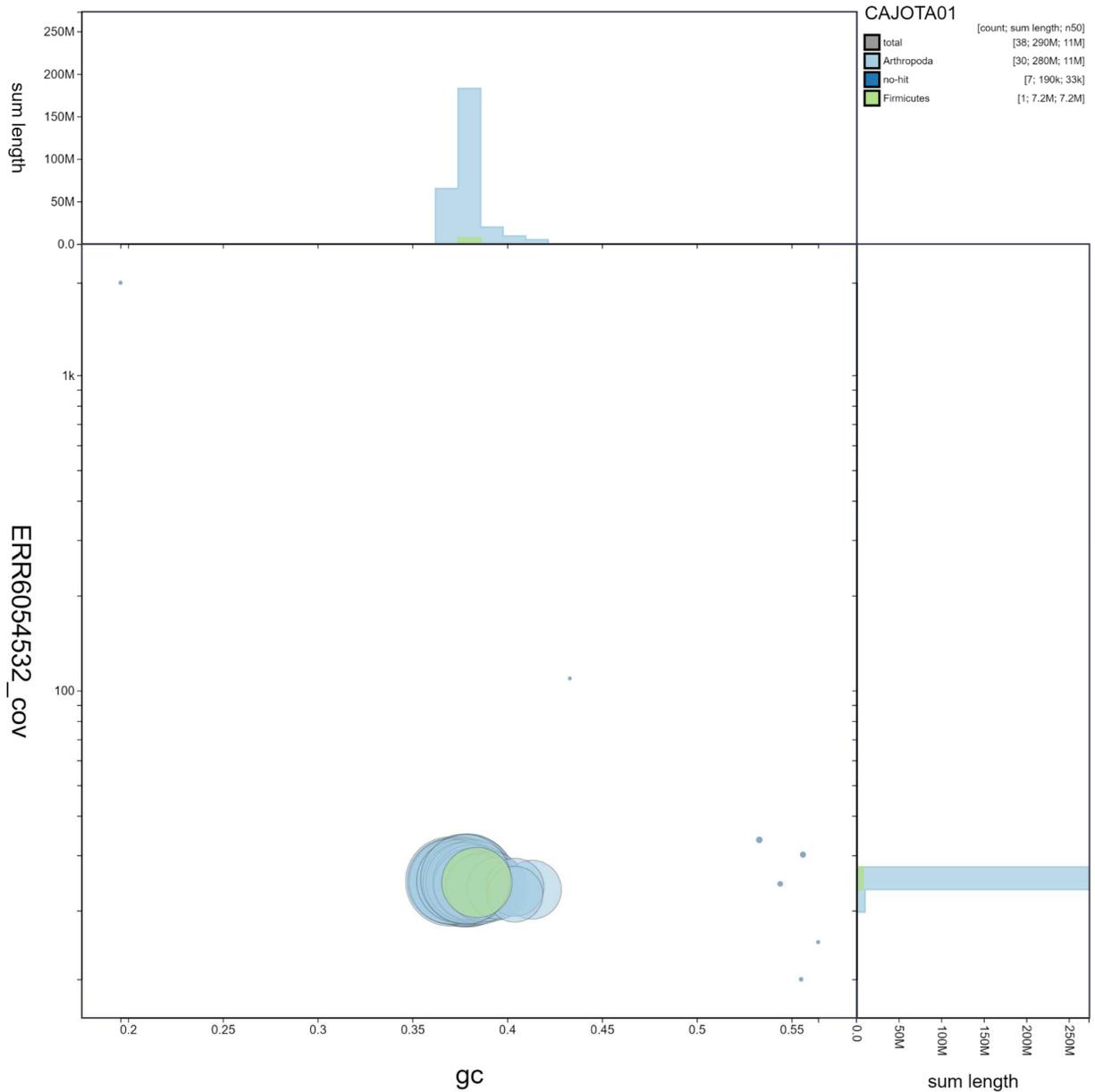
**Figure 2. Genome assembly of *Pheosia tremula*, ilPheTrem1.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 290,221,673 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (12,641,679 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (10,632,747 and 7,102,514 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/ilPheTrem1.1/dataset/CAJOTA01/snail>.

## Methods

### Sample acquisition and DNA extraction

A single male *P. tremula* (ilPheTrem1) was collected from Wytham Woods, Oxfordshire, UK (latitude 51.768, longitude -1.337) by Douglas Boyes, UKCEH, using a light trap. The specimen was identified by the same individual and preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ilPheTrem1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Thorax/abdomen tissue was cryogenically disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. Fragment size analysis of 0.01–0.5 ng of DNA was then performed using an Agilent



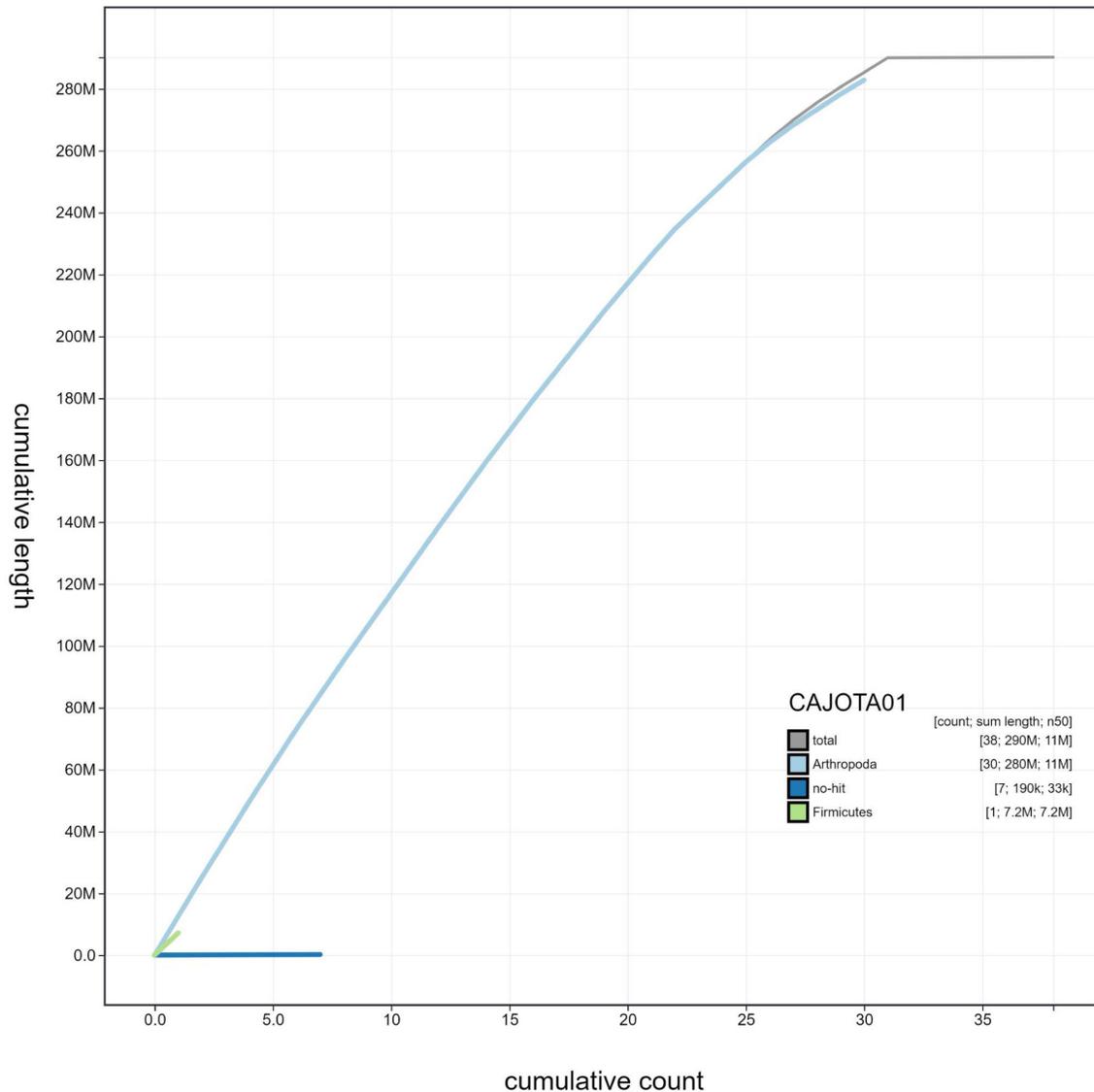
**Figure 3. Genome assembly of *Pheosia tremula*, ilPheTrem1.1: GC coverage.** BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilPheTrem1.1/dataset/CAJOTA01/blob>.

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 DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size between 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.

### Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was performed



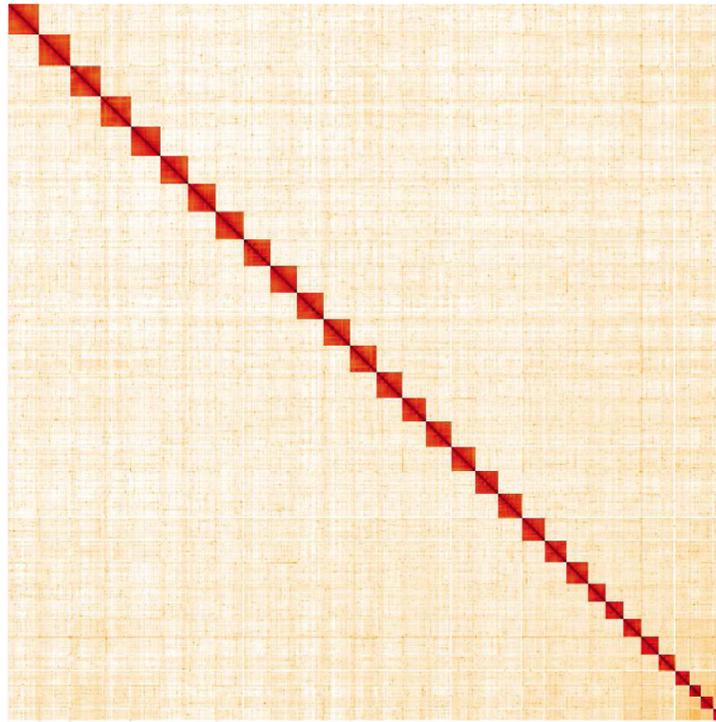
**Figure 4. Genome assembly of *Pheosia tremula*, ilPheTrem1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilPheTrem1.1/dataset/CAJOTA01/cumulative>.

by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II and Illumina HiSeq X instruments. Hi-C data were generated from thorax/abdomen tissue using the Arima Hi-C+ kit and sequenced on HiSeq X.

#### Genome assembly

Assembly was carried out with HiCanu (Nurk *et al.*, 2020). Haplotypic duplication was identified and removed with purge\_dups (Guan *et al.*, 2020). Scaffolding with Hi-C data (Rao *et al.*, 2014) was carried out with SALSA2 (Ghurye *et al.*, 2019). The Hi-C scaffolded assembly was polished with the 10X

Genomics Illumina data by aligning to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012). One round of the Illumina polishing was applied. The mitochondrial genome was assembled with MitoHiFi (Uliano-Silva *et al.*, 2021) and annotated using MitoFinder (Allio *et al.*, 2020). 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 (Howe *et al.*, 2021) was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext. The genome was analysed within the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of all software tool versions used, where appropriate.



**Figure 5. Genome assembly of *Pheosia tremula*, ilPheTrem1.1: Hi-C contact map.** Hi-C contact map of the ilPheTrem1.1 assembly, visualised in HiGlass. Chromosomes are given in order of size from left to right and top to bottom.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Pheosia tremula*, ilPheTrem1.1.**

INSDC accession	Chromosome	Size (Mb)	GC%
HG995397.1	1	12.64	37.9
HG995398.1	2	12.62	37.7
HG995399.1	3	12.25	38
HG995400.1	4	12.19	37.9
HG995402.1	5	11.69	37
HG995403.1	6	11.25	37.4
HG995404.1	7	10.95	37.5
HG995405.1	8	10.89	37.1
HG995406.1	9	10.71	37.7
HG995407.1	10	10.70	37.1
HG995408.1	11	10.69	37
HG995409.1	12	10.63	37.8
HG995410.1	13	10.31	37.7
HG995411.1	14	10.09	37.9
HG995412.1	15	10.01	37.4

INSDC accession	Chromosome	Size (Mb)	GC%
HG995413.1	16	9.62	38
HG995414.1	17	9.57	37.7
HG995415.1	18	9.47	38.2
HG995416.1	19	9.08	37.8
HG995417.1	20	8.96	38.5
HG995418.1	21	8.62	38.1
HG995419.1	22	7.33	38
HG995420.1	23	7.21	38.4
HG995421.1	24	7.20	39.1
HG995422.1	25	7.10	38.7
HG995423.1	26	6.30	38.5
HG995424.1	27	5.58	39.5
HG995425.1	28	5.08	41.3
HG995426.1	29	4.91	40.4
HG995427.1	30	4.57	40.4
HG995401.1	Z	11.80	37.8
HG995428.1	MT	0.02	19.5
-	Unplaced	0.17	53.9

**Table 3. Software tools used.**

Software tool	Version	Source
HiCanu	2.1	<a href="#">Nurk et al., 2020</a>
purge_dups	1.2.3	<a href="#">Guan et al., 2020</a>
SALSA2	2.2	<a href="#">Ghurye et al., 2019</a>
longranger align	2.2.2	<a href="https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines">https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines</a>
freebayes	v1.3.1-17-gaa2ace8	<a href="#">Garrison &amp; Marth, 2012</a>
MitoHiFi	1.0	<a href="#">Uliano-Silva et al., 2021</a>
gEVAL	N/A	<a href="#">Chow et al., 2016</a>
HiGlass	1.11.6	<a href="#">Kerpedjiev et al., 2018</a>
PretextView	0.1.x	<a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>
BlobToolKit	2.6.2	<a href="#">Challis et al., 2020</a>

### 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.

### Data availability

European Nucleotide Archive: *Pheosia tremula* (swallow prominent). Accession number [PRJEB43537](#); <https://www.ebi.ac.uk/ena/browser/view/PRJEB43537>.

The genome sequence is released openly for reuse. The *P. tremula* 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.5746938>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.5744972>.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: <https://doi.org/10.5281/zenodo.5744840>.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: <https://doi.org/10.5281/zenodo.5746904>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.5743293>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.5638618>.

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[Publisher Full Text](#)

# Open Peer Review

Current Peer Review Status:  

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## Version 1

Reviewer Report 08 December 2022

<https://doi.org/10.21956/wellcomeopenres.19334.r53442>

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 **Yuttapong Thawornwattana** 

Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, USA

Boyes & Holland report a chromosome-level genome assembly of *Pheosia tremula* (Clerck, 1759) (Lepidoptera: Notodontidae) based on high-coverage PacBio HiFi long reads from a single male individual. The assembly was scaffolded with Hi-C data and polished with 10X Genomics Illumina reads, produced as part of the Darwin Tree of Life project.

The assembly is of high-quality, with both scaffold N50 and N90 in a megabase range, and high BUSCO assembly completeness (>98% for single-copy orthologs in Lepidoptera). However, genome annotation was not mentioned. The status of the W chromosome was also not mentioned. There was notable a presence of firmicutes bacteria from taxonomic annotation of scaffolds could be due to contamination or symbiosis.

**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:** phylogenetics, evolutionary 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 19 January 2022

<https://doi.org/10.21956/wellcomeopenres.19334.r47665>

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**Simon H. Martin** 

Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

The authors present a highly contiguous and complete haploid genome assembly for a male *Pheosia tremula*. This excellent assembly would be of value as a reference genome and for comparative genomics projects.

I have only a few minor requests for improved clarity:

1. Please specify the read length for the Illumina sequencing.
2. Prior to scaffolding with SLASA2, I believe it is necessary to align the HI-C reads to the contigs. As far as I could tell, the alignment approach is not described.
3. The large contig that is labelled as Firmicutes in Figures 3 and 4 is not mentioned in the main text. Was this interpreted as contamination and excluded from the final assembly?

**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?**

Partly

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

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Evolution, genomics and population genetics

**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.**

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