DATA NOTE

The genome sequence of the Powdered Quaker, *Orthosia gracilis* (Schiffermüller, 1775) [version 1; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual male *Orthosia gracilis* (the powdered quaker; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 715.5 megabases in span. Most of the assembly is scaffolded into 14 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.43 kilobases in length.

Keywords

Orthosia gracilis, powdered quaker, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 07 Jul 2023	✓ view	✓ view

1. **Niklas Wahlberg**, Lund university, Lund, Sweden

2. **Joel Vizueta**, University of Copenhagen, Copenhagen, Denmark

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; **Boyes C**: Writing – Original Draft Preparation;

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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; Noctuoidea; Noctuidae; Hadeninae; Orthosia (Schiffermüller, 1775) (NCBI:txid997542).

Background

Orthosia gracilis (Powdered Quaker) is a common and widespread Noctuid moth found throughout Europe and eastwards to China (Leraut, 2019). Its distribution and abundance in Great Britain and Ireland decreased significantly over the last 50 years, although it has shown some recovery in the last 20 years (Randle *et al.*, 2019).

The common name Powdered Quaker is a reference to the plain clothing traditionally worn by Quakers (Marren, 2019). In this species, the wings have a dusting of tiny black flecks which distinguish it from other spring-flying species in the genus. There is significant variation in the ground colour of the moth with dark forms usually occurring on bogs where the larvae feed on bog-myrtle. These dark forms can occur in the same locations as paler forms, but the two forms do not mix as the dark form occurs earlier in all of its life stages (Heath & Emmet, 1983).

The Powdered Quaker has one generation a year, flying between March and May. It is found in habitats that include damp woodland and gardens, but it favours marshland. The adult moth regularly comes to light, is attracted to sugar, and feeds on sallow catkins and blackthorn flowers. The larvae feed at night on a range of herbaceous and woody plants (Waring *et al.*, 2017).

A genome sequence from *O. gracilis* will be useful for research into colour variation in moths, and more generally for comparative studies across the Lepidoptera. The genome of *O. gracilis* 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. Here we present a chromosomally complete genome sequence for *O. gracilis* based on a male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Orthosia gracilis* (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 was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected six missing joins or mis-joins and removed two haplotypic duplications, reducing the assembly length by 0.29% and increasing the scaffold count by one.

Figure 1. Photograph of the *Orthosia gracilis* (ilOrtGrac1) specimen used for genome sequencing.

The final assembly has a total length of 715.5 Mb in 19 sequence scaffolds with a scaffold N50 of 52.0 Mb (Table 1). Most (99.98%) of the assembly sequence was assigned to 14 chromosomal-level scaffolds, representing13 autosomes and the Z sex chromosome. 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 68.8 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 completeness of 98.8% (single = 97.9%, duplicated = 0.8%), 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/997542.

Methods

Sample acquisition and nucleic acid extraction

A male *Orthosia gracilis* (specimen ID Ox001092, individual ilOrtGrac1) was collected from Wytham Woods, Oxfordshire (biological vice-county Berkshire), UK (latitude 51.77, longitude –1.34) on 2021-03-31 using a light trap. The specimen was identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilOrtGrac1 sample was weighed and dissected on dry ice with head tissue set aside for Hi-C

Table 1. Genome data for *Orthosia gracilis*, ilOrtGrac1.1.

Project accession data		
Assembly identifier	ilOrtGrac1.1	
Species	*Orthosia gracilis*	
Specimen	ilOrtGrac1	
NCBI taxonomy ID	997542	
BioProject	PRJEB56737	
BioSample ID	SAMEA10107015	
Isolate information	ilOrtGrac1, male: thorax (DNA sequencing), head (Hi-C scaffolding)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	68.8	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.8%[S:97.9%,D:0.8%],F:0.3%, M:1.0%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.98%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10395972	
Hi-C Illumina	ERR10378050	
Genome assembly		
Assembly accession	GCA_947562075.1	
Accession of alternate haplotype	GCA_947563345.1	
Span (Mb)	715.5	
Number of contigs	92	
Contig N50 length (Mb)	14.1	
Number of scaffolds	20	
Scaffold N50 length (Mb)	52.0	
Longest scaffold (Mb)	75.5	

* 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/Orthosia%20gracilis/dataset/CANOAQ01/busco.

sequencing. Thorax tissue was cryogenically disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. 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

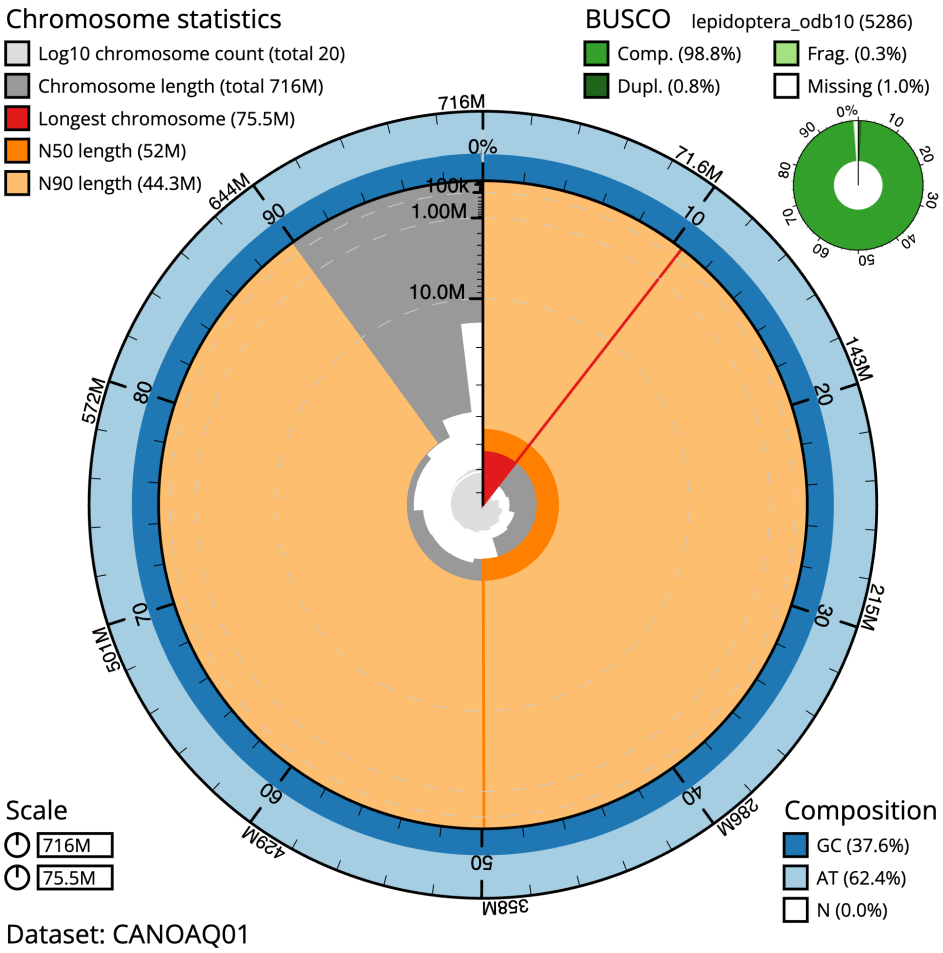

Figure 2. Genome assembly of *Orthosia gracilis*, ilOrtGrac1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 715,548,976 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (75,520,589 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (52,000,109 and 44,255,424 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/Orthosia%20gracilis/dataset/CANOAQ01/snail.

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 DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on a Pacific Biosciences SEQUEL II (HiFi) instrument. Hi-C data were also generated from head tissue of ilOrtGrac1 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 (Zhou *et al.*, 2023). The assembly was checked for contamination and corrected as described previously (Howe *et al.*, 2021).

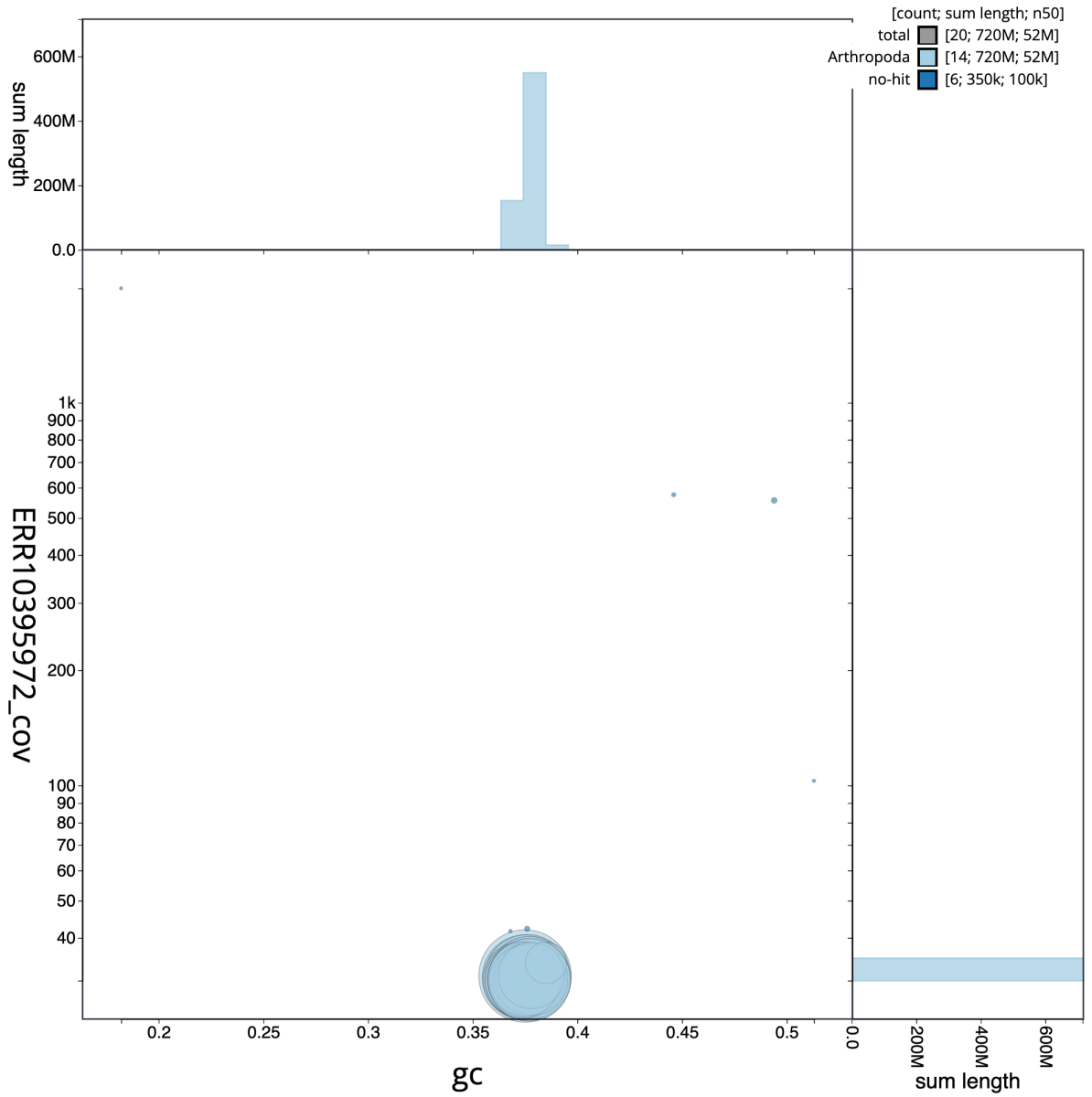

Figure 3. Genome assembly of *Orthosia gracilis*, ilOrtGrac1.1: BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Orthosia%20gracilis/dataset/CANOAQ01/blob.

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.*, 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 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.

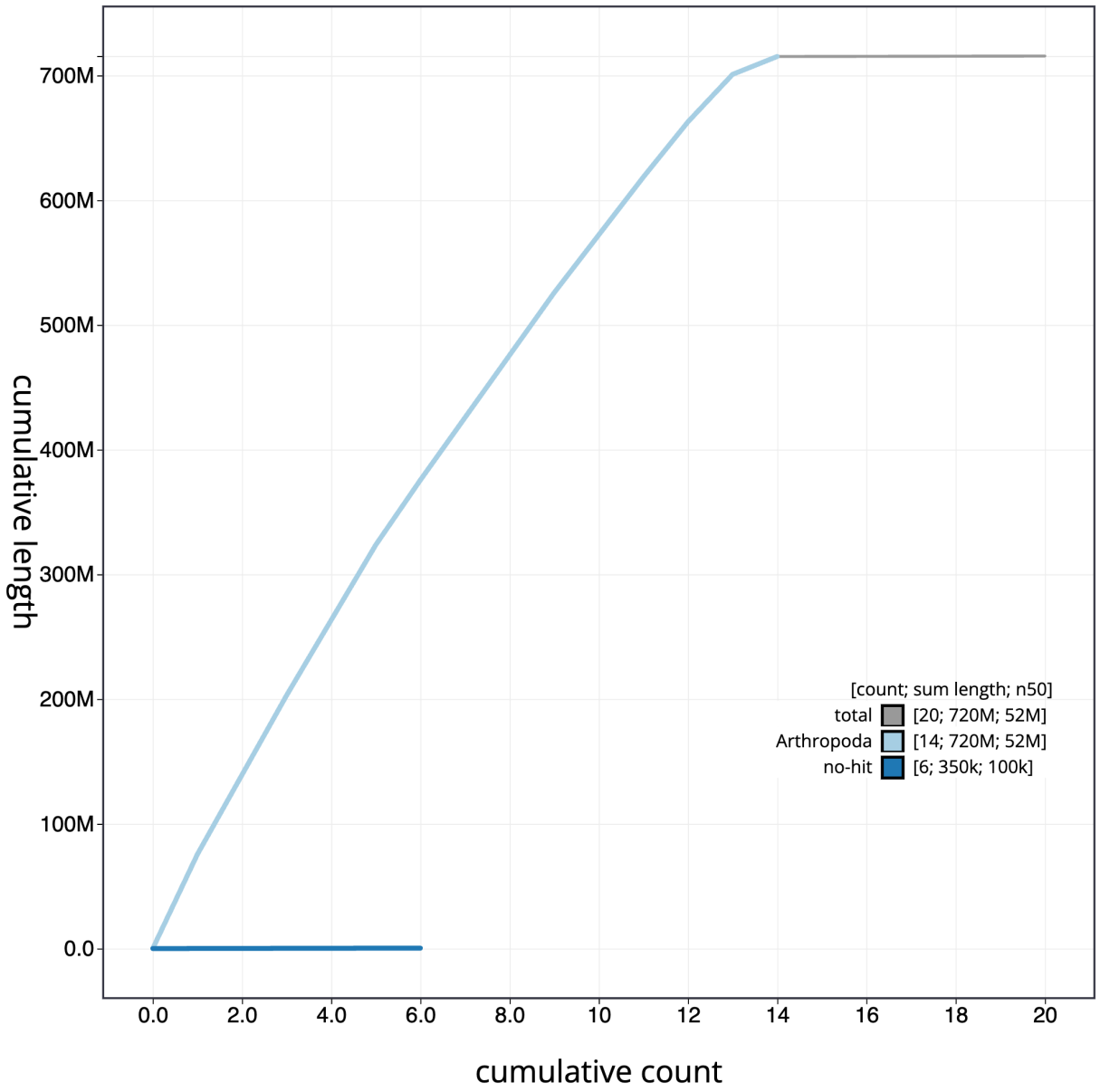

Figure 4. Genome assembly of *Orthosia gracilis*, ilOrtGrac1.1: BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/Orthosia%20gracilis/dataset/CANOAQ01/cumulative.

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

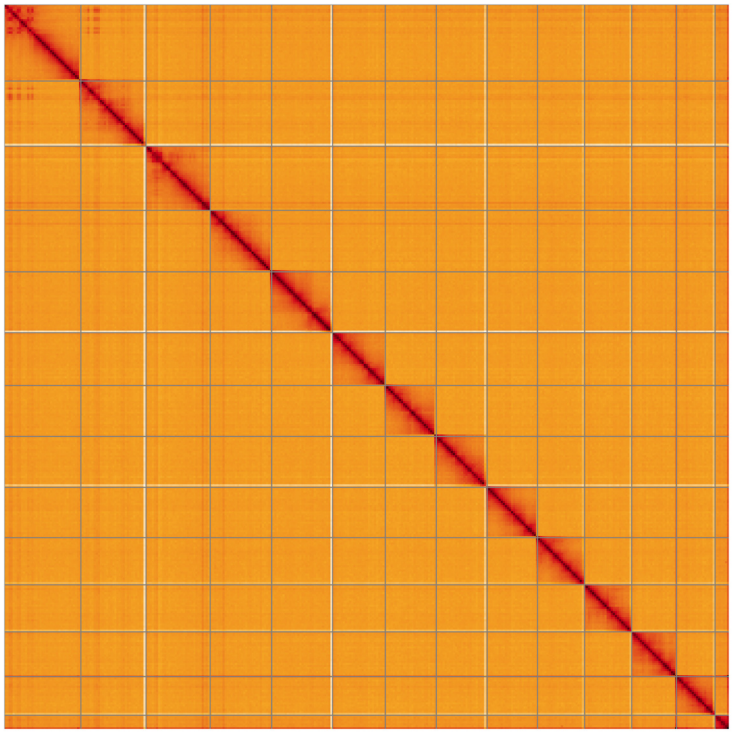

Figure 5. Genome assembly of *Orthosia gracilis*, ilOrtGrac1.1: Hi-C contact map of the ilOrtGrac1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=disBjOz7ROa-mijGcPkDjw.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Orthosia gracilis*, ilOrtGrac1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX387264.1	1	75.52	37.5
OX387265.1	2	64.17	37.5
OX387266.1	3	63.29	37.5
OX387267.1	4	60.73	37.5
OX387269.1	5	52.0	37.5
OX387270.1	6	50.3	37.5
OX387271.1	7	50.11	37.5
OX387272.1	8	49.78	37.5
OX387273.1	9	46.43	37.5
OX387274.1	10	46.34	38.0
OX387275.1	11	44.26	37.5
OX387276.1	12	38.02	38.0
OX387277.1	13	14.3	38.5
OX387268.1	Z	59.94	37.5
OX387278.1	MT	0.02	18.5

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
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

Data availability

European Nucleotide Archive: *Orthosia gracilis* (powdered quaker). Accession number PRJEB56737; https://identifiers.org/ena.embl/PRJEB56737 (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Orthosia gracilis* 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 using available RNA-Seq data 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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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 23 August 2023

https://doi.org/10.21956/wellcomeopenres.21761.r65317

✔ **Joel Vizueta**
University of Copenhagen, Copenhagen, Capital Region of Denmark, Denmark

The authors describe in this genome report the chromosome level assembly of the powdered quaker *Orthosia gracilis*. The sequenced genome is very high quality based on the reported metrics, and the methods are robust. This genomic resource will be interesting in comparative genomics analyses within Lepidoptera, and the presented data is already publicly available.

Based on current genome reports published in Wellcome Open Research from the Darwin Tree of Life, this article follows the same structure and contents, therefore I would recommend this manuscript to be accepted as it is.

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: Ecology and evolution; Comparative 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 22 August 2023

https://doi.org/10.21956/wellcomeopenres.21761.r64227

Niklas Wahlberg
Lund university, Lund, Sweden

This is yet one more Lepidoptera genome put together by the Darwin Tree of Life project. As there are already several hundred such genomes and genome reports, I expect that the major wrinkles in protocols and how to report them have been ironed out. And so it would seem with this genome report. The genome of *Orthosia gracilis* falls within the expected size of species in the so-called "pest clade" of Noctuidae. With 50 Noctuidae genomes already published, and another 90 or so publicly available, this family is poised to become a standard for studying genome evolution in insects. Not much else to say, other than the *Orthosia gracilis* genome looks ready to be used in further analyses!

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: Phylogenomics of Lepidoptera, systematics, molecular phylogenetics.

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.