DATA NOTE

The genome sequence of the brimstone moth, *Opisthograptis luteolata* (Linnaeus, 1758) [version 1; peer review: 2 approved]

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**Abstract**
We present a genome assembly from an individual male *Opisthograptis luteolata* (the brimstone moth; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 363 megabases in span. The majority of the assembly (99.99%) is scaffolded into 31 chromosomal pseudomolecules with the Z sex chromosome assembled. The complete mitochondrial genome was also assembled and is 16.7 kilobases in length.

**Keywords**
Opisthograptis luteolata, brimstone moth, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.
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Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Ennominae; Opisthograptis; Opisthograptis luteolata (Linnaeus, 1758) (NCBI:txid934882).

Background
The brimstone moth, Opisthograptis luteolata (Linnaeus, 1758), is a common, brightly coloured, yellow moth with markings along the leading edge of its wings and on each forewing tip; it is sometimes confused with the Brimstone butterfly due to their similar appearance. Very rare white forms of this species have occasionally been reported. O. luteolata is a nocturnal species found in Western Asia and across the Palearctic region and overwinters as part-grown larvae or in cocoons as pupae. The larvae mostly feed on plants in the Rosaceae family and emerge in two to three generations each year, with some authors suggesting a three-generation pattern over two years (Waring & Townsend, 2017). In The colours of animals, the green form of O. luteolata larvae is used as an example to describe counter-shading in insects (Poulton, 1890). This defensive method was more recently confirmed to be an effective form of crypsis in caterpillars (Rowland et al., 2008). Alternatively, the darker larval forms mimic twigs present on host plant species.

The genome of O. luteolata, 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 O. luteolata, based on the ilOpiLute1 specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report
The genome was sequenced from a single male O. luteolata collected from near Chalet, Wytham, Berkshire, UK (Figure 1). A total of 61-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 93-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 2 missing joins, reducing the assembly size by 0.56% and the scaffold number by 23.26%.

The final assembly has a total length of 363 Mb in 33 sequence scaffolds with a scaffold N50 of 13.2 Mb (Table 1). The majority, 99.99%, of the assembly sequence 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 v5.3.2 (Manni et al., 2021) completeness of 98.3% (single 98.0%, duplicated 0.3%) 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 O. luteolata specimen (ilOpiLute1; genome assembly) was collected using a light trap from near Chalet, Wytham, Berkshire, UK (latitude 51.772, longitude -1.337).
Figure 2. Genome assembly of *Opisthograptis luteolata*, ilOpiLute1.2: 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 363,201,500 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 (16,907,887 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (13,236,533 and 8,601,474 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.genomeweb.hub.org/view/CAKNUS02/dataset/CAKNUS02/snail.

by Douglas Boyes (University of Oxford). The specimen was identified by Douglas Boyes and snap-frozen on dry ice.

A single *O. luteolata* specimen of unknown sex (iLOpiLute2; Hi-C) was collected using a light trap from Wytham Woods, Berkshire, UK (latitude 51.771, longitude -1.337) by Douglas Boyes (University of Oxford). The specimen was identified by Douglas Boyes and snap-frozen on dry ice.

DNA was extracted at the Scientific Operations Core, Wellcome Sanger Institute. The ilOpiLute1 sample was weighed and dissected on dry ice. Whole organism tissue was disrupted by manual grinding in lysis buffer with a disposable 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 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 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 HiSeq (10X) instruments. Hi-C data were generated in the Tree of Life laboratory from abdomen tissue of ilOpiLute2 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

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**Figure 3. Genome assembly of Opisthograpta luteolata, ilOpiLute1.2: 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/CAKNUS02/dataset/CAKNUS02/blob.
Figure 4. Genome assembly of *Opisthograptis luteolata*, ilOpiLute1.2: 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/CAKNUS02/dataset/CAKNUS02/cumulative.

was checked for contamination as described previously (Howe et al., 2021). Manual curation was performed using HiGlass (Kerpedjiev et al., 2018) and Pretext. 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 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
Table 2. Chromosomal pseudomolecules in the genome assembly of *Opisthograptis luteolata*, iiOpiLute1.2.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| OV928034.1      | 1          | 15.55     | 38.3|
| OV928035.1      | 2          | 15.45     | 38.3|
| OV928036.1      | 3          | 14.94     | 38.5|
| OV928037.1      | 4          | 14.88     | 38.5|
| OV928038.1      | 5          | 14.19     | 37.9|
| OV928039.1      | 6          | 14.15     | 38  |
| OV928040.1      | 7          | 13.82     | 38.1|
| OV928041.1      | 8          | 13.61     | 38.1|
| OV928042.1      | 9          | 13.6      | 37.9|
| OV928043.1      | 10         | 13.56     | 37.8|
| OV928044.1      | 11         | 13.33     | 38.5|
| OV928045.1      | 12         | 13.24     | 38.5|
| OV928046.1      | 13         | 13.1      | 38.1|
| OV928047.1      | 14         | 12.78     | 38.1|
| OV928048.1      | 15         | 12.59     | 38.6|
| OV928049.1      | 16         | 12.36     | 38.3|
| OV928050.1      | 17         | 12.03     | 38.5|
| OV928051.1      | 18         | 11.9      | 38.7|
| OV928052.1      | 19         | 11.54     | 38.2|
| OV928053.1      | 20         | 11.06     | 38.8|
| OV928054.1      | 21         | 10.92     | 38.8|
| OV928055.1      | 22         | 9.21      | 38.9|
| OV928056.1      | 23         | 9.1       | 38.5|
| OV928057.1      | 24         | 9.06      | 38.6|
| OV928058.1      | 25         | 8.6       | 38.4|
| OV928059.1      | 26         | 8.1       | 39  |
| OV928060.1      | 27         | 6.58      | 39.3|
| OV928061.1      | 28         | 5.76      | 39.7|
| OV928062.1      | 29         | 5.64      | 40.7|
| OV928063.1      | 30         | 5.53      | 40  |
| OV928033.1      | Z          | 16.91     | 38.1|
| OV928065.1      | MT         | 0.02      | 19  |

*Note: Unplaced pseudomolecules are not shown.*

Figure 5. Genome assembly of *Opisthograptis luteolata*, iiOpiLute1.2: Hi-C contact map. Hi-C contact map of the iiOpiLute1.2 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=caRV68qHQE5sJAWXe18Fg](https://genome-note-higlass.tol.sanger.ac.uk/l/?d=caRV68qHQE5sJAWXe18Fg).
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: Opisthograptis luteolata (brimstone moth). Accession number PRJEB48397; https://identifiers.org/ena embrl/PRJEB48397.

The genome sequence is released openly for reuse. The *O. luteolata* 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.6418202.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.6866293.

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.6125046.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.6418363.

**Table 3. Software tools used.**

| Software tool | Version | Source |
|---------------|---------|--------|
| Hifiasm      | 0.15.3  | Cheng et al., 2021 |
| purge_dups   | 1.2.3   | Guan et al., 2020 |
| YaHS         | 1.0     | Zhou et al., 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 et al., 2021 |
| HiGlass      | 1.11.6  | Kerpedjiev et al., 2018 |
| PretextView  | 0.2.x   | https://github.com/wtsi-hpag/ PretextView |
| BlobToolKit  | 3.2.6   | Challis et al., 2020 |

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Challis R, Richards E, Rajan J, et al.: BlobToolKit-Interactive Quality Assessment of Genome Assemblies. *G3 (Bethesda)*, 2020; 10(4): 1:861–74. PubMed Abstract | Publisher Full Text | Free Full Text

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PretextView | Free Full Text

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Zhou C, McCarthy SA, Durbin R: YaHS: Yet Another Hi-C Scaffolding Tool. bioRxiv. 2022. Publisher Full Text

| Reference Sourc | Reference Source |
|-----------------|------------------|
| https://doi.org/10.5281/zenodo.6418202 | Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.6418202. |
| https://doi.org/10.5281/zenodo.6866293 | Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.6866293. |
| https://doi.org/10.5281/zenodo.5746904 | Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.5746904. |
| https://doi.org/10.5281/zenodo.6125046 | Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.6125046. |
| https://doi.org/10.5281/zenodo.6418363 | Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.6418363. |
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In this article, the genomes of *Opisthograptis luteolata* (Linnaeus, 1758) were sequenced. The nuclear genome is 363 megabases and mitochondrial genome is 16.7 kilobases in length. Most of the assembly sequence were assigned to 31 chromosomal-level scaffolds which were corresponding to 30 autosomes and one sex chromosome. These are helpful for us to understand the moth.

**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:** Animal 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.
The genome sequence of the brimstone moth, *Opisthograptis luteolata* (Linnaeus, 1758)

The authors present a genome assembly from *Opisthograptis luteolata* (Geometridae), commonly known as the brimstone moth. The genome was sequenced from a fresh male sample collected near Chalet, Wytham, Berkshire, UK. The DNA extraction, library preparation and sequencing procedures followed the latest protocols in genome processing. The genome assembly was carried out with HiFiasm and following well known protocols. The genome sequencing resulted in 363 MB in 33 sequence scaffolds. As stated by the authors, the majority of the assembly (99.99%) is scaffolded into 31 chromosomal pseudomolecules with the Z sex chromosome assembled. The complete mitochondrial genome was also assembled and is 16.7 kilobases in length. Moreover, the data was compared to the lepidoptera_obd10 reference set from BUSCO dataset and they retrieved 98.3% of completeness. In figure 2, the authors present a great summary of the results. The plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 363,201,500 bp assembly. I do not have any further comments, and I encourage the publication of this research. I personally congratulate the authors for the initiative and collaborative effort that will be available to researchers and public interested in moths.

**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:** Molecular systematics

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.