DATA NOTE

# The genome sequence of the Seraphim, *Lobophora halterata* (Hufnagel, 1767) [version 1; peer review: 2 approved]

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## Abstract

We present a genome assembly from an individual female *Lobophora halterata* (the Seraphim; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 315 megabases in span. The complete assembly is scaffolded into 32 chromosomal pseudomolecules with the Z and W sex chromosomes assembled. The mitochondrial genome has also been assembled and is 15.7 kilobases in length.

## Keywords

Lobophora halterata, the Seraphim, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

**Open Peer Review**

**Approval Status** ✓ ✓

| | 1 | 2 |
|---|---|---|
| **version 1** 23 Dec 2022 | ✓ view | ✓ view |

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2. **Satoshi Yamamoto**, Institute for Agro-Environmental Sciences, NARO (NIAES), Tsukuba, Japan

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

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

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## Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Geometroidea; Geometridae; Larentiinae; *Lobophora; Lobophora halterata* (Hufnagel, 1767) (NCBI: txid934876).

## Background

*Lobophora halterata* (the Seraphim) is a delicately patterned moth in the family Geometridae. The adult has a series of wavy grey and brown stripes across broad white forewings, which at rest form effective camouflage against tree bark. The moth is found across central and northern Europe, with scattered records from Russia and Japan (GBIF Secretariat, 2021). *L. halterata* has a single generation per year in the UK: larvae feed on aspen and polar, overwintering occurs as a pupa, and adults have a short flight period in May and June (Waring & Townsend, 2017). Males of this species have a particularly unusual hindwing feature that explains the origin of the common name and the scientific name. The common name refers to a type of angel in Jewish, Christian and Islamic texts; the seraphim are generally described as six-winged angels that act as guardians of the throne of God (Holland, 2012). The Seraphim moth does not actually have six wings, but there is a 'concertina' or Z-fold on the trailing edge of hind wing giving the impression of a small third pair of wings lying on top of the hindwings. There is also error in entomological etymology: in religious texts seraphim is the plural of seraph, whereas in the moth the plural term has become singular (Holland, 2012). The genus name *Lobophora* refers to this 'lobe' of wing tissue, as does the specific name *halterata* which draws comparison to the lobe-like halteres of Diptera (Maitland Emmet, 1991). The function of the unusual hindwing lobe of *L. halterata* is unknown, although the fact it is restricted to males suggests it likely to have a sex-specific role, potentially associated with a scent organ (Hobby, 2009). The developmental genetic basis of the morphological feature is entirely unknown.

The genome of *L. halterata* was sequenced as part of the Darwin Tree of Life Project, a collaborative effort to sequence all named eukaryotic species in the Atlantic Archipelago of Britain and Ireland. Here we present a chromosomally complete genome sequence for *L. halterata*, based on the ilLobHalt1 specimen from Wytham Woods, Oxfordshire, UK.

### Genome sequence report

The genome was sequenced from an individual female *L. halterata* (Figure 1) collected from Wytham Woods, Berkshire, UK (51.77, –1.34). A total of 72-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 four missing or mis-joins, reducing the scaffold number by 10.53%.

The final assembly has a total length of 314.9 Mb in 34 sequence scaffolds with a scaffold N50 of 10.9 Mb


**Figure 1. Photograph of the *Lobophora halterata* (ilLobHalt1) specimen used for genome sequencing.**

(Table 1). The complete assembly sequence was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes and the W and Z sex chromosomes. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size. (Figure 2–Figure 5; Table 2). The mitochondrial genome was also assembled. 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. Evaluation of the assembly shows a consensus quality value (QV) of 71.9 and *k*-mer completeness of 100%. 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 male *L. halterata* (ilLobHalt1) was collected using a light trap from Wytham Woods, Berkshire, UK (latitude 51.77, longitude –1.34) by Douglas Boyes (University of Oxford). The sample was identified by Douglas Boyes and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ilLobHalt1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Abdomen 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 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.

**Table 1. Genome data for *Lobophora halterata*, ilLobHalt1.1.**

| **Project accession data** | | |
|---|---|---|
| Assembly identifier | ilLobHalt1.1 | |
| Species | *Lobophora halterata* | |
| Specimen | ilLobHalt1 | |
| NCBI taxonomy ID | 934876 | |
| BioProject | PRJEB50743 | |
| BioSample ID | SAMEA7520514 | |
| Isolate information | female; thorax (PacBio sequencing), head (Hi-C) | |
| **Assembly metrics*** | | ***Benchmark*** |
| Consensus quality (QV) | 71.9 | *≥ 50* |
| *k*-mer completeness | 100% | *≥ 95%* |
| BUSCO** | C:98.3%[S:98.0%,D:0.3%], F:0.4%,M:1.3%,n:5286 | *C ≥ 95%* |
| Percentage of assembly mapped to chromosomes | 100% | *≥ 95%* |
| Sex chromosomes | Z and W sex chromosomes | *localised homologous pairs* |
| Organelles | Mitochondrial genome assembled | *complete single alleles* |
| **Raw data accessions** | | |
| PacificBiosciences SEQUEL II | ERR8575379 | |
| Hi-C Illumina | ERR8571665 | |
| **Genome assembly** | | |
| Assembly accession | GCA_945859715.1 | |
| *Accession of alternate haplotype* | GCA_945859755.1 | |
| Span (Mb) | 314.9 | |
| Number of contigs | 38 | |
| Contig N50 length (Mb) | 10.9 | |
| Number of scaffolds | 34 | |
| Scaffold N50 length (Mb) | 10.9 | |
| Longest scaffold (Mb) | 12.9 | |

*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/ilLobHalt1.1/dataset/CAKOAX01/busco.

## Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on the Pacific Biosciences SEQUEL II (HiFi) instrument. Hi-C data were also generated from head/thorax tissue of ilLobHalt1 using the Arima v2 kit and sequenced on the Illumina HiSeq X Ten instrument.

## Genome assembly

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 scaffolded

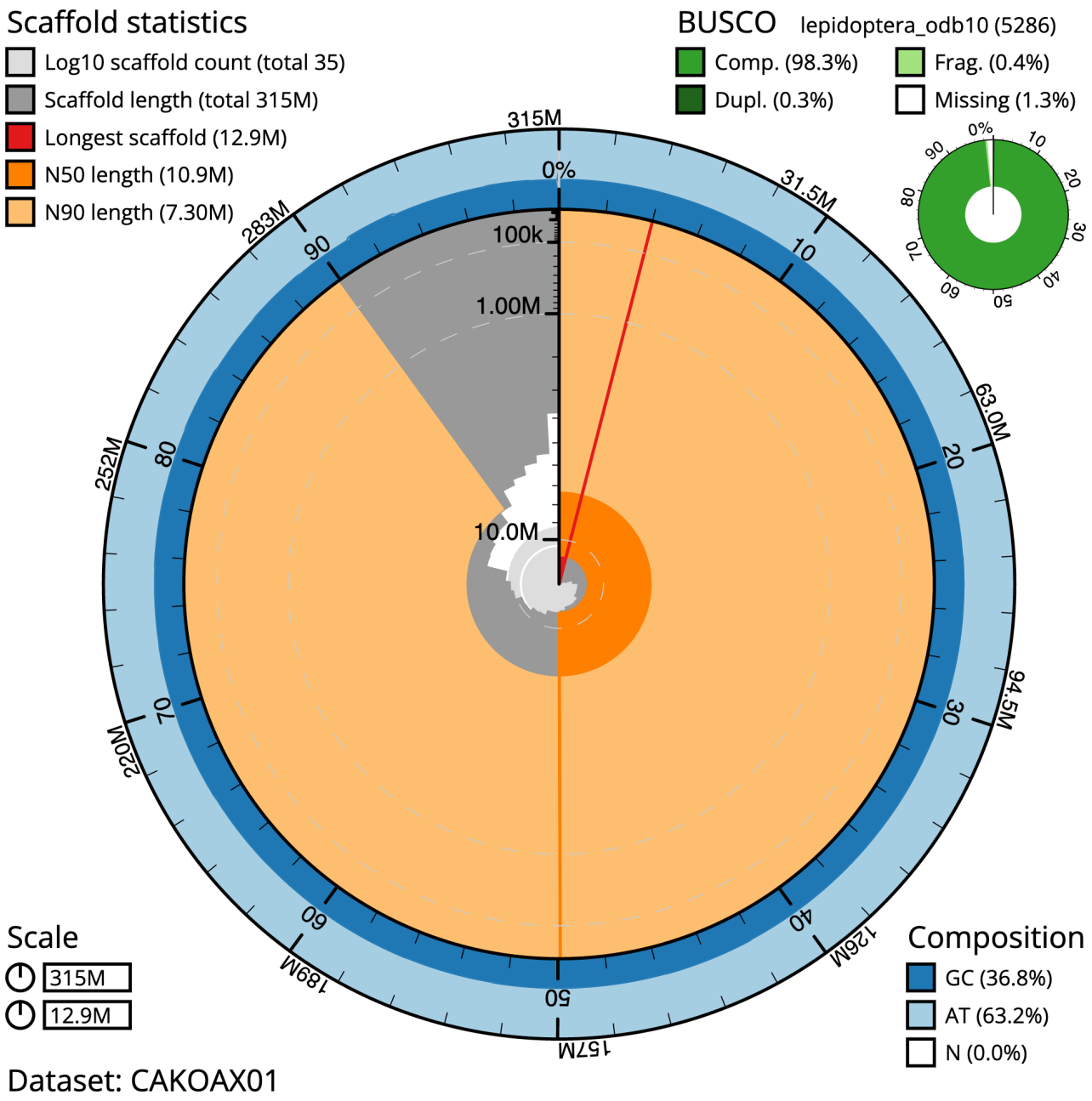


**Figure 2. Genome assembly of *Lobophora halterata*, ilLobHalt1.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 314,876,238 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (12,870,039 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (10,931,577 and 7,303,528 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/ilLobHalt1.1/dataset/CAKOAX01/snail.

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 Pretext (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021), which performed

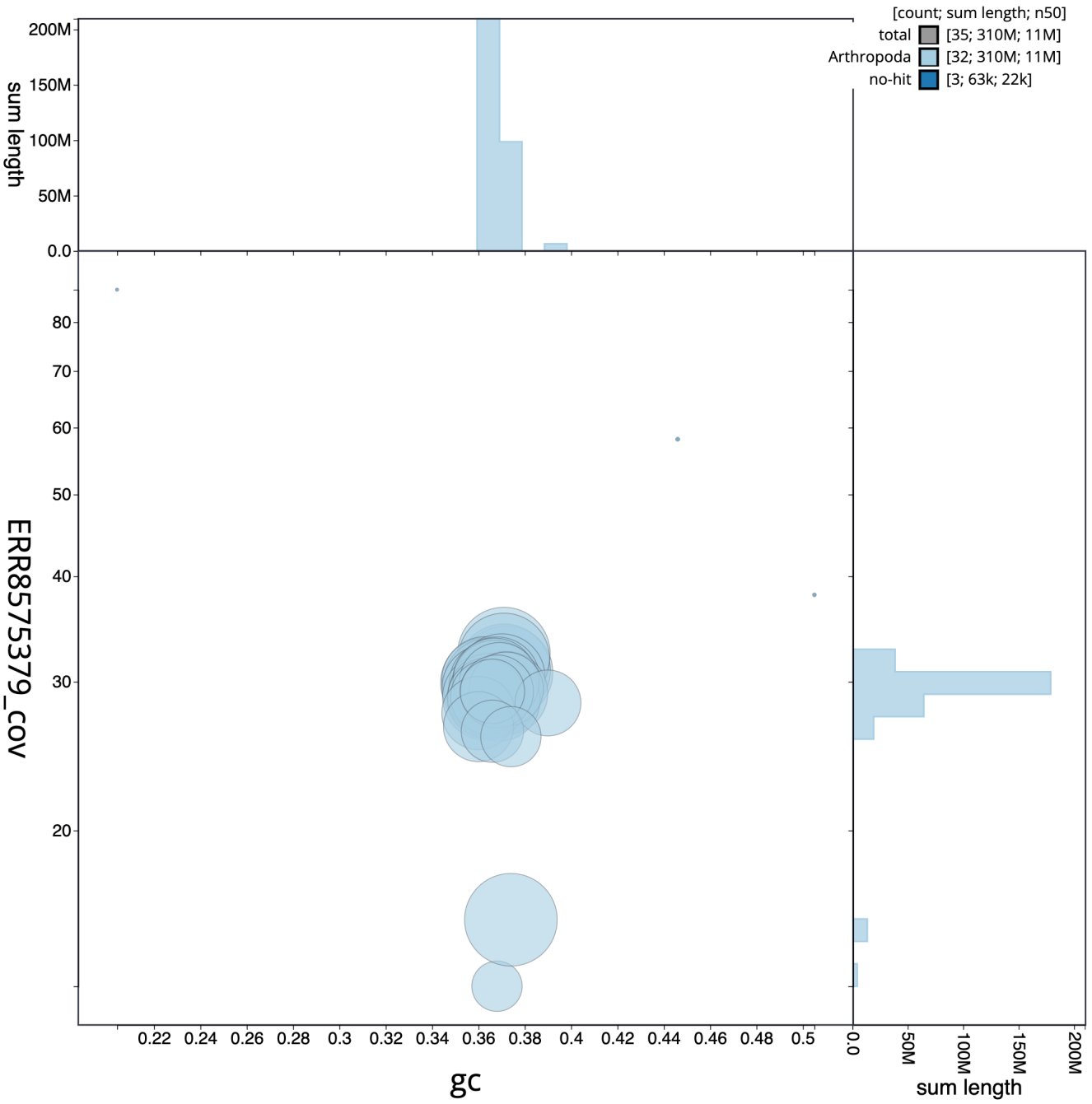


**Figure 3. Genome assembly of *Lobophora halterata*, ilLobHalt1.1: GC coverage.** BlobToolKit GC-coverage plot. Chromosomes are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilLobHalt1.1/dataset/CAKOAX01/blob.

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

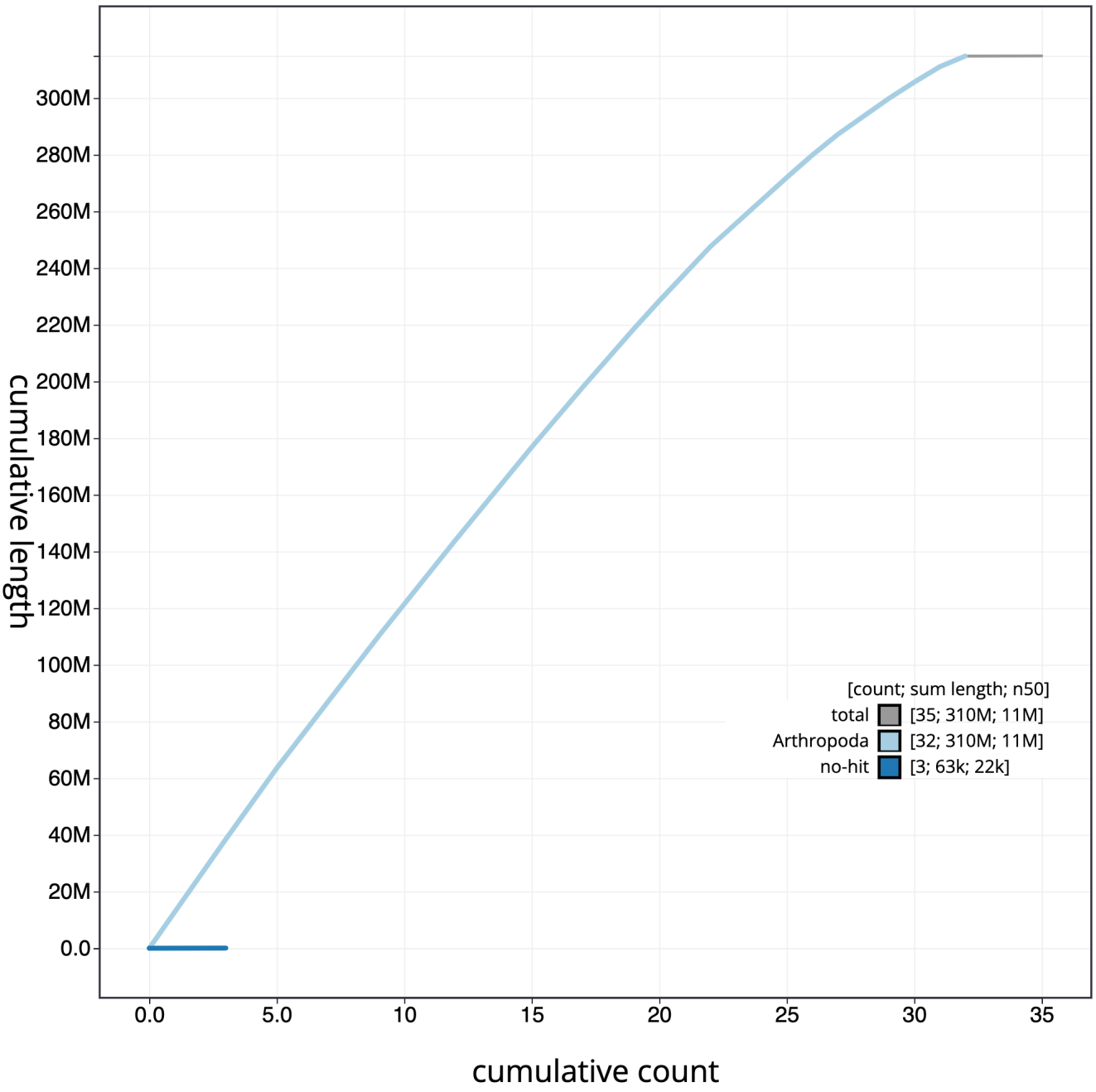


**Figure 4. Genome assembly of *Lobophora halterata*, ilLobHalt1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilLobHalt1.1/dataset/CAKOAX01/cumulative.

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.

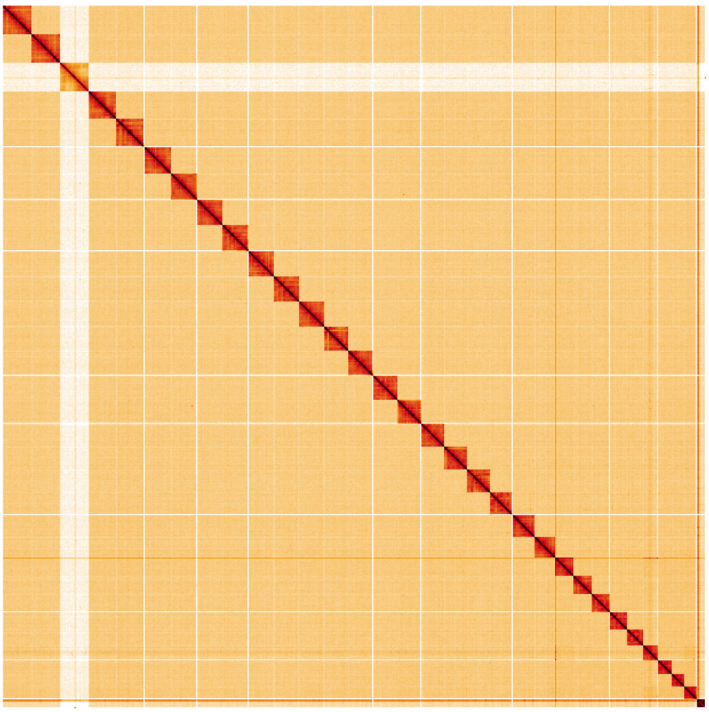

**Figure 5. Genome assembly of *Lobophora halterata*, ilLobHalt1.1: Hi-C contact map.** Hi-C contact map of the ilLobHalt1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=T1L-6HBOROe3dhzQyJ_IRQ.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Lobophora halterata*, ilLobHalt1.**

| INSDC accession | Chromosome | Size (Mb) | GC% |
|---|---|---|---|
| OW052001.1 | 1 | 12.87 | 37.2 |
| OW052002.1 | 2 | 12.81 | 37.1 |
| OW052004.1 | 3 | 12.7 | 37.1 |
| OW052005.1 | 4 | 12.41 | 37.1 |
| OW052006.1 | 5 | 11.92 | 36.3 |
| OW052007.1 | 6 | 11.58 | 36.8 |
| OW052008.1 | 7 | 11.57 | 36.7 |
| OW052009.1 | 8 | 11.53 | 36.4 |
| OW052010.1 | 9 | 11.28 | 36.3 |
| OW052011.1 | 10 | 11.24 | 36.3 |
| OW052012.1 | 11 | 11.24 | 37 |
| OW052013.1 | 12 | 11.05 | 36.6 |
| OW052014.1 | 13 | 10.93 | 36.8 |
| OW052015.1 | 14 | 10.93 | 36.3 |
| OW052016.1 | 15 | 10.61 | 36.7 |
| OW052017.1 | 16 | 10.54 | 36.5 |
| OW052018.1 | 17 | 10.3 | 36.7 |
| OW052019.1 | 18 | 10.26 | 37.2 |
| OW052020.1 | 19 | 9.87 | 36.9 |
| OW052021.1 | 20 | 9.71 | 36.4 |
| OW052022.1 | 21 | 9.41 | 36.9 |
| OW052023.1 | 22 | 8.24 | 36.4 |
| OW052024.1 | 23 | 8.19 | 37.2 |
| OW052025.1 | 24 | 8 | 36 |
| OW052026.1 | 25 | 7.92 | 36.8 |
| OW052027.1 | 26 | 7.3 | 36 |
| OW052028.1 | 27 | 6.47 | 39 |
| OW052029.1 | 28 | 6.2 | 36.6 |
| OW052030.1 | 29 | 5.77 | 36.6 |
| OW052031.1 | 30 | 5.42 | 37.4 |
| OW052032.1 | W | 3.78 | 36.8 |
| OW052003.1 | Z | 12.77 | 37.4 |
| OW052033.1 | MT | 0.02 | 20.5 |
| - | - | 0.05 | 47.3 |

**Table 3. Software tools and versions used.**

| Software tool | Version | Source |
|---|---|---|
| BlobToolKit | 3.4.0 | Challis *et al.*, 2020 |
| Hifiasm | 0.16.1-r375 | Cheng *et al.*, 2021 |
| HiGlass | 1.11.6 | Kerpedjiev *et al.*, 2018 |
| MitoHiFi | 2 | Uliano-Silva *et al.*, 2021 |
| PretextView | 0.2 | Harry, 2022 |
| purge_dups | 1.2.3 | Guan *et al.*, 2020 |
| YaHS | yahs-1.1.91eebc2 | Zhou *et al.*, 2022 |

## Data availability

European Nucleotide Archive: *Lobophora halterata* (the seraphim). Accession number PRJEB50743; https://identifiers.org/ena.embl/PRJEB50743 (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Lobophora halterata* 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 16 January 2023

https://doi.org/10.21956/wellcomeopenres.20751.r53775



✔ **Satoshi Yamamoto**
Institute for Agro-Environmental Sciences, NARO (NIAES), Tsukuba, Japan

This study presents a *de novo* genome assembly of *Lobophora halterata* (Lepidoptera; Geometridae) using a high coverage HiFi long read dataset. Scaffolding with Hi-C data subsequently yielded 32 chromosome-scale scaffolds. The assembly techniques employed conform to standard genome assembly methods, and the resulting chromosomal count is consistent with previous reports on chromosomes of geometrid species (for example, Suomalainen 1965[1]). As such, the assembly can be considered credible.

However, the methodology section does not sufficiently detail the methods employed for quality control of raw reads, the options and parameters utilized in the assembly program, or the approach for identifying sex chromosomes.

All data generated in this study have been deposited in public databases for accessibility. This study is deemed valuable given the relative scarcity of chromosome-scale genome assemblies for geometrid species.

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

**Are the protocols appropriate and is the work technically sound?**
Partly

**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:*** Evolutionary biology

**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 10 January 2023

https://doi.org/10.21956/wellcomeopenres.20751.r53776



**Yandong Ren**
College of Life Sciences, Shaanxi Normal University, Xi'an, China

This manuscript provided us a high-quality *Lobophora halterata* genome. The protocols are well described and the datasets are clearly presented. However, the details of methods and materials are not sufficient. How was the library constructed? What's the version of the software used in this manuscript? More detail should be added in the revised manuscript. Besides, I know this manuscript is a data note paper, the following analysis still need added:

1. The authors performed k-mer analysis. So what software was used in this analysis. The k-mer results should also be showed in the results part.

2. How about the N50 and the mean length of the raw reads.

3. How about the genome quality of other Geometridae species? The authors should added a table with the genome quality of all the published Geometridae genome.

**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:*** 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.**