




DATA NOTE

# The genome sequence of the Scorched Carpet, *Ligdia adustata* (Denis & Schiffermüller, 1775) [version 1; peer review: awaiting peer review]

Douglas Boyes<sup>1+</sup>, Owen T. Lewis <sup>2</sup>,  
University of Oxford and Wytham Woods Genome Acquisition Lab,  
Darwin Tree of Life Barcoding collective,  
Wellcome Sanger Institute Tree of Life Management, Samples and Laboratory  
team,  
Wellcome Sanger Institute Scientific Operations: Sequencing Operations,  
Wellcome Sanger Institute Tree of Life Core Informatics team,  
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

<sup>1</sup>UK Centre for Ecology & Hydrology, Wallingford, England, UK

<sup>2</sup>University of Oxford, Oxford, England, UK

+ Deceased author

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## Open Peer Review

**Approval Status** AWAITING PEER REVIEW

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

## Abstract

We present a genome assembly from an individual female *Ligdia adustata* (the Scorched Carpet; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 399.7 megabases in span. Most of the assembly is scaffolded into 31 chromosomal pseudomolecules, including the Z sex chromosome. The mitochondrial genome has also been assembled and is 15.43 kilobases in length. Gene annotation of this assembly on Ensembl identified 17,979 protein coding genes.

## Keywords

*Ligdia adustata*, Scorched Carpet, genome sequence, chromosomal, Lepidoptera



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

**Corresponding author:** Darwin Tree of Life Consortium ([mark.blaxter@sanger.ac.uk](mailto:mark.blaxter@sanger.ac.uk))

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## Species taxonomy

Eukaryota; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphimesenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Ennominae; *Ligdia*; *Ligdia adustata* (Denis & Schiffermuller, 1775) (NCBI:txid934875).

## Background

The Scorched Carpet (*Ligdia adustata*) is a distinctive geometrid moth and is the only representative of its genus found in Britain and Ireland. Its larvae are monophagous on the foliage of spindle (*Euonymus europaeus*).

Adults of the Scorched Carpet have two generations in most parts of Britain and Ireland, and it overwinters as a pupa (Waring *et al.*, 2017). The first generation of this moth now flies several weeks earlier in the year than in the 1970s (Randle *et al.*, 2019).

The Scorched Carpet is found in the southern half of Britain, and across much of Ireland, where it has a more thinly scattered distribution. Its range extends across much of Europe to the eastern Mediterranean and central Asia (GBIF Secretariat, 2023).

The genome of the scorched carpet, *Ligdia adustata*, 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 *Ligdia adustata*, based on one female specimen from Wytham Woods, Oxfordshire.

## Genome sequence report

The genome was sequenced from one female *Ligdia adustata* (Figure 1) collected from Wytham Woods, Oxfordshire, UK (51.77, -1.32). A total of 49-fold coverage in Pacific



**Figure 1.** Photograph of the *Ligdia adustata* (ilLigAdus1) specimen used for genome sequencing.

Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 10 missing joins or mis-joins and removed one haplotypic duplication, reducing the scaffold number by 5.26%.

The final assembly has a total length of 399.7 Mb in 35 sequence scaffolds with a scaffold N50 of 14.3 Mb (Table 1). The snailplot in Figure 2 provides a summary of the assembly statistics, while the distribution of assembly scaffolds on GC proportion and coverage is shown in Figure 3. The cumulative assembly plot in Figure 4 shows curves for subsets of scaffolds assigned to different phyla. Most (99.97%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Read coverage across Z chromosome is approximately half average, no W chromosome can be found, so the specimen is likely to be female ZO karyotype. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (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 65.2 with *k*-mer completeness of 200%, and the assembly has a BUSCO v5.3.2 completeness of 98.6% (single = 98.1%, duplicated = 0.5%), using the lepidoptera\_odb10 reference set ( $n = 5,286$ ).

Metadata for specimens, barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at <https://links.tol.sanger.ac.uk/species/934875>.

## Genome annotation report

The *Ligdia adustata* genome assembly (GCA\_947049295.1) was annotated using the Ensembl rapid annotation pipeline (Table 1; [https://rapid.ensembl.org/Ligdia\\_adustata\\_GCA\\_947049295.1/Info/Index](https://rapid.ensembl.org/Ligdia_adustata_GCA_947049295.1/Info/Index)). The resulting annotation includes 18,142 transcribed mRNAs from 17,979 protein-coding genes.

## Methods

### Sample acquisition and nucleic acid extraction

A female *Ligdia adustata* (specimen ID Ox001877, ToLID ilLigAdus1) was collected from Wytham Woods, Oxfordshire (biological vice-country Berkshire), UK (latitude 51.77, longitude -1.32) on 2021-05-28 using a light trap. The specimen was collected and identified by Douglas Boyes (University of Oxford) and preserved on dry ice.

The workflow for high molecular weight (HMW) DNA extraction at the Wellcome Sanger Institute (WSI) includes a sequence of core procedures: sample preparation; sample homogenisation; DNA extraction; HMW DNA fragmentation; and fragmented DNA clean-up. The sample was prepared for DNA extraction at the WSI Tree of Life core laboratory: the ilLigAdus1 sample was weighed and dissected on dry ice

**Table 1. Genome data for *Ligdia adustata*, iLigAdus1.1.**

| <b>Project accession data</b>                |  |                                   |
|--|--|-----------------------------------|
| Assembly identifier                          | iLigAdus1.1  |                                   |
| Species                                      | <i>Ligdia adustata</i>   |                                   |
| Specimen                                     | iLigAdus1  |                                   |
| NCBI taxonomy ID                             | 934875   |                                   |
| BioProject                                   | PRJEB55023   |                                   |
| BioSample ID                                 | SAMEA10979137  |                                   |
| Isolate information                          | iLigAdus1, female: head and thorax (DNA and Hi-C), abdomen (RNA) |                                   |
| <b>Assembly metrics*</b>                     |  | <b>Benchmark</b>                  |
| Consensus quality (QV)                       | 65.2   | ≥ 50                              |
| <i>k</i> -mer completeness                   | 100%   | ≥ 95%                             |
| BUSCO**                                      | C:98.6%[S:98.1%,D:0.5%],F:0.4%,M:1.0%,n:5,286                    | C ≥ 95%                           |
| Percentage of assembly mapped to chromosomes | 99.97%   | ≥ 95%                             |
| Sex chromosomes                              | Z chromosome   | <i>localised homologous pairs</i> |
| Organelles                                   | Mitochondrial genome assembled                                   | <i>complete single alleles</i>    |
| <b>Raw data accessions</b>                   |  |                                   |
| PacificBiosciences SEQUEL II                 | ERR10008904  |                                   |
| Hi-C Illumina                                | ERR10015061  |                                   |
| PolyA RNA-Seq Illumina                       | ERR10890700  |                                   |
| <b>Genome assembly</b>                       |  |                                   |
| Assembly accession                           | GCA_947049295.1  |                                   |
| <i>Accession of alternate haplotype</i>      | GCA_947049285.1  |                                   |
| Span (Mb)                                    | 399.7  |                                   |
| Number of contigs                            | 53   |                                   |
| Contig N50 length (Mb)                       | 12.9   |                                   |
| Number of scaffolds                          | 35   |                                   |
| Scaffold N50 length (Mb)                     | 14.3   |                                   |
| Longest scaffold (Mb)                        | 18.4   |                                   |
| <b>Genome annotation</b>                     |  |                                   |
| Number of protein-coding genes               | 17,979   |                                   |
| Number of gene transcripts                   | 18,142   |                                   |

\* 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).

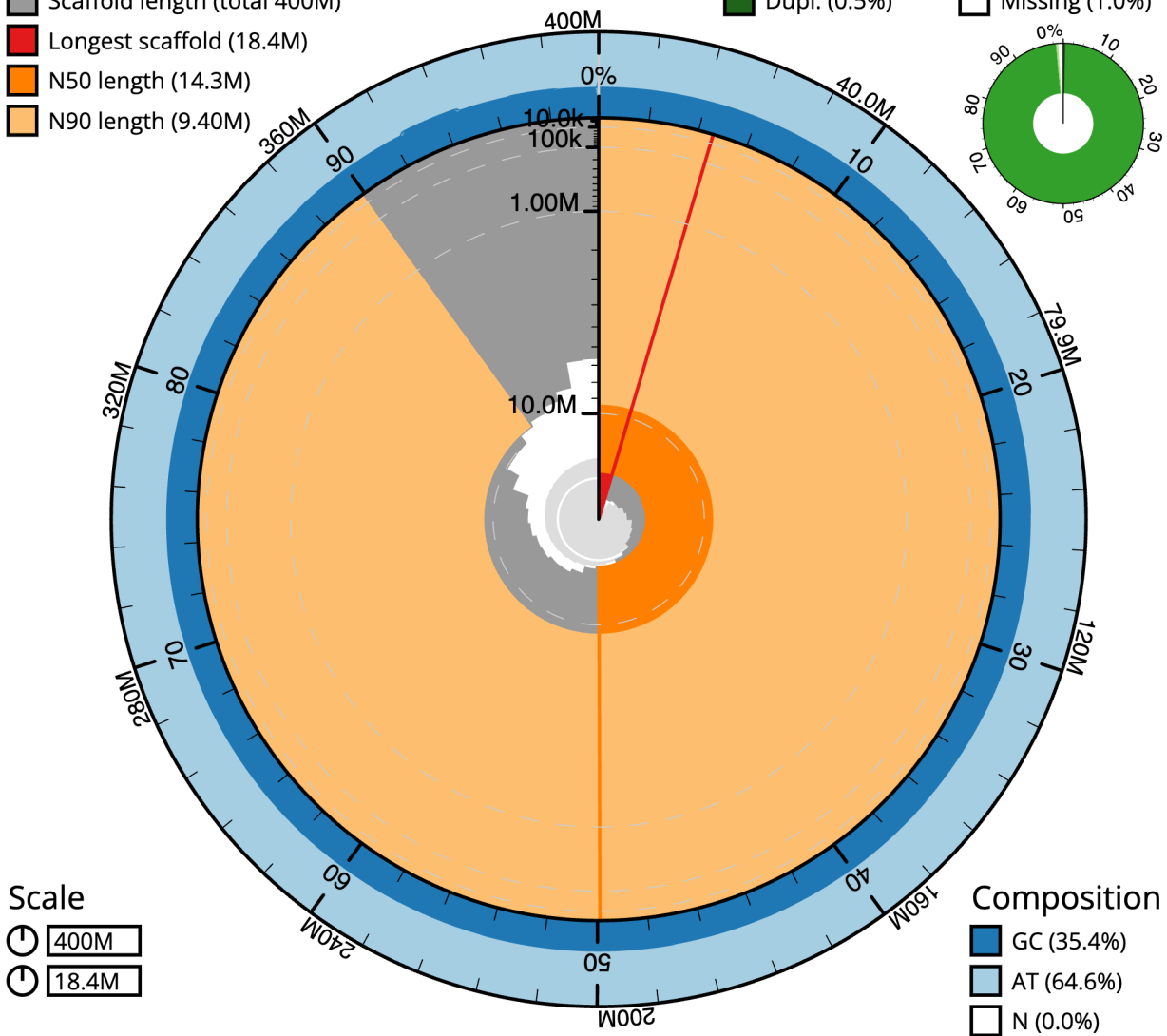
\*\* BUSCO scores based on the lepidoptera\_odb10 BUSCO set using v5.3.2. C = complete [S = single copy, D = duplicated], F = fragmented, M = missing, n = number of orthologues in comparison. A full set of BUSCO scores is available at <https://blobtoolkit.genomehubs.org/view/iLigAdus1.1/dataset/CAMRIO01/busco>.

## Scaffold statistics

- Log10 scaffold count (total 36)
- Scaffold length (total 400M)
- Longest scaffold (18.4M)
- N50 length (14.3M)
- N90 length (9.40M)

## BUSCO lepidoptera\_odb10 (5286)

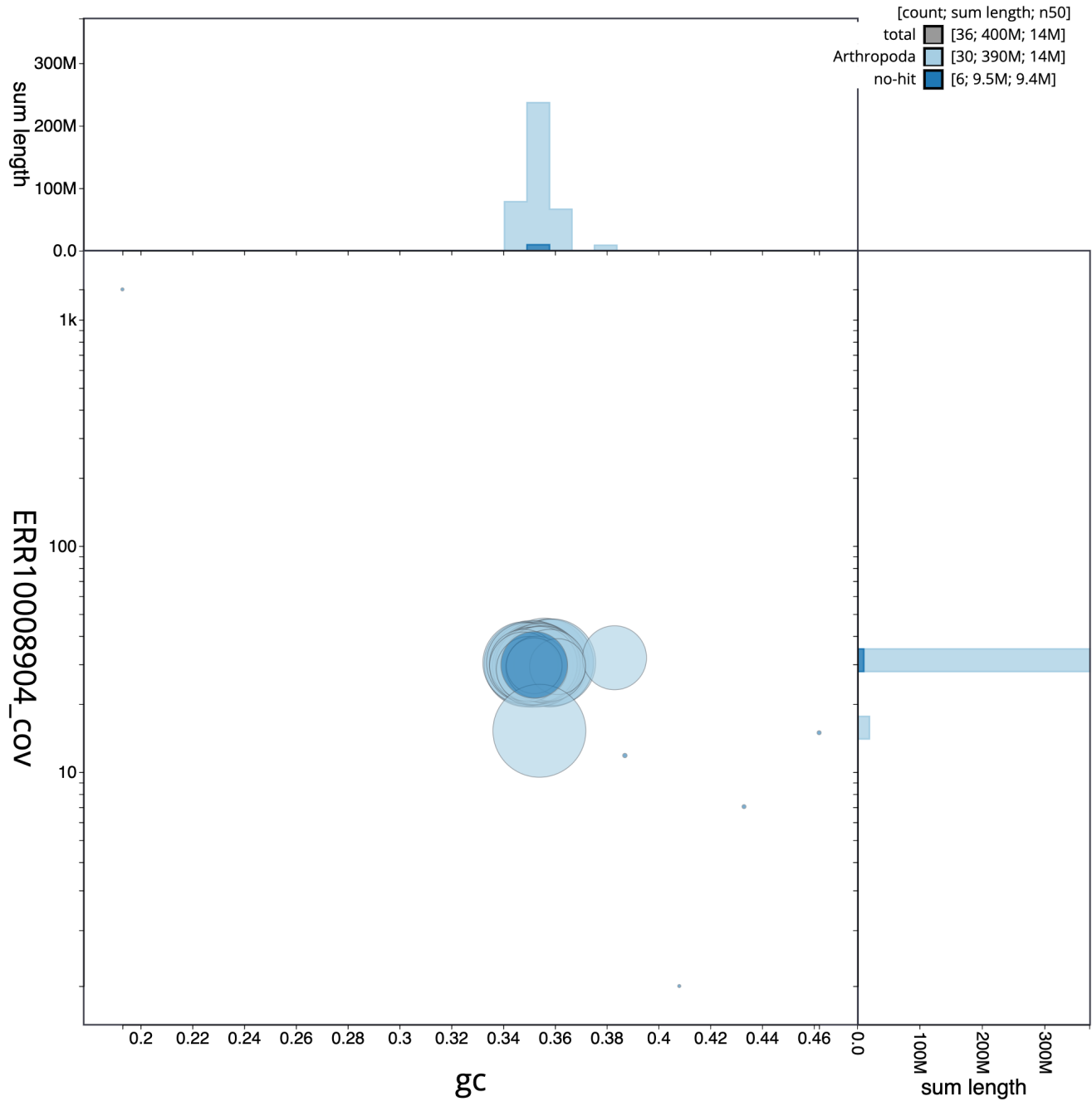
- Comp. (98.6%)
- Frag. (0.4%)
- Dupl. (0.5%)
- Missing (1.0%)



**Figure 2. Genome assembly of *Ligdia adustata*, iLigAdus1.1: 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 399,726,061 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 (18,410,582 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (14,256,869 and 9,403,506 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/iLigAdus1.1/dataset/CAMRIO01/snail>.

with tissue set aside for RNA and Hi-C sequencing (Jay *et al.*, 2023). Tissue from the head and thorax was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted in the WSI Scientific Operations core using the Automated MagAttract v2 protocol (Oatley *et al.*, 2023). The DNA was sheared into an average

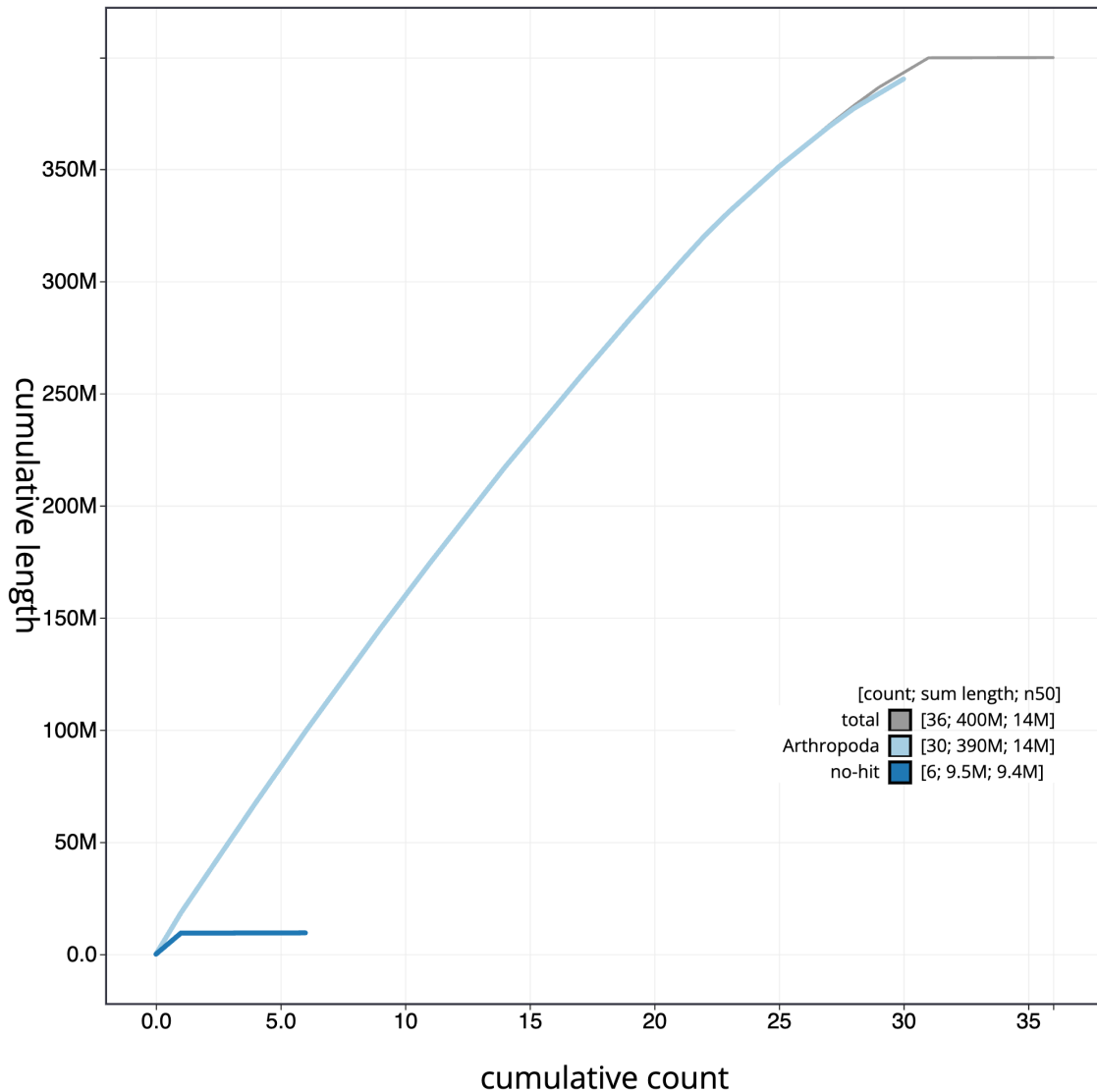
fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 31 (Bates *et al.*, 2023). Sheared DNA was purified by solid-phase reversible immobilisation (Strickland *et al.*, 2023): in brief, the method employs a 1.8X ratio of AMPure PB beads to sample to eliminate shorter fragments and concentrate the DNA. The concentration of the sheared and



**Figure 3. Genome assembly of *Ligdia adustata*, iLigAdus1.1: 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/iLigAdus1.1/dataset/CAMRIO01/blob>.

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.

RNA was extracted from abdomen tissue of iLigAdus1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana protocol (do Amaral *et al.*, 2023). The RNA concentration was assessed using a Nanodrop



**Figure 4. Genome assembly of *Ligdia adustata*, iLigAdus1.1: 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/iLigAdus1.1/dataset/CAMRIO01/cumulative>.

spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Protocols developed by the WSI Tree of Life laboratory are publicly available on protocols.io (Denton *et al.*, 2023b).

#### Sequencing

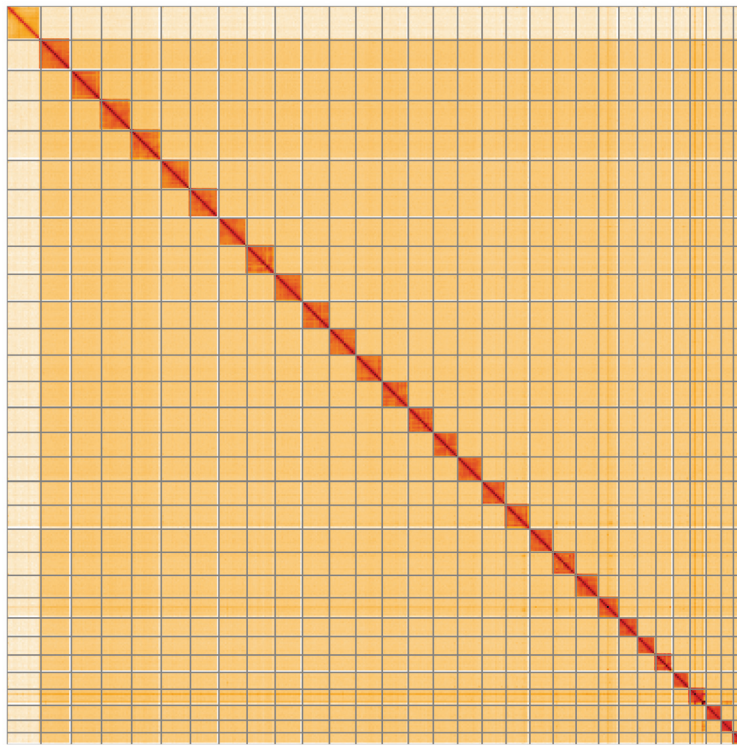
Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed

using the NEB Ultra II RNA Library Prep kit. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) and Illumina NovaSeq 6000 (RNA-Seq) instruments. Hi-C data were also generated from remaining head and thorax tissue of iLigAdus1 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





**Figure 5. Genome assembly of *Ligdia adustata*, iLigAdus1.1: Hi-C contact map of the iLigAdus1.1 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/I/?d=FvbNo4trTae9zL6sf7WRgQ>.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Ligdia adustata*, iLigAdus1.**

| INSDC accession | Chromosome | Length (Mb) | GC%  |
|-----------------|------------|-------------|------|
| OX346214.1      | 1          | 16.46       | 35.5 |
| OX346215.1      | 2          | 16.31       | 36.0 |
| OX346216.1      | 3          | 16.3        | 36.0 |
| OX346217.1      | 4          | 16.0        | 36.0 |
| OX346218.1      | 5          | 15.82       | 35.0 |
| OX346219.1      | 6          | 15.39       | 35.0 |
| OX346220.1      | 7          | 15.25       | 35.0 |
| OX346221.1      | 8          | 15.15       | 35.0 |
| OX346222.1      | 9          | 14.79       | 35.5 |
| OX346223.1      | 10         | 14.58       | 35.0 |
| OX346224.1      | 11         | 14.31       | 35.0 |
| OX346225.1      | 12         | 14.26       | 35.5 |
| OX346226.1      | 13         | 14.06       | 35.5 |
| OX346227.1      | 14         | 13.54       | 35.0 |
| OX346228.1      | 15         | 13.23       | 35.0 |

| INSDC accession | Chromosome | Length (Mb) | GC%  |
|-----------------|------------|-------------|------|
| OX346229.1      | 16         | 13.19       | 35.5 |
| OX346230.1      | 17         | 12.97       | 35.0 |
| OX346231.1      | 18         | 12.9        | 35.0 |
| OX346232.1      | 19         | 12.6        | 35.5 |
| OX346233.1      | 20         | 12.41       | 35.5 |
| OX346234.1      | 21         | 12.17       | 35.5 |
| OX346235.1      | 22         | 10.96       | 36.0 |
| OX346236.1      | 23         | 10.04       | 35.0 |
| OX346237.1      | 24         | 10.0        | 35.0 |
| OX346238.1      | 25         | 9.4         | 35.0 |
| OX346239.1      | 26         | 9.08        | 35.0 |
| OX346240.1      | 27         | 8.7         | 38.5 |
| OX346241.1      | 28         | 8.11        | 35.5 |
| OX346242.1      | 29         | 6.63        | 36.0 |
| OX346243.1      | 30         | 6.58        | 35.0 |
| OX346213.1      | Z          | 18.41       | 35.5 |
| OX346244.1      | MT         | 0.02        | 19.5 |



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). 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.*, 2023), 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.

#### Genome annotation

The BRAKER2 pipeline (Brûna *et al.*, 2021) was used in the default protein mode to generate annotation for the *Ligdia adustata* assembly (GCA\_947049295.1) in Ensembl Rapid Release.

#### 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 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            | <a href="https://github.com/blobtoolkit/blobtoolkit">https://github.com/blobtoolkit/blobtoolkit</a>                     |
| BUSCO                  | 5.3.2            | <a href="https://gitlab.com/ezlab/busco">https://gitlab.com/ezlab/busco</a>   |
| Hifiasm                | 0.16.1-r375      | <a href="https://github.com/chhylp123/hifiasm">https://github.com/chhylp123/hifiasm</a>                                 |
| HiGlass                | 1.11.6           | <a href="https://github.com/higlass/higlass">https://github.com/higlass/higlass</a>                                     |
| Merqury                | MerquryFK        | <a href="https://github.com/thegenemyers/MERQURY.FK">https://github.com/thegenemyers/MERQURY.FK</a>                     |
| MitoHiFi               | 2                | <a href="https://github.com/marcelauliano/MitoHiFi">https://github.com/marcelauliano/MitoHiFi</a>                       |
| PretextView            | 0.2              | <a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>                         |
| purge_dups             | 1.2.3            | <a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a>                                 |
| sanger-tol/genomenote  | v1.0             | <a href="https://github.com/sanger-tol/genomenote">https://github.com/sanger-tol/genomenote</a>                         |
| sanger-tol/readmapping | 1.1.0            | <a href="https://github.com/sanger-tol/readmapping/tree/1.1.0">https://github.com/sanger-tol/readmapping/tree/1.1.0</a> |
| YaHS                   | yahs-1.1.91eebc2 | <a href="https://github.com/c-zhou/yahs">https://github.com/c-zhou/yahs</a>   |

## Data availability

European Nucleotide Archive: *Ligdia adustata* (scorched carpet). Accession number PRJEB55023; <https://identifiers.org/ena.embl/PRJEB55023> (Wellcome Sanger Institute, 2022). The genome sequence is released openly for reuse. The *Ligdia adustata* 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. 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.7125292>.

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 Management, Samples and Laboratory team are listed here: <https://doi.org/10.5281/zenodo.10066175>.

Members of Wellcome Sanger Institute Scientific Operations: Sequencing Operations are listed here: <https://doi.org/10.5281/zenodo.10043364>.

Members of the Wellcome Sanger Institute Tree of Life Core Informatics team are listed here: <https://doi.org/10.5281/zenodo.10066637>.

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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