



## DATA NOTE

# The genome sequence of the snout, *Hypena proboscidalis* (Linnaeus, 1758) [version 1; peer review: 2 approved, 1 approved with reservations]

Douglas Boyes <sup>1</sup>, Peter W.H. Holland <sup>2</sup>,  
University of Oxford and Wytham Woods Genome Acquisition Lab,  
Darwin Tree of Life Barcoding collective,  
Wellcome Sanger Institute Tree of Life programme,  
Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective,  
Tree of Life Core Informatics collective, Darwin Tree of Life Consortium

<sup>1</sup>UK Centre for Ecology & Hydrology, Wallingford, Oxfordshire, OX10 8BB, UK

<sup>2</sup>Department of Zoology, University of Oxford, Oxford, OX1 3SZ, UK

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## Abstract

We present a genome assembly from an individual female *Hypena proboscidalis* (the snout; Arthropoda; Insecta; Lepidoptera; Erebidæ). The genome sequence is 637 megabases in span. The majority of the assembly is scaffolded into 31 chromosomal pseudomolecules, with the Z sex chromosome assembled.

## Keywords

*Hypena proboscidalis*, the snout, genome sequence, chromosomal



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

## Open Peer Review

Approval Status ? ✓ ✓

|                  | 1                    | 2                    | 3                    |
|------------------|----------------------|----------------------|----------------------|
| <b>version 1</b> | ?                    | ✓                    | ✓                    |
| 15 Sep 2021      | <a href="#">view</a> | <a href="#">view</a> | <a href="#">view</a> |

1. **Angela McGaughran** , University of Waikato, Hamilton, New Zealand
2. **Zdenek F Fric** , Institute of Entomology,, Branisovska, Czech Republic
3. **Kuppusamy Sivasankaran** , Loyola Collège, Chennai, Tamilnadu, India

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

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

**Author roles:** **Boyes D:** Formal Analysis, Investigation, Methodology; **Holland PWH:** Formal Analysis, Investigation, Supervision, Writing – Original Draft Preparation;

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

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

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## Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Erebidae; Hypeninae; Hypena; *Hypena proboscidalis* Linnaeus 1758 (NCBI:txid753189).

## Introduction

Caterpillars of *Hypena proboscidalis* (the snout) are specialised herbivores of nettle plants; the common and binomial names reference the prominent labial palps of the adult. The genome of *H. proboscidalis* 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 *H. proboscidalis*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

## Genome sequence report

The genome was sequenced from a single female *H. proboscidalis* collected from Wytham Woods, Oxfordshire, UK (latitude 51.772, longitude -1.338). A total of 23-fold coverage in Pacific Biosciences single-molecule long reads (N50 18 kb) and 55-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 34 missing/misjoins and removed 14 haplotypic duplications, reducing the assembly length by 2.17% and the scaffold number by 28.57%, and increasing the scaffold N50 by 14.21%. The final assembly has a total length of 637 Mb in 56 sequence scaffolds with a scaffold N50 of 22 Mb (Table 1). The majority, 98.3%, of assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes (numbered by sequence length), and the Z sex chromosome (Figure 1–Figure 4; Table 2). The assumed sex chromosome karyotype is ZO. The assembly has a BUSCO v5.1.2 (Simão *et al.*, 2015) completeness of 98.7% 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.

## Methods

A female *H. proboscidalis*, ilHypProb1, and a second specimen of unknown sex, ilHypProb2, were collected from Wytham Woods, Oxfordshire, UK (latitude 51.772, longitude -1.338) by Douglas Boyes, University of Oxford using a light trap. The specimens were snap-frozen in dry ice using a Cool-Rack before transferring to the Wellcome Sanger Institute (WSI).

DNA was extracted from head and thorax tissue of ilHypProb1 by the Scientific Operations core at the WSI using the Qiagen MagAttract HMW DNA kit, according to the manufacturer's instructions. RNA was extracted from ilHypProb2 in the Tree of Life Laboratory at the WSI using TRIzol (Invitrogen), according to the manufacturer's instructions. RNA was then eluted in 50 µl RNase-free water and its concentration RNA

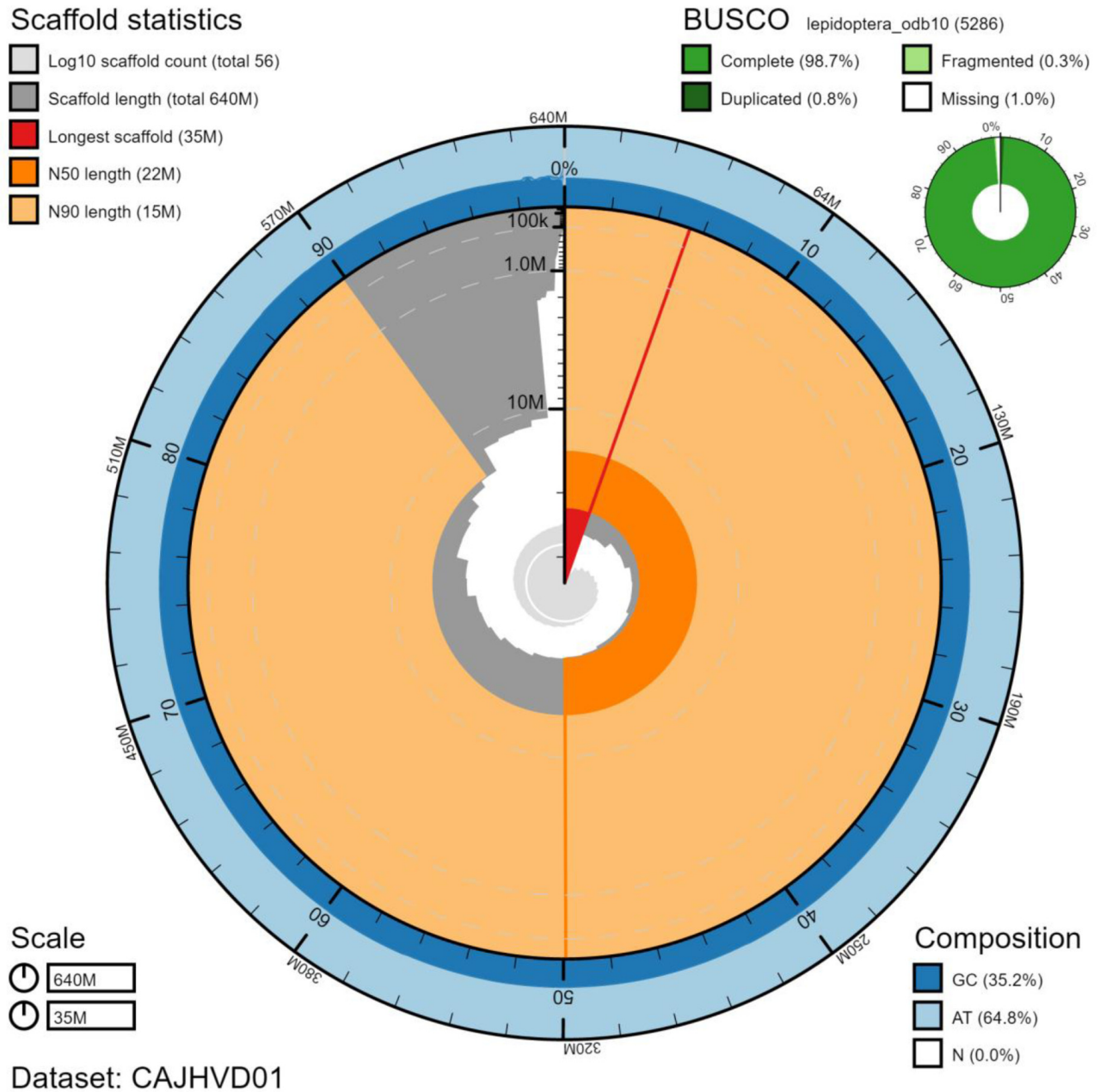
**Table 1. Genome data for *Hypena proboscidalis*, ilHypProb1.1.**

| Project accession data           |  |
|----------------------------------|--|
| Assembly identifier              | ilHypProb1.1                                   |
| Species                          | <i>Hypena proboscidalis</i>                    |
| Specimen                         | ilHypProb1                                     |
| NCBI taxonomy ID                 | NCBI:txid753189                                |
| BioProject                       | PRJEB42129                                     |
| BioSample ID                     | SAMEA7520188                                   |
| Isolate information              | Female, head/abdomen/thorax                    |
| Raw data accessions              |  |
| PacificBiosciences SEQUEL II     | ERR6406200                                     |
| 10X Genomics Illumina            | ERR6002650, ERR6002651, ERR6003040, ERR6003041 |
| Hi-C Illumina                    | ERR6002652                                     |
| Illumina PolyA RNA-Seq           | ERR6002653                                     |
| Genome assembly                  |  |
| Assembly accession               | GCA_905147285.1                                |
| Accession of alternate haplotype | GCA_905147305.1                                |
| Span (Mb)                        | 637  |
| Number of contigs                | 86   |
| Contig N50 length (Mb)           | 21   |
| Number of scaffolds              | 56   |
| Scaffold N50 length (Mb)         | 22   |
| Longest scaffold (Mb)            | 26   |
| BUSCO* genome score              | C:98.7%[S:98.0%,D:0.8%],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/ilHypProb1.1/dataset/CAJHVD01/busco>.

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

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries, in addition to PolyA RNA-Seq libraries, were constructed according to the manufacturers' instructions. Sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) Illumina HiSeq X (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were

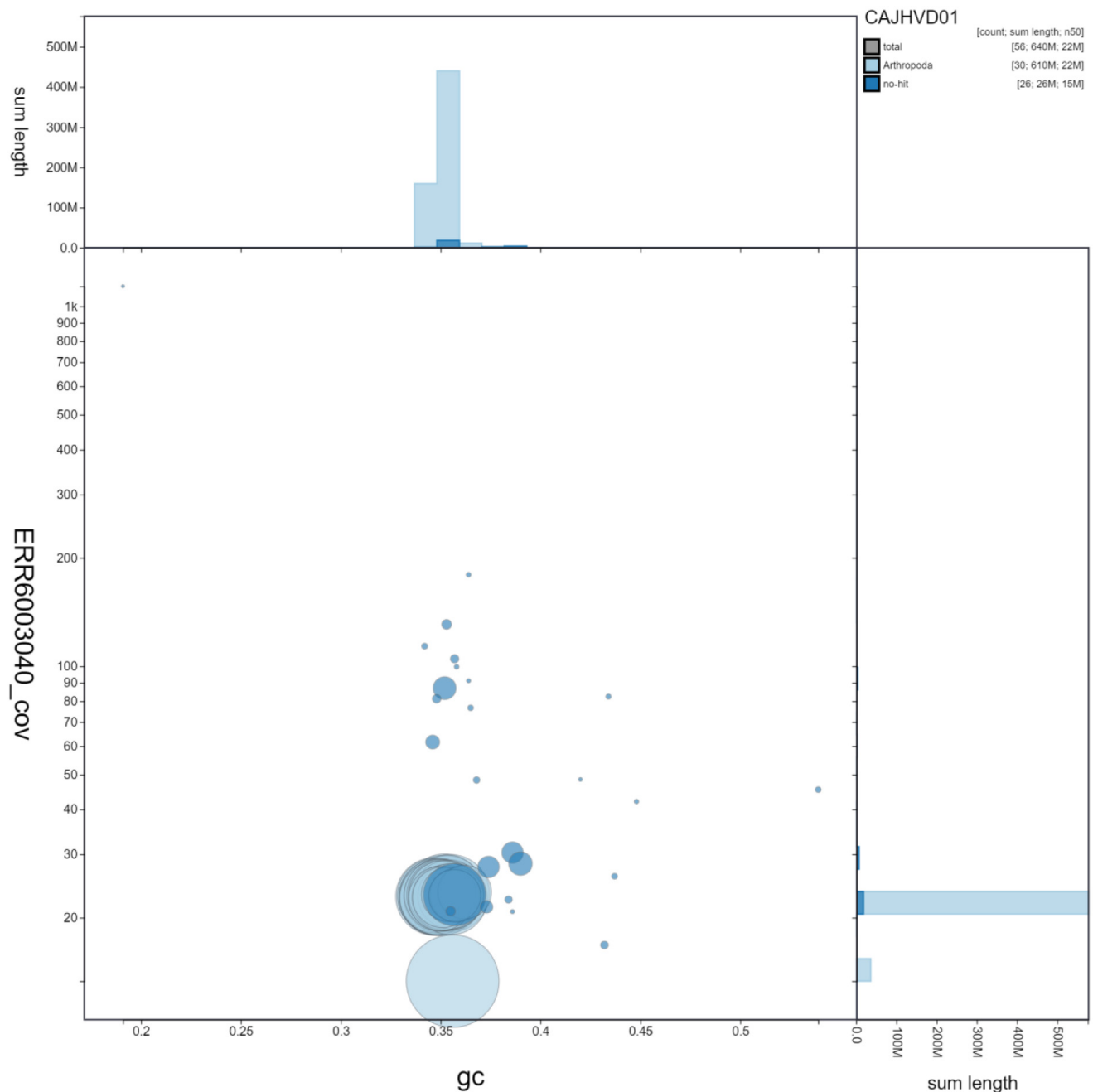


**Figure 1. Genome assembly of *Hypena proboscidalis*, ilHypProb1.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilHypProb1.1/dataset/CAJHVD01/snail>.

generated from abdomen tissue using the Qiagen EpiTect Hi-C kit and sequenced on HiSeq X.

Assembly was carried out with Hifiasm (Cheng *et al.*, 2021); haplotypic duplication was identified and removed with purge\_dups

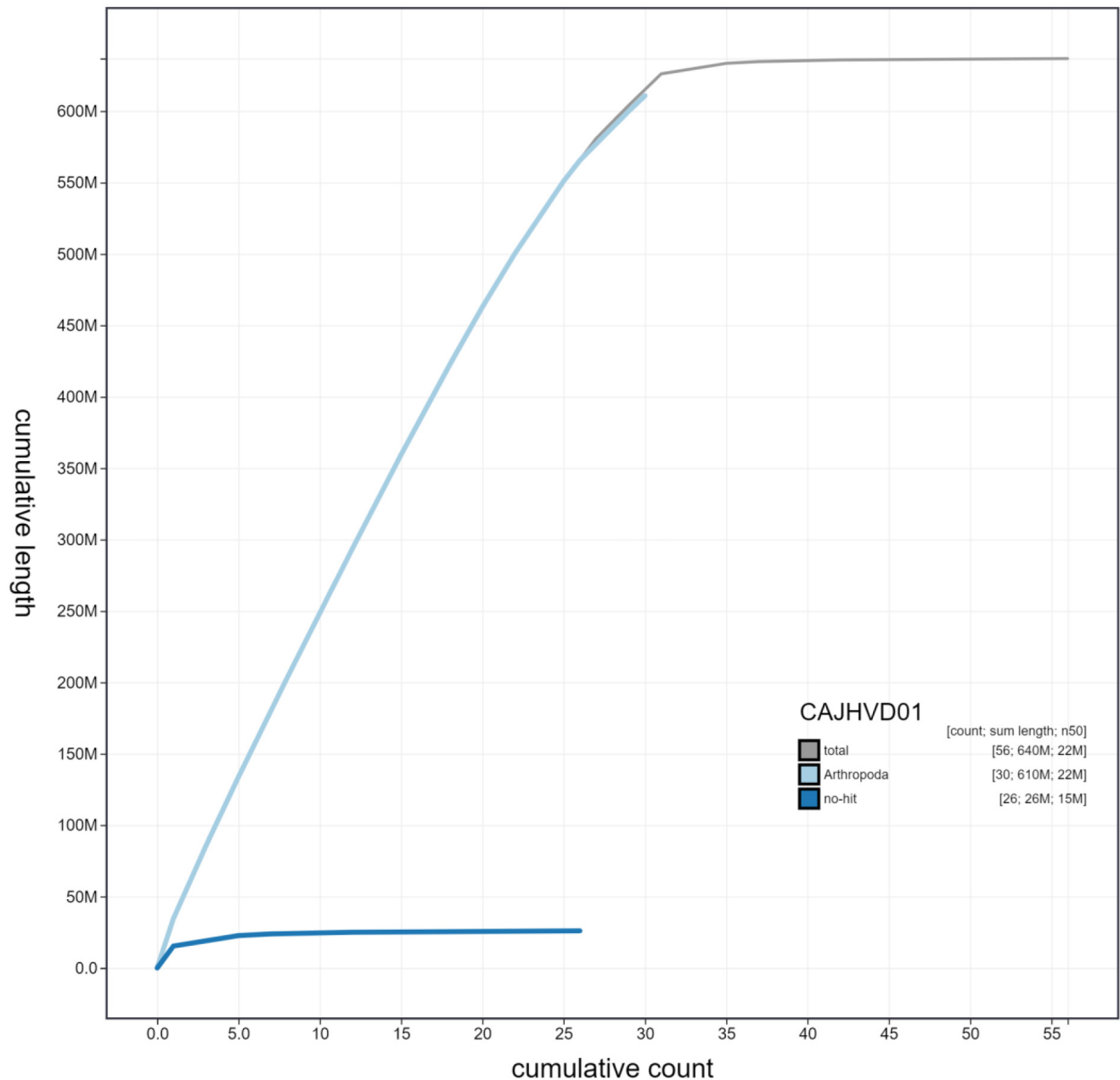
(Guan *et al.*, 2020). The assembly was polished with the 10X Genomics Illumina data by aligning to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012). One round of the Illumina polishing was applied. Scaffolding with Hi-C data (Rao *et al.*, 2014) was carried



**Figure 2. Genome assembly of *Hypena proboscidalis*, ilHypProb1.1: 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/ilHypProb1.1/dataset/CAJHVD01/blob>.

out with SALSA2 (Ghurye *et al.*, 2019). 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 was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext. The mitochondrial

genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021). 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.

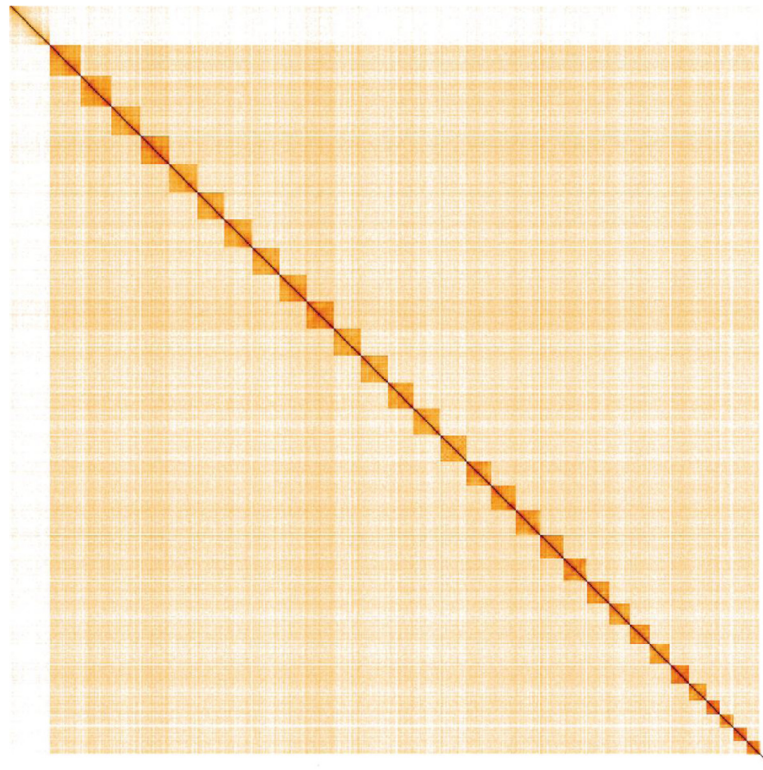


**Figure 3. Genome assembly of *Hypena proboscidalis*, ilHypProb1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all chromosomes. Coloured lines show cumulative lengths of chromosomes assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilHypProb1.1/dataset/CAJHVD01/cumulative>.

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





**Figure 4. Genome assembly of *Hypena proboscidalis*, ilHypProb1.1: Hi-C contact map.** Hi-C contact map of the ilHypProb1.1 assembly, visualised in HiGlass.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Hypena proboscidalis*, ilHypProb1.1.**

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|-----------------|------------|-----------|------|
| LR990127.1      | 1          | 25.59     | 35.1 |
| LR990128.1      | 2          | 25.33     | 35.2 |
| LR990129.1      | 3          | 24.21     | 34.7 |
| LR990130.1      | 4          | 24.04     | 35.5 |
| LR990131.1      | 5          | 23.26     | 34.8 |
| LR990132.1      | 6          | 23.15     | 34.8 |
| LR990133.1      | 7          | 23.08     | 34.9 |
| LR990134.1      | 8          | 22.66     | 34.8 |
| LR990135.1      | 9          | 22.65     | 35   |
| LR990136.1      | 10         | 22.64     | 35.3 |
| LR990137.1      | 11         | 22.30     | 34.7 |
| LR990138.1      | 12         | 22.17     | 34.8 |
| LR990139.1      | 13         | 21.97     | 35   |
| LR990140.1      | 14         | 21.88     | 34.8 |
| LR990141.1      | 15         | 21.39     | 35.5 |

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|-----------------|------------|-----------|------|
| LR990142.1      | 16         | 21.18     | 35   |
| LR990143.1      | 17         | 20.50     | 35.1 |
| LR990144.1      | 18         | 20.12     | 35.2 |
| LR990145.1      | 19         | 19.97     | 35.4 |
| LR990146.1      | 20         | 18.83     | 35.3 |
| LR990147.1      | 21         | 18.67     | 35.1 |
| LR990148.1      | 22         | 17.10     | 35.2 |
| LR990149.1      | 23         | 17.06     | 35.5 |
| LR990150.1      | 24         | 16.60     | 35   |
| LR990151.1      | 25         | 15.36     | 35.6 |
| LR990152.1      | 26         | 14.56     | 35.1 |
| LR990153.1      | 27         | 11.71     | 35.4 |
| LR990154.1      | 28         | 11.47     | 35.8 |
| LR990155.1      | 29         | 11.35     | 36.2 |
| LR990156.1      | 30         | 10.71     | 35.7 |
| LR990126.1      | Z          | 34.62     | 35.6 |
| LR990157.1      | MT         | 0.02      | 19.3 |
| -               | Unplaced   | 10.64     | 37.4 |

**Table 3. Software tools used.**

| Software tool    | Version           | Source  |
|------------------|-------------------|---|
| Hifiasm          | 0.12              | <a href="#">Cheng et al., 2021</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        | 1.3.1-17-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>  |

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 WSI), and in some circumstances other Darwin Tree of Life collaborators.

### Data availability

European Nucleotide Archive: *Hypena proboscoidalis* (the snout). Accession number PRJEB42129: <https://identifiers.org/ena.embl:PRJEB42129>.

The genome sequence is released openly for reuse. The *H. proboscoidalis* 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 the 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](#).

### Acknowledgements

Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: <https://doi.org/10.5281/zenodo.4789929>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.4893704>.

Members of the Wellcome Sanger Institute Tree of Life programme collective are listed here: <https://doi.org/10.5281/zenodo.5377053>.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: <https://doi.org/10.5281/zenodo.4790456>.

Members of the Tree of Life Core Informatics collective are listed here: <https://doi.org/10.5281/zenodo.5013542>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.4783559>.

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[Publisher Full Text](#)

# Open Peer Review

Current Peer Review Status: ? ✓ ✓

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## Version 1

Reviewer Report 10 February 2023

<https://doi.org/10.21956/wellcomeopenres.18992.r54363>

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**Kuppusamy Sivasankaran** 

Loyola Collège, Chennai, Tamilnadu, India

### Review report

I appreciate the authors for assembling the whole genome sequence of Snout *Hypena proboscidalis*. The appropriate software, genome assembling process, and whole genome sequencing technologies were used.

#### **The few comments on the manuscripts.**

- Total sequence lengths (636,786,269 bp) were not mentioned in the text.
- Non-nuclear genome size and Scaffold count not mentioned in the text.
- How many nuclear genes were established in the assembling of the entire genome? The text makes no mention of the nuclear gene numbers.
- The maximum and minimum contig length was not indicated. Total contigs length was not indicated (18,834,925 bp).
- Gene recovery statistics can be given in the text.
- Overall, the manuscript is written well and it can be accepted for indexing.

#### **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:** Phylogenetic analysis of the superfamily Noctuoidea moths using mitochondrial genome sequence

**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 08 February 2023

<https://doi.org/10.21956/wellcomeopenres.18992.r54390>

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**Zdenek F Fric** 

Institute of Entomology,, Branisovska, Czech Republic

The paper introduces a newly published genome of another British moth, continuing in the Darwin Tree of Life Project, where the authors try to sequence all known species occurring in British islands. The aim is clear, the methodology is identical to all previous projects of the DTLP and the exact pipeline commands are in the same way obscured. However, the initiative is an important step in knowledge and the resulting DTLP sequences are a very useful tool for other genomic projects as they are easy to use as a reference genome for all other 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:** Lepidoptera phylogeny and evolution

**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 30 September 2021

<https://doi.org/10.21956/wellcomeopenres.18992.r45888>

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**Angela McGaughran** 

Te Aka Mātuatua/School of Science, University of Waikato, Hamilton, New Zealand

The rationale for the study is not particularly defined - there is a very brief introduction to the species and mention that the sequencing is part of the DTLF, but some more information could be provided about the species. E.g., is it a pest, is it endangered, is it important in ecosystem function, etc. This would help place the study a bit better contextually.

The Methods section outlines all of the software used and version numbers are provided in Table 3. But there is no detail of the actual commands used, so the study cannot be replicated. Provision of the bioinformatic coding in Supplementary material would address this.

The figures are attractive to look at, but the legends are very brief and therefore the figures are not easy to interpret. For example, in Fig 4 the axes are not labelled; in Fig 1, the relevant percentages or other values seem to all be stated in the associated keys, so, though the figure looks great, it's not clear what it's adding. There is also no description of what the figures mean in the text.

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

Partly

**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?**

Partly

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

**Reviewer Expertise:** Genomics, population 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, however I have significant reservations, as outlined above.**

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