



DATA NOTE

The genome sequence of the spectacle, *Abrostola tripartita*

Hufnagel, 1766 [version 1; peer review: 3 approved]

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V1 First published: 06 Dec 2021, 6:330
<https://doi.org/10.12688/wellcomeopenres.17355.1>

Latest published: 06 Dec 2021, 6:330
<https://doi.org/10.12688/wellcomeopenres.17355.1>

Abstract

We present a genome assembly from an individual male *Abrostola tripartita* (the spectacle; Arthropoda; Insecta; Lepidoptera; Noctuidae). The genome sequence is 381 megabases in span. The majority of the assembly (99.99%) is scaffolded into 31 chromosomal pseudomolecules, with the Z sex chromosome assembled.

Keywords

Abrostola tripartita, the spectacle, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status ✓ ✓ ✓

	1	2	3
version 1	✓	✓	✓
06 Dec 2021	view	view	view

1. **Youngik Yang** , National Marine Biodiversity Institute of Korea, Janghang-eup, South Korea
2. **Xingtian Zhang** , Fujian Agriculture and Forestry University, Fuzhou, China
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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; **Crowley LM:** Writing – Original Draft Preparation, Writing – Review & Editing; **Holland PWH:** Supervision, Writing – Original Draft Preparation, Writing – Review & Editing;

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Wellcome Trust through core funding to the Wellcome Sanger Institute (206194) and the Darwin Tree of Life Discretionary Award (218328).

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

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How to cite this article: Boyes D, Crowley LM, Holland PWH *et al.* **The genome sequence of the spectacle, *Abrostola tripartita* Hufnagel, 1766 [version 1; peer review: 3 approved]** Wellcome Open Research 2021, 6:330 <https://doi.org/10.12688/wellcomeopenres.17355.1>

First published: 06 Dec 2021, 6:330 <https://doi.org/10.12688/wellcomeopenres.17355.1>

Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Noctuidae; Plusiinae; *Abrostola*; *Abrostola tripartita* Hufnagel, 1766 (NCBI:txid938171).

Background

Abrostola tripartita (the spectacle) is a grey and white noctuid moth recorded from across the Palaearctic region. It is found commonly across the UK where it has increased significantly in abundance in recent decades (Sterling & Henwood, 2020). The common name derives from two rings of grey hairs on the thorax with the appearance of a pair of goggles, visible when the moth is viewed from anterior; these hairs are on the thorax and are not associated with the head or eyes. The larvae feed on nettle (*Urtica dioica*) and may be found across any habitat where the food plant is in abundance. Adults often feed at flowers including red valerian (*Centranthus ruber*) and sage (*Salvia*). In the UK the adult flight period occurs as a single generation in the north (May to July) and as two generations in the south (May-July and July-September) (Waring *et al.*, 2003). Overwintering occurs as a pupa among plant litter on the ground or under bark.

Genome sequence report

The genome was sequenced from one male *A. tripartita* (Figure 1) collected from Wytham Woods, Oxfordshire

(biological vice-county: Berkshire), UK (latitude 51.769, longitude -1.339). A total of 54-fold coverage in Pacific Biosciences single-molecule long reads and 102-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 30 missing/misjoins and removed 3 haplotypic duplications, reducing the assembly size by 0.44% and the scaffold number by 20.00%.

The final assembly has a total length of 381 Mb in 32 sequence scaffolds with a scaffold N50 of 13.6 Mb (Table 1). The majority of the assembly sequence (99.99%) 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 v5.1.2 (Manni *et al.*, 2021) completeness of 99.0% (single 98.8%, duplicated 0.2%) 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 single male *A. tripartita* (ilAbrTrip1) was collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.769, longitude -1.339) by Douglas Boyes, UKCEH, using a light trap. The sample was identified by the same individual, and preserved on dry ice.



Figure 1. Image of the ilAbrTrip1 specimen taken prior to preservation and processing. Specimen shown next to FluidX storage tube, 43.9 mm in length.

Table 1. Genome data for *Abrostola tripartita*, ilAbrTrip1.1.

Project accession data	
Assembly identifier	ilAbrTrip1.1
Species	<i>Abrostola tripartita</i>
Specimen	ilAbrTrip1
NCBI taxonomy ID	NCBI:txid938171
BioProject	PRJEB43740
BioSample ID	SAMEA7520667
Isolate information	Male, thorax (genome assembly), head (Hi-C), abdomen (RNA-Seq)
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6412364
10X Genomics Illumina	ERR6054553-ERR6054556
Hi-C Illumina	ERR6054552
Illumina polyA RNA-Seq	ERR6054557
Genome assembly	
Assembly accession	GCA_905340225.1
Accession of alternate haplotype	GCA_905340255.1
Span (Mb)	381
Number of contigs	34
Contig N50 length (Mb)	13.6
Number of scaffolds	32
Scaffold N50 length (Mb)	13.6
Longest scaffold (Mb)	16.0
BUSCO* genome score	C:99.0%[S:98.8%,D:0.2%],F:0.3%,M:0.7%,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/ilAbrTrip1.1/dataset/CAJPHX01/busco>.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ilAbrTrip1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C and RNA sequencing. Thorax tissue was cryogenically disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. 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.

RNA was extracted from abdomen tissue 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 assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA Broad-Range (BR) Assay kit. Analysis of the

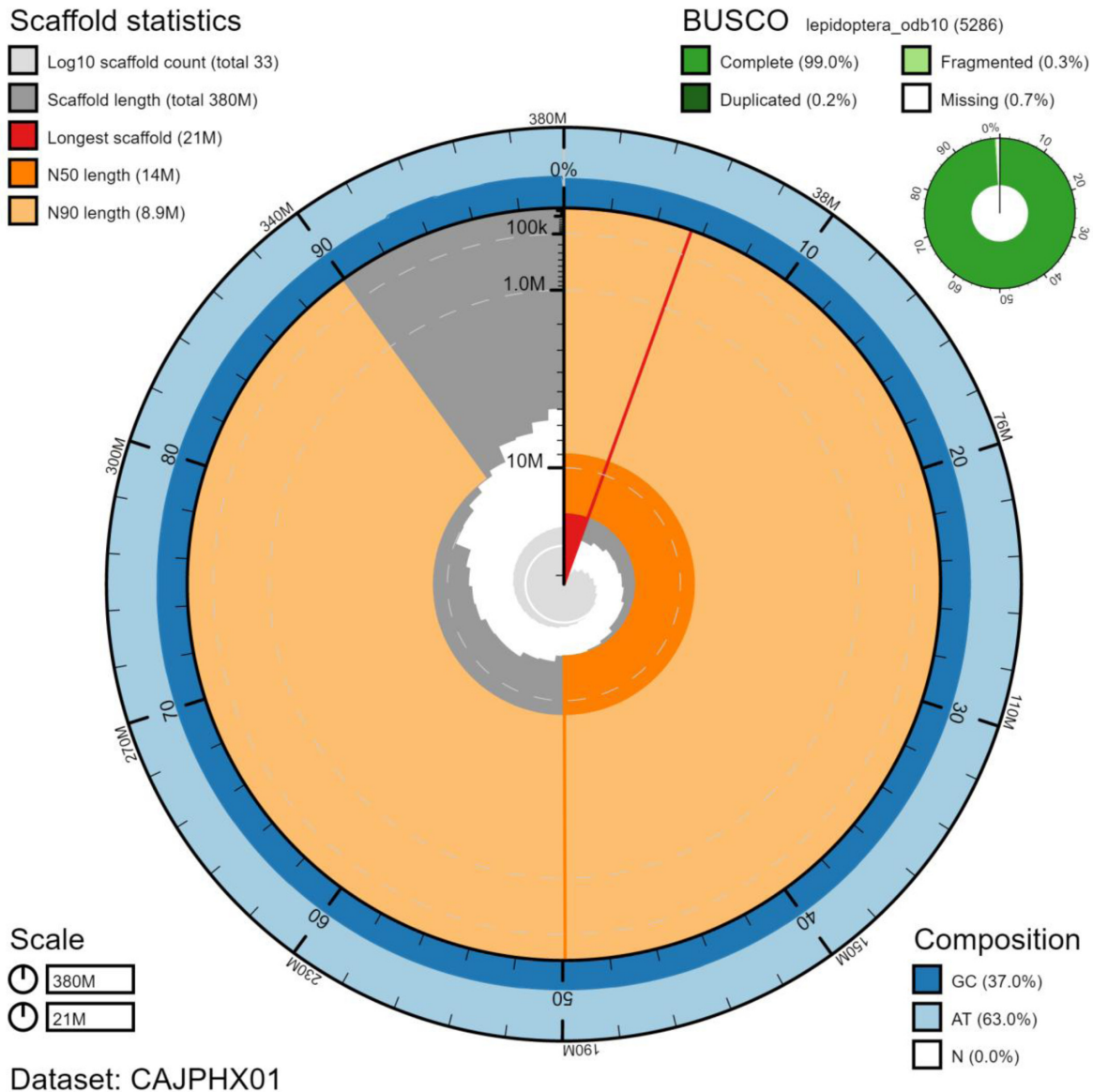


Figure 2. Genome assembly of *Abrostola tripartita*, ilAbrTrip1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 381,057,457 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (20,963,991 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (13,645,312 and 8,916,110 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.genomehubs.org/view/ilAbrTrip1.1/dataset/CAJPHX01/snail>.

integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud 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. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq X (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were generated from head tissue using the Arima Hi-C+ kit and sequenced on HiSeq X.

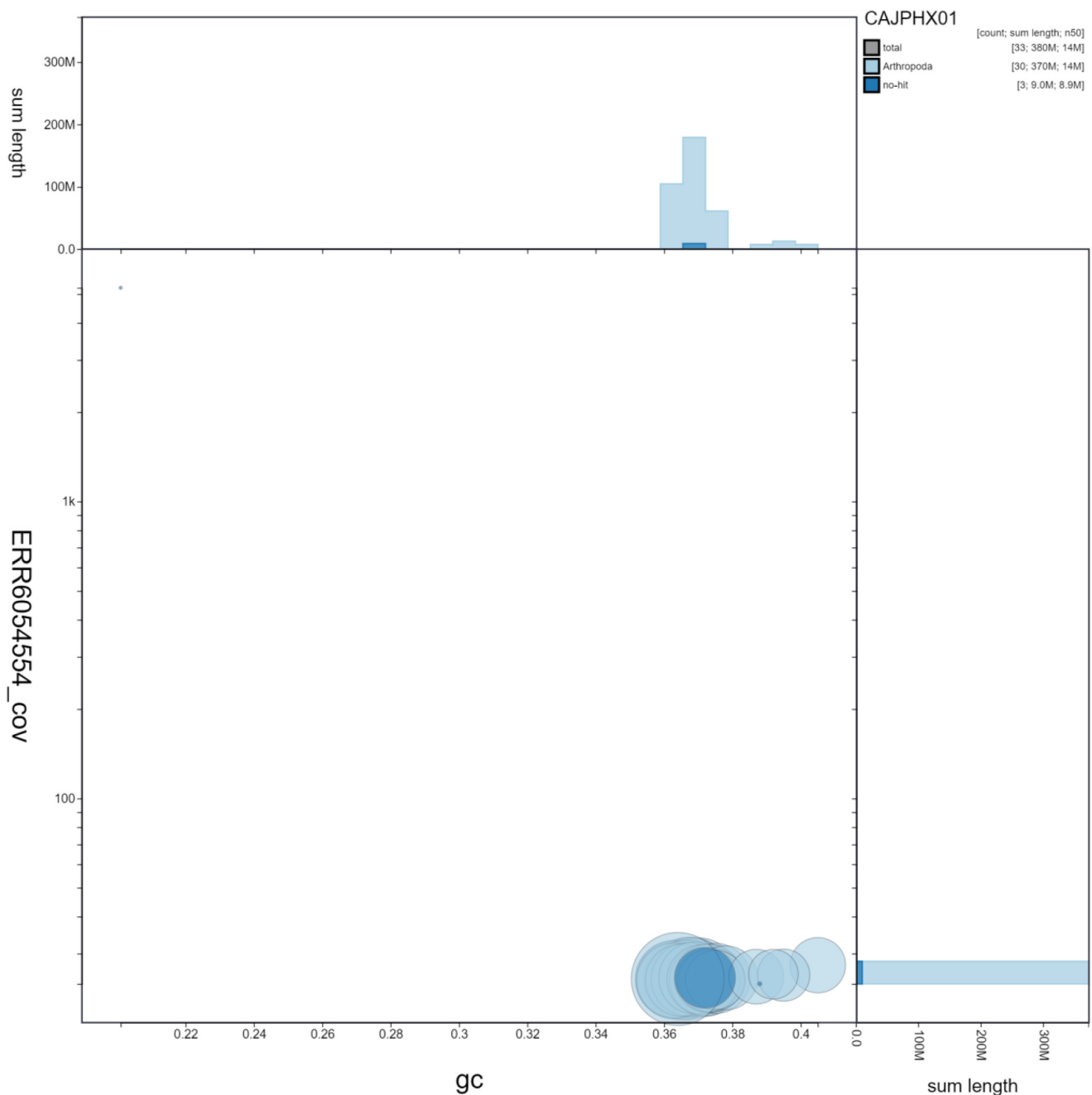


Figure 3. Genome assembly of *Abrostola tripartita*, ilAbrTrip1.1: GC coverage. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilAbrTrip1.1/dataset/CAJPHX01/blob>.

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 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 (Howe *et al.*, 2021) was performed using gEVAL, HiGlass

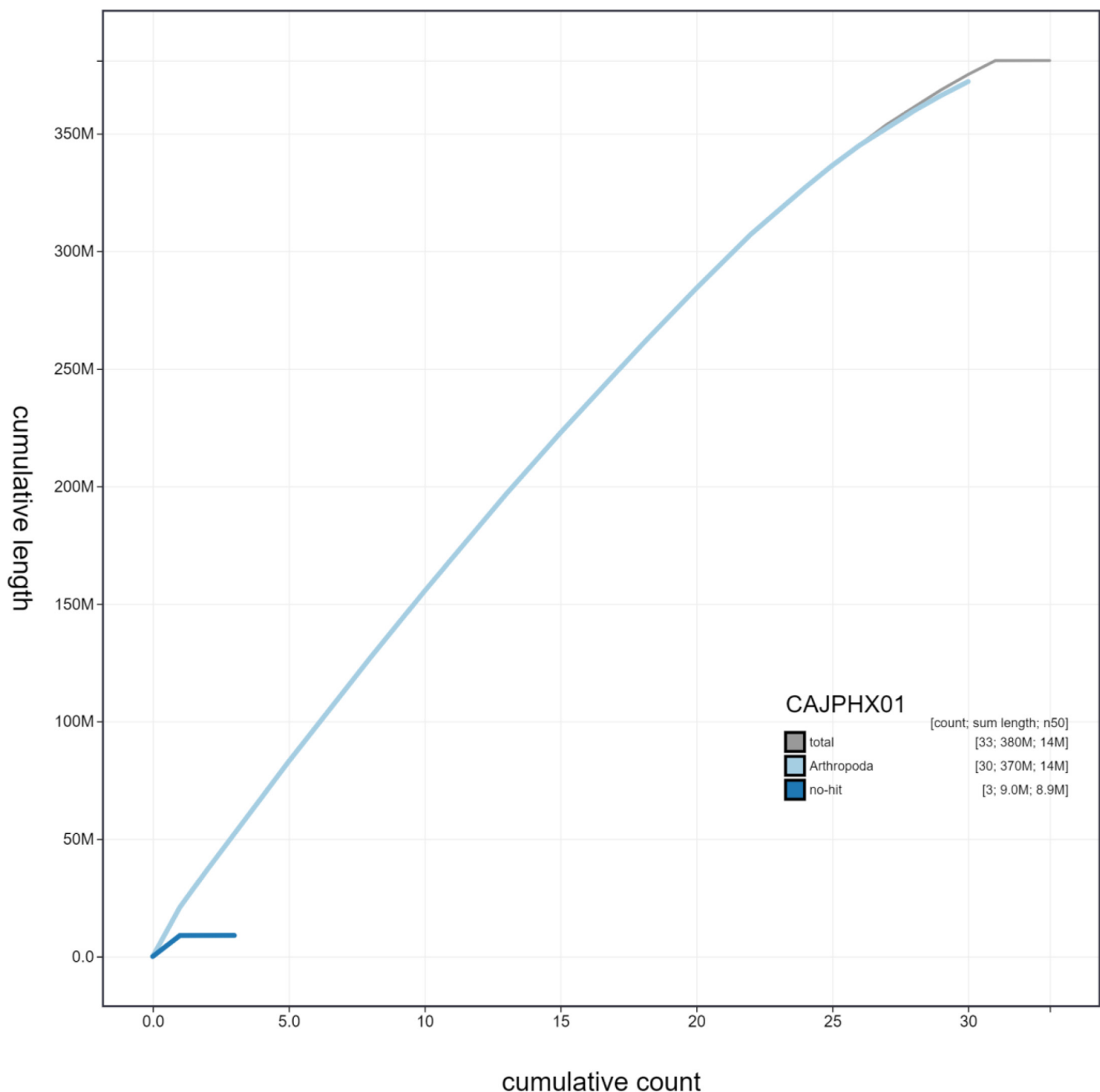


Figure 4. Genome assembly of *Abrostola tripartita*, ilAbrTrip1.1: cumulative sequence. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilAbrTrip1.1/dataset/CAJPHX01/cumulative>.

(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.

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,

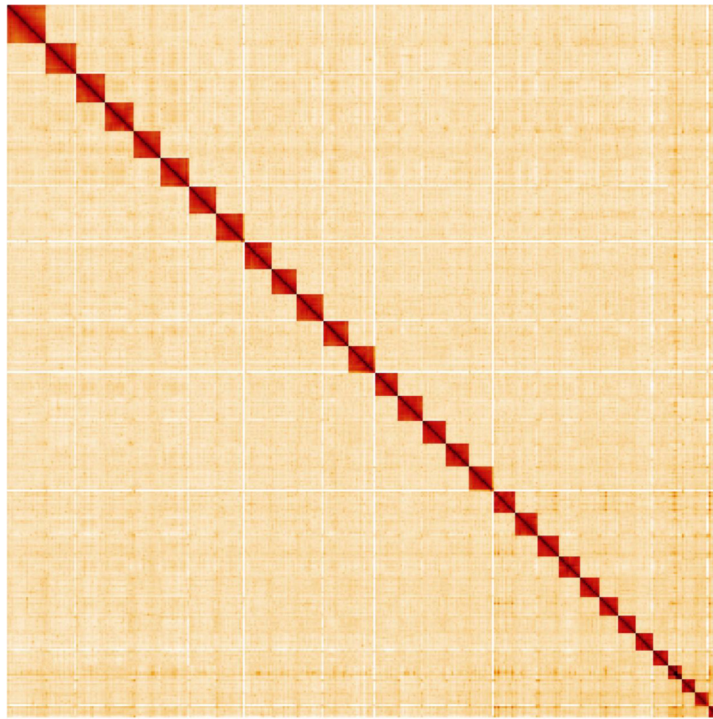


Figure 5. Genome assembly of *Abrostola tripartita*, ilAbrTrip1.1: Hi-C contact map. Hi-C contact map of the ilAbrTrip1.1 assembly, visualised in HiGlass.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Abrostola tripartita*, ilAbrTrip1.1.

INSDC accession	Chromosome	Size (Mb)	GC%
HG996487.1	1	15.98	36.7
HG996488.1	2	15.59	36.8
HG996489.1	3	15.22	37
HG996490.1	4	15.13	36.7
HG996491.1	5	14.91	36.8
HG996492.1	6	14.74	36.3
HG996493.1	7	14.47	36.5
HG996494.1	8	14.45	36.3
HG996495.1	9	14.04	36.6
HG996496.1	10	13.84	36.3
HG996497.1	11	13.71	36.7
HG996498.1	12	13.65	36.3
HG996499.1	13	13.10	36.7
HG996500.1	14	12.99	36.7
HG996501.1	15	12.52	36.5

INSDC accession	Chromosome	Size (Mb)	GC%
HG996502.1	16	12.52	36.9
HG996503.1	17	12.43	37.2
HG996504.1	18	12.03	37.2
HG996505.1	19	11.91	37.5
HG996506.1	20	11.64	37.1
HG996507.1	21	11.29	37.3
HG996508.1	22	10.01	37.4
HG996509.1	23	9.94	37.8
HG996510.1	24	9.43	37.3
HG996511.1	25	8.92	37.2
HG996512.1	26	8.52	37.5
HG996513.1	27	7.37	40.5
HG996514.1	28	7.24	38.7
HG996515.1	29	6.58	39.5
HG996516.1	30	5.91	39.2
HG996486.1	Z	20.96	36.4
HG996517.1	MT	0.02	19.9
-	Unplaced	0.03	38.5

Table 3. Software tools used.

Software tool	Version	Source
Hifiasm	0.142	Cheng et al., 2021
purge_dups	1.2.3	Guan et al., 2020
SALSA2	2.2	Ghurye et al., 2019
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	1.0	Uliano-Silva et al., 2021
gEVAL	N/A	Chow et al., 2016
HiGlass	1.11.6	Kerpedjiev et al., 2018
PretextView	0.1.x	https://github.com/wtsi-hpag/PretextView
BlobToolKit	2.6.2	Challis et al., 2020

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.

Data availability

European Nucleotide Archive: *Abrostola tripartita* (the spectacle). Accession number [PRJEB43740](#); <https://identifiers.org/ena.embl/PRJEB43740>.

The genome sequence is released openly for reuse. The *A. tripartita* 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](#).

Author information

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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[Reference Source](#)

Open Peer Review

Current Peer Review Status:   

Version 1

Reviewer Report 25 January 2022

<https://doi.org/10.21956/wellcomeopenres.19185.r47467>

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Uma Rao

Division of Nematology, ICAR- Indian Agricultural Research Institute, New Delhi, Delhi, India

The paper is an announcement and is written succinctly. All methods are adequate and results are sufficient to explain the findings. It is recommend for indexing with one minor edit:

There is one mismatch between data in text and figure. Table 1 and text report the longest scaffold as 16.0 Mb, whereas figure 2 shows it as 21 Mb, which should be corrected.

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: Plant nematode interactions, genomics, transgenics

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 11 January 2022

<https://doi.org/10.21956/wellcomeopenres.19185.r47462>

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Xingtang Zhang 

State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Institute of Applied Ecology, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, China

The authors provide a high-quality chromosome-scale genome assembly of *Abrostola tripartita*. I believe these resources will be useful for the community. I only have two comments on this manuscript:

1. The authors used Arima Hi-C+ kit to construct the Hi-C but provide limited methods. What is the difference between Arima Hi-C and other related Hi-C methods? Similarly, the SALSA scaffolder was used without details. More information should be added.
2. The quality of genome assembly was only accessed using BUSCO and heatmap. I recommend adding more analysis to valid the genome assembly. For instance, the authors could map sequencing reads to their assembly and check the distribution of coverage along each chromosome.

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.

Reviewer Report 05 January 2022

<https://doi.org/10.21956/wellcomeopenres.19185.r47465>

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**Youngik Yang** 

National Marine Biodiversity Institute of Korea, Janghang-eup, South Korea

The data note by Boyes *et al.* presented a high-quality genome assembly of *Abrostola tripartita*, a grey and white noctuid moth. They applied state-of-the-art sequencing technologies and bioinformatics methods. The resulting assembly contained 32 pseudo-chromosomes and BUSCO was 99%. I believe that this high-quality chromosome-level assembly will be a valuable resource for further studies of this species as well as comparative studies with other species.

I have some recommendations for improving the article.

1. Specimen image would be better to be shown next to the ruler not the FluidX storage.
2. Please, provide the exact number of chromosomes and references if available.
3. MitoHiFi requires a close-related species for input. Please, provide what it was.
4. Figure 2 and 3 are not very informative in my opinion. The information in the figures can be combined in table 2.

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:** Bioinformatics, 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.
