



DATA NOTE

REVISED

The genome sequence of the Buff-tip, *Phalera bucephala* (Linnaeus, 1758)

[version 2; peer review: 1 approved, 1 approved with reservations]

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Abstract

We present a genome assembly from an individual female *Phalera bucephala* (the buff-tip; Arthropoda; Insecta; Lepidoptera; Notodontidae). The genome sequence is 933 megabases in span. Most of the assembly (99.27%) is scaffolded into 31 chromosomal pseudomolecules, with the W and Z sex chromosome assembled. The assembly achieves the Earth BioGenome Project reference standard of **6.C.Q58**. Gene annotation of this assembly on Ensembl identified 12,540 protein-coding genes. The mitochondrial genome has also been assembled, with a length of 15.51 kilobases. This assembly was generated as part of the Darwin Tree of Life project, which produces reference genomes for eukaryotic species found in Britain and Ireland.

Keywords

Phalera bucephala, buff-tip, genome sequence, chromosomal, Lepidoptera



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

Open Peer Review

Approval Status

	1	2
version 2 (revision) 26 Sep 2025		
version 1 27 Jan 2022	 view	 view

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Agricultural Research Service, Beltsville, USA
2. **Luc Swevers**, National Center for Scientific
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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; Holland PWH: Supervision, Writing – Original Draft Preparation, Writing – Review & Editing;

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REVISED Amendments from Version 1

Version 2 of this data note includes information about the genome annotation performed by Ensembl at the European Bioinformatics Institute.

We have also expanded the rationale for sequencing in the Background section.

Any further responses from the reviewers can be found at the end of the article

Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Notodontidae; Phalerinae; *Phalera*; *Phalera bucephala* (Linnaeus, 1758) (NCBI:txid753216).

Background

Phalera bucephala (Buff-tip) exhibits one of the most striking examples of camouflage amongst UK moths: the yellow-tipped forewings held tent-like along the body give the convincing appearance of a broken birch twig. The moth is nocturnal and found across the UK, mainland Europe and parts of Asia. The larvae are polyphagous, feeding on the leaves of several deciduous trees including birch, beech and oak. Ford (1967) comments that the larvae can produce a pungent smell, presumably as a defence mechanism. The species can become a transient pest; for example, defoliating trees along the Maidenhead bypass in the UK in the 1970s (Port & Thompson, 1980) and apple trees in Lithuania (Molis, 1970). The species has also been used in studies to assess the effect of multiple stressors (herbivores, powdery mildew and aphids) on oak trees, revealing complex plant-pathogen-insect interactions (van Dijk *et al.*, 2020).

The genome of *P. bucephala*, 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. Our reference genome enables tests of the genetic architecture of crypsis and twig mimicry (e.g. pigmentation, patterning, behaviour) and supports comparative analyses of chemosensory and detoxification gene families linked to host choice and the species' occasional pest status. Here we present a chromosomally complete genome sequence for *P. bucephala*, based on one female specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from a single female *P. bucephala* (Figure 1) collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.764, longitude -1.327). A total of 34-fold coverage in Pacific Biosciences single-molecule circular consensus HiFi long reads (N50 15 kb) and 51-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with

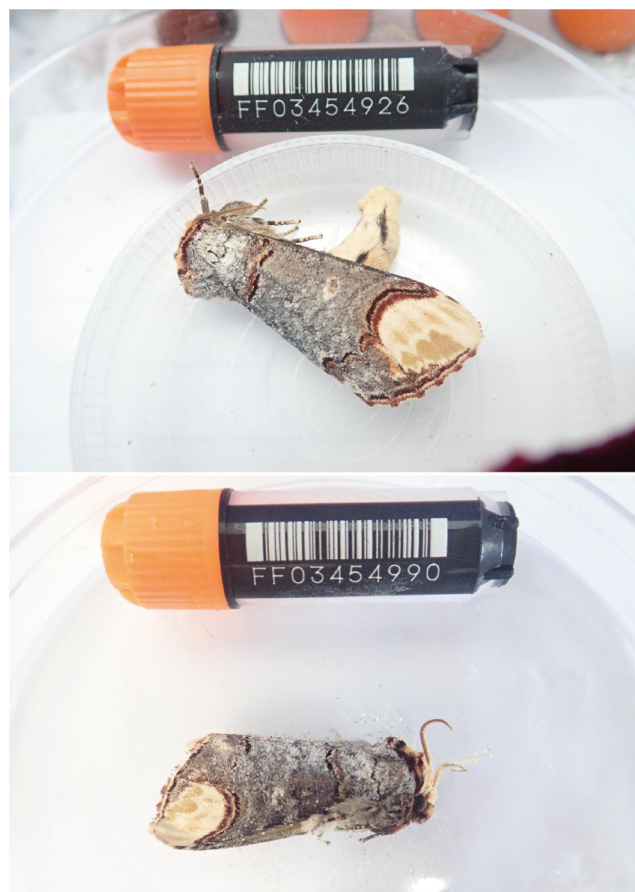


Figure 1. Image of the *Phalera bucephala* specimens taken prior to preservation and processing. Above, ilPhaBuce1, used for genome and Hi-C sequencing; below, ilPhaBuce2, used for RNA-Seq.

chromosome conformation Hi-C data. Manual assembly curation corrected 155 missing/misjoins and removed 4 haplotypic duplications, reducing the assembly size by 0.22% and scaffold number by 45.28%, and increasing the scaffold N50 by 40.20%.

The final assembly has a total length of 933 Mb in 116 sequence scaffolds with a scaffold N50 of 34 Mb (Table 1). Of the assembly sequence, 99.27% was assigned to 31 chromosomal-level scaffolds, representing 29 autosomes (numbered by sequence length), and the W and Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.1.2 (Manni *et al.*, 2021) completeness of 98.9% (single 97.8%, duplicated 1.0%) 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.

The Earth BioGenome Project metric, calculated for the primary assembly, is **6.C.Q58**, meeting the recommended reference standard.

Table 1. Genome data for *Phalera bucephala*, ilPhaBuce1.2.

Project accession data	
Assembly identifier	ilPhaBuce1.2
Species	<i>Phalera bucephala</i>
Specimen	ilPhaBuce1
NCBI taxonomy ID	NCBI:txid753216
BioProject	PRJEB42140
BioSample ID	SAMEA7519921
Isolate information	Female, head/abdomen/thorax
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6594494, ERR6594495
10X Genomics Illumina	ERR6002720-ERR6002727
Hi-C Illumina	ERR6002728-ERR6002730
Illumina polyA RNA-Seq	ERR6002731
Genome assembly	
Assembly accession	GCA_905147815.2
Accession of alternate haplotype	GCA_905147805.2
Span (Mb)	933
Number of contigs	295
Contig N50 length (Mb)	8.5
Number of scaffolds	116
Scaffold N50 length (Mb)	34.1
Longest scaffold (Mb)	43.5
BUSCO* genome score	C:98.9%[S:97.8%,D:1.0%],F:0.3%,M:0.8%,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/ilPhaBuce1.2/dataset/CAJHXA02/busco>.

Genome annotation report

The *Phalera bucephala* genome assembly (GCA_905147815.2) was annotated by Ensembl at the European Bioinformatics Institute (EBI). This annotation includes 22,530 transcribed mRNAs from 12,540 protein-coding and 2 181 non-coding genes. The average transcript length is 19,937.40 bp, with an average of 11.53 coding transcripts per gene and 6.98 exons per transcript. For further information about the annotation, please refer to the [annotation page](#) on Ensembl.

Methods

Sample acquisition and nucleic acid extraction

A female *P. bucephala* (ilPhaBuce1) and a second specimen of unknown sex (ilPhaBuce2) were collected from Wytham

Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.764, longitude -1.327) by Douglas Boyes, UKCEH, using a net. The samples were formally identified by Douglas Boyes and snap-frozen on dry ice.

DNA was extracted from whole organism tissue of ilPhaBuce1 at the Wellcome Sanger Institute (WSI) Scientific Operations core from the whole organism using the Qiagen MagAttract HMW DNA kit, according to the manufacturer's instructions. RNA was extracted from thorax/abdomen tissue of ilPhaBuce2 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

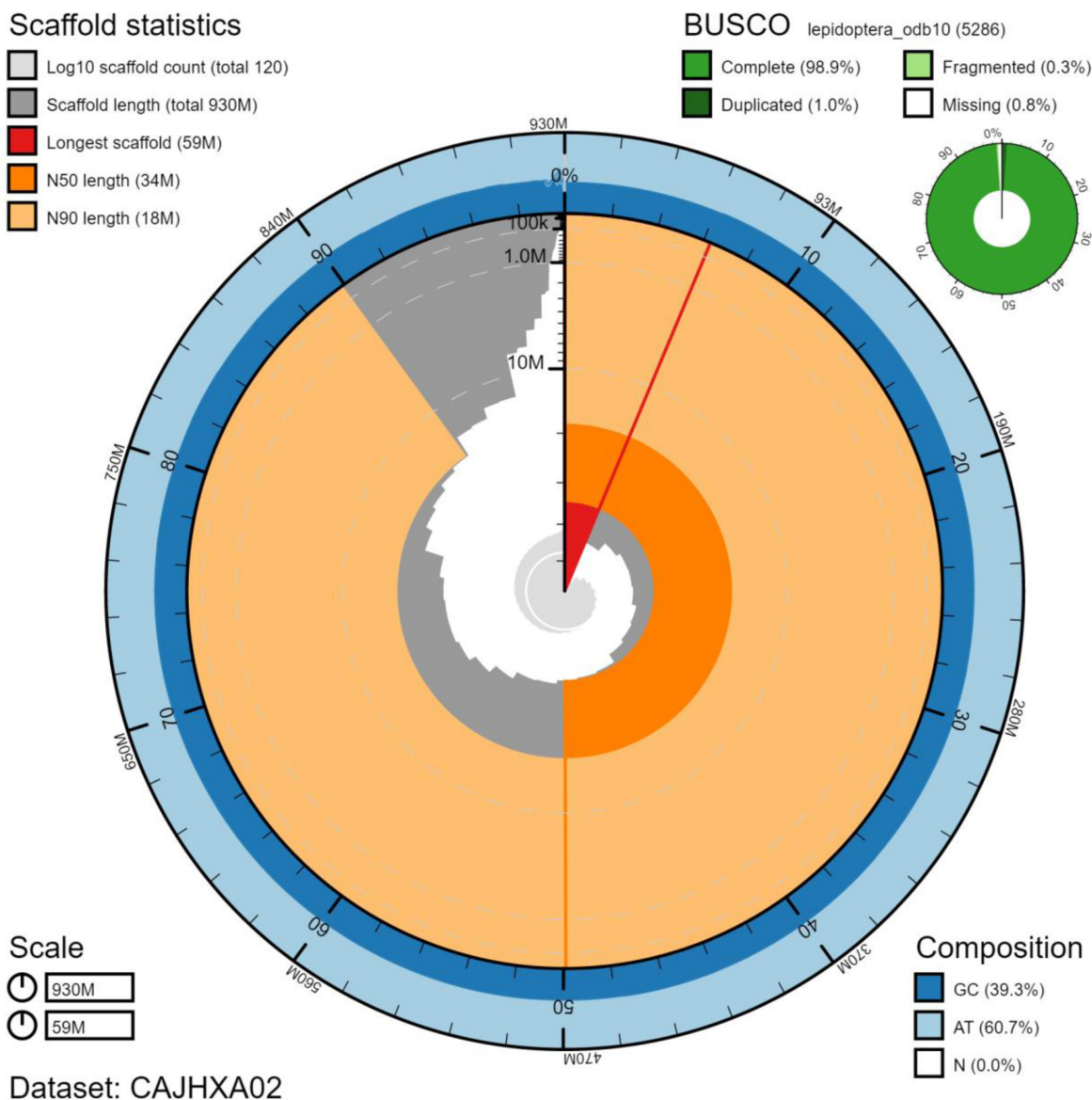


Figure 2. Genome assembly of *Phalera bucephala*, ilPhaBuce1.2: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 933,147,695 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (59,027,677 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (34,116,407 and 18,324,721 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/ilPhaBuce1.2/dataset/CAJHXA02/snail>.

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.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud sequencing libraries were constructed

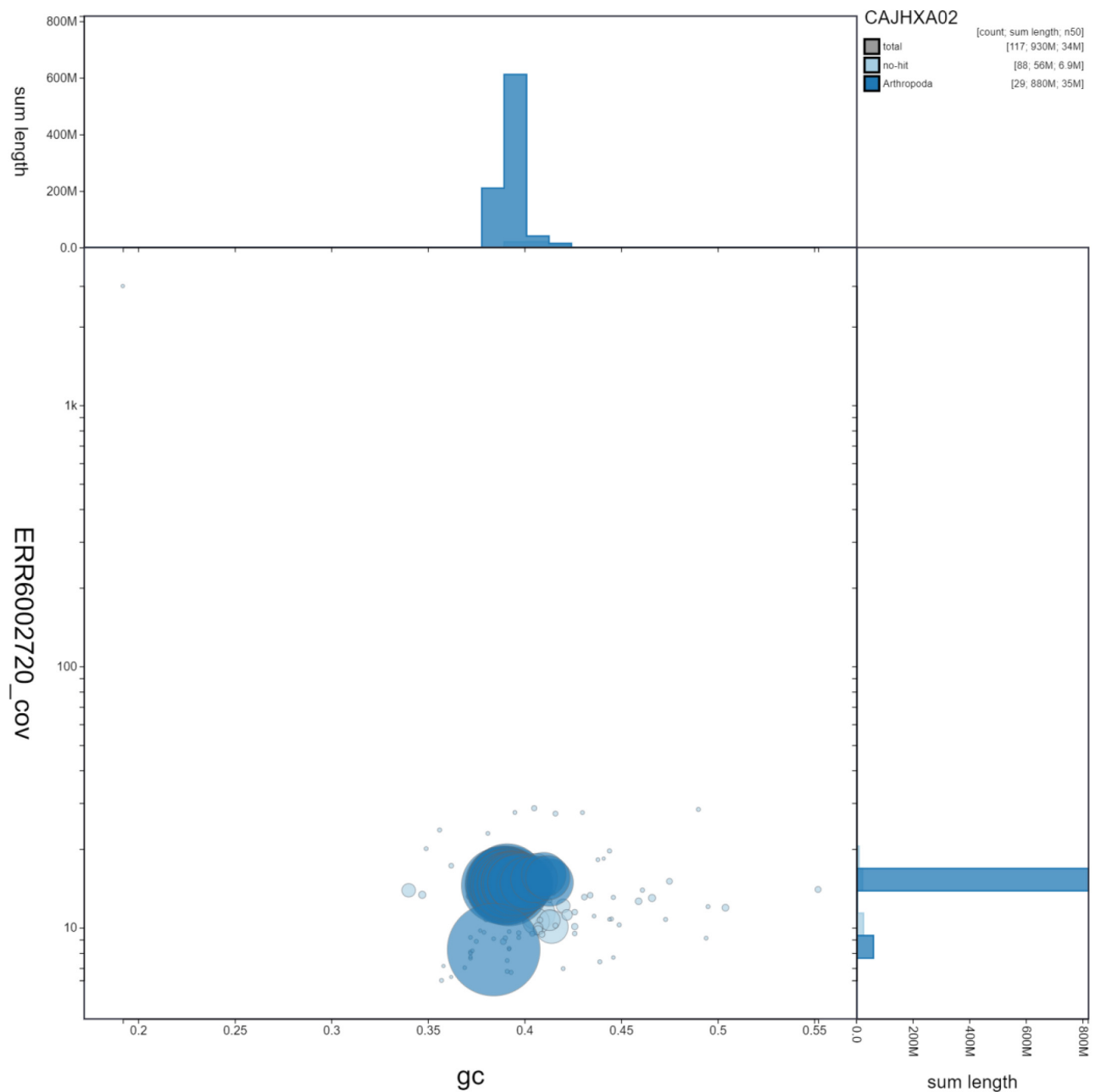


Figure 3. Genome assembly of *Phalera bucephala*, ilPhaBuce1.2: GC coverage. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilPhaBuce1.2/dataset/CAJHXA02/blob>.

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 Qiagen EpiTect Hi-C kit and sequenced on HiSeq X.

Genome assembly

Assembly was carried out with HiCanu (Nurk *et al.*, 2020). Haplotypic duplication was identified and removed with purge_dups

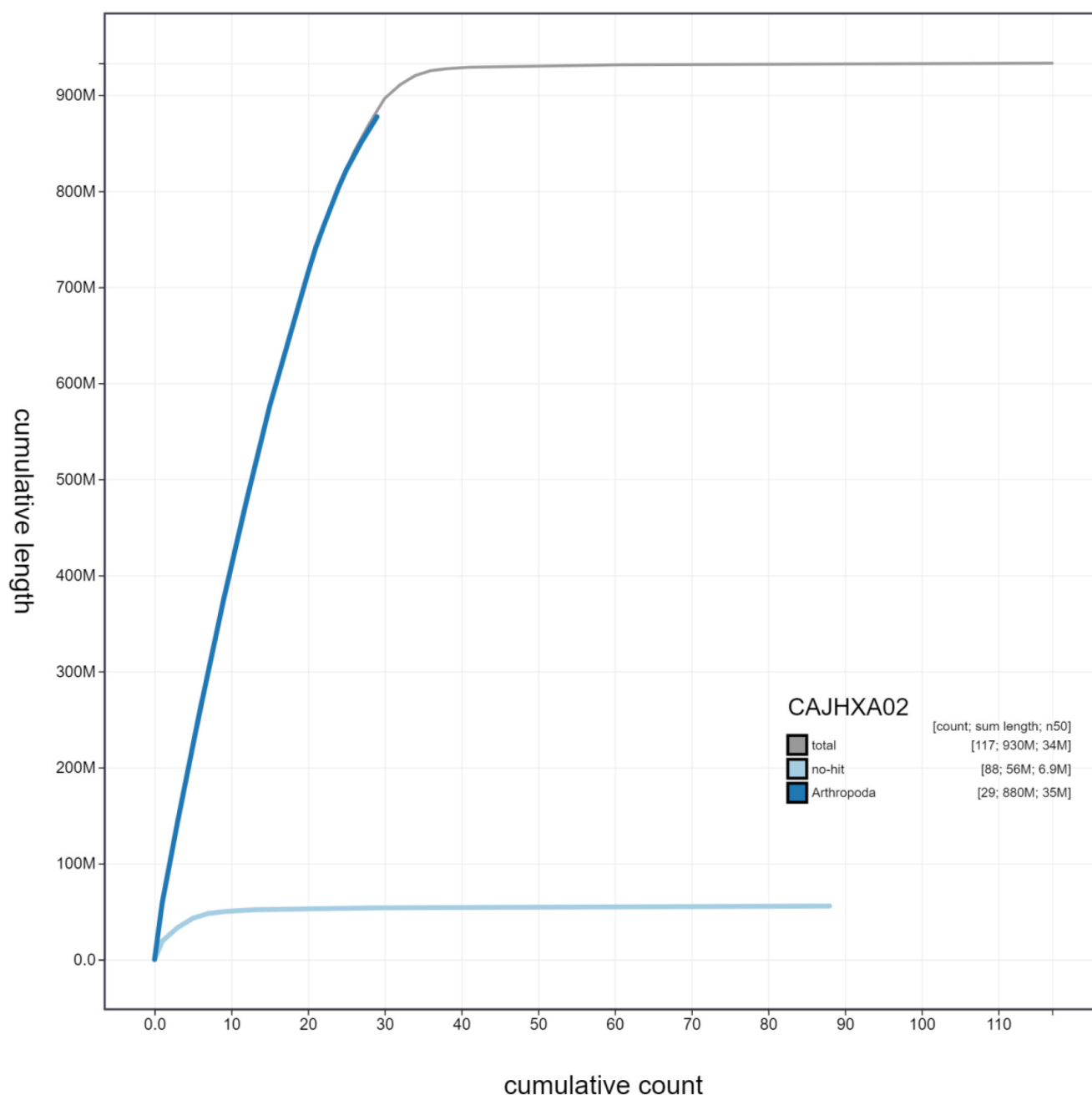


Figure 4. Genome assembly of *Phalera bucephala*, ilPhaBuce1.2: cumulative sequence. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ilPhaBuce1.2/dataset/CAJHXA02/cumulative>.

(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 was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext.

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.

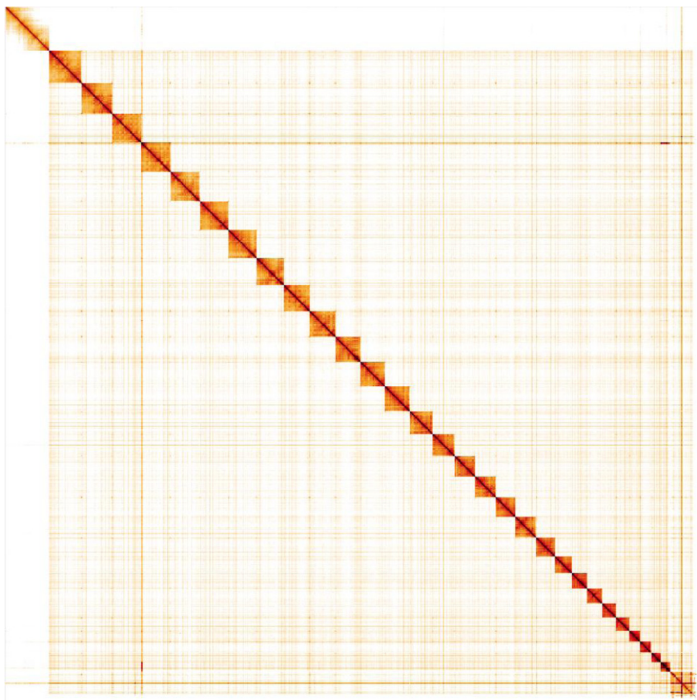


Figure 5. Genome assembly of *Phalera bucephala*, ilPhaBuce1.2: Hi-C contact map. Hi-C contact map of the ilPhaBuce1.2 assembly, visualised in HiGlass. Chromosomes are given in order of size from left to right and top to bottom.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Phalera bucephala*, ilPhaBuce1.2.

INSDC accession	Chromosome	Size (Mb)	GC%
LR990610.1	1	43.49	39.2
LR990611.1	2	40.87	39.1
LR990612.1	3	39.81	38.9
LR990613.1	4	39.67	39
LR990614.1	5	39.31	38.7
LR990615.1	6	38.13	39.1
LR990616.1	7	37.74	38.9
LR990617.1	8	37.02	39.2
LR990618.1	9	34.85	39.1
LR990619.1	10	34.54	39.3
LR990620.1	11	34.12	38.9
LR990621.1	12	33.31	39.1
LR990622.1	13	33.06	39
LR990623.1	14	31.29	39.1
LR990624.1	15	29.29	39.3

INSDC accession	Chromosome	Size (Mb)	GC%
LR990625.1	16	27.79	39.2
LR990626.1	17	27.27	39.4
LR990627.1	18	27.25	39.4
LR990628.1	19	26.99	39.5
LR990629.1	20	26.05	39.8
LR990630.1	21	22.17	39.6
LR990631.1	22	20.78	40
LR990632.1	23	20.08	39.5
LR990633.1	24	19.01	40
LR990634.1	25	18.32	40.1
LR990635.1	26	14.81	41.3
LR990636.1	27	14.56	40.5
LR990637.1	28	12.96	41
LR990638.1	29	12.86	41.2
LR990639.1	W	7.37	40.7
LR990609.1	Z	59.03	38.4
LR990640.1	MT	0.02	19.3

Table 3. Software tools used.

Software tool	Version	Source
HiCanu	1.0	Nurk <i>et al.</i>, 2020
purge_dups	1.2.3	Guan <i>et al.</i>, 2020
SALSA2	2.2	Ghurye <i>et al.</i>, 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
gEVAL	N/A	Chow <i>et al.</i>, 2016
PretextView	0.1.x	https://github.com/wtsi-hpag/PretextView
HiGlass	1.11.6	Kerpedjiev <i>et al.</i>, 2018
BlobToolKit	2.6.4	Challis <i>et al.</i>, 2020

Data availability

European Nucleotide Archive: *Phalera bucephala* (buff-tip) genome assembly, ilPhaBuce1. Accession number [PRJEB42140](#); <https://identifiers.org/ena.embl/PRJEB42140>.

The genome sequence is released openly for reuse. The *Phalera bucephala* genome sequencing initiative is part of the Darwin Tree of Life Project (PRJEB40665), Sanger Institute Tree of Life Programme (PRJEB43745) and Project Psyche (PRJEB71705). 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.5746938>.

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Members of the Wellcome Sanger Institute Tree of Life programme are listed here: <https://doi.org/10.5281/zenodo.5744840>.

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.5743293>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.5638618>.

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Reviewer Report 23 November 2023

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As a general impression, the sequencing data acquisition and analysis were performed well and very professionally.

The rationale for sequencing this species could be more elaborated, e.g. for studies of the mechanism of mimicry or pest control.

Two individuals were processed, for genome sequencing and RNA-seq respectively. For the assembly of the genome, were these two sets of data integrated? A workflow for both sequencing strategies (DNA versus RNA) (and its potential integration) would be useful.

Can an estimation be provided of levels of sequence identity between the two samples (at the level of mRNA)?

Since the sex of the second individual was not determined morphologically, could its sex be predicted by sequence analysis (based on sex-specific genes such as vitellogenin (female) or sperm-specific tubulin (male))?

For a non-bioinformatician, Figure 3 is puzzling. More explanation is needed to describe the utility of this figure and the significance of the analysis.

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: Insect molecular biology and biotechnology

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.

Reviewer Report 15 February 2023

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The authors present a chromosomally complete genome sequence for the buff-tip moth, *Phalera bucephala*. The genome is sequenced from a single female, with a total of 34X coverage from HiFi long reads, 51X coverage from 10X Genomics read clouds and chromosome confirmation Hi-C data. Manual assembly curations are performed to improve the assembly. Standard bioinformatics pipelines are followed, with all softwares clearly indicated.

The main issue with the report stems from a comment mentioned on page 4 that “Poly(A) RNA-Seq libraries were constructed”, suggesting that multiple libraries were generated and sequenced, however in Table 1, it is apparent that there was only one library sequenced.

Following up on this, it is not clear, in the methods describing “Genome Assembly” if the RNA-Seq library was at all utilized during assembly process, for example, during manual curation or otherwise.

Finally, a cosmetic note concerning Figure 5, it may be useful to the reader if Chromosome labels are indicated, at least for the W and Z chromosomes as these are specifically mentioned in the abstract, and there are otherwise no clear indications in the figure which plots correlate specifically to these chromosomes. (This is mentioned because in another similar Data Note in this journal, there is an a similar Hi-C contact plot with an online interactive version, in which the chromosome accessions are clearly shown for each box).

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: insect molecular biology, genetics and genomics/transcriptomics

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.
