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DATA NOTE

The genome sequence of the Sycamore Piercer, *Pammene aurita* (Razowski, 1991) [version 1; peer review: 3 approved]

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Abstract

We present a genome assembly from an individual male *Pammene aurita* (the Sycamore Piercer; Arthropoda; Insecta; Lepidoptera; Tortricidae). The genome sequence is 1,041.8 megabases in span. Most of the assembly is scaffolded into 28 chromosomal pseudomolecules, including the assembled Z sex chromosome. The mitochondrial genome has also been assembled and is 16.7 kilobases in length.

Keywords

Pammene aurita, Sycamore Piercer, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.

Open Peer Review

Approval Status ✓ ✓ ✓

	1	2	3
version 1 12 Apr 2023	✓ view	✓ view	✓ view

1. **Panagiotis Ioannidis**, Foundation for Research & Technology Hellas, Crete, Greece
2. **Niklas Wahlberg**, Lund university, Lund, Sweden
3. **Yash Sondhi**, University of Florida, Florida, USA

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

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Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Tortricoidea; Tortricidae; Olethreutinae; Grapholitini; *Pammene; Pammene aurita* (Razowski, 1991) (NCBI:txid1870148).

Background

The Sycamore Piercer *Pammene aurita* (Razowski, 1992) is a moth of the Tortricidae family. The adults exhibit attractive light orange markings on the forewings that fade to a dull brown towards the head. There is little variation in these markings (Bradley *et al.*, 1979). The English vernacular name of this species is derived from the feeding habits of the larvae, which feed internally in the seeds of Sycamore (*Acer pseudoplatanus),* 'piercing' the seed and leaving a neat round hole when the larva exits the seed to pupate (Bradley *et al.*, 1979; Elliott *et al.*, 2018; Hancock *et al.*, 2015). Larvae feed between August and September, and the moth overwinters as a pupa, choosing dead wood or bark as a pupation site in captivity (Bradley *et al.*, 1979; Elliott *et al.*, 2018; Hancock *et al.*, 2015). Adults can be found between June and August, flying around Sycamores in the afternoon, and coming to light after dark (Elliott *et al.*, 2018).

Globally, *P. aurita* is confined to northern and central Europe (GBIF Secretariat, 2022; Hancock *et al.*, 2015). This species is a relatively recent addition to the British and Irish fauna, apparently colonising south-eastern England in the mid-20th century (Bradley *et al.*, 1979; Hancock *et al.*, 2015). Since then, the moth has spread across England and Wales, and has been recorded in eastern Ireland (Elliott *et al.*, 2018).

The genome of *Pammene aurita* 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 *Pammene aurita*, based on one male specimen from Wytham Woods, Oxfordshire, UK.

Genome sequence report

The genome was sequenced from one male *Pammene aurita* specimen (Figure 1) collected from Wytham Woods, Oxfordshire, UK (latitude 51.77, longitude –1.34). A total of 24-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 76 missing joins or mis-joins and removed 19 haplotypic duplications, reducing the assembly length by 1.13% and the scaffold number by 32.47%.

The final assembly has a total length of 1,041.8 Mb in 52 sequence scaffolds with a scaffold N50 of 37.5 Mb (Table 1). Most (99.81%) of the assembly sequence was assigned to 28 chromosomal-level scaffolds, representing 27 autosomes, and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 2–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 estimated Quality Value (QV) of the final assembly is 62.5 with *k*-mer completeness of 100%, and the assembly has a BUSCO v5.3.2 (Manni *et al.*, 2021) completeness of 98.1% (single 97.3%, duplicated 0.8%) using the lepidoptera_odb10 reference set (n = 5,286).

Methods

Sample acquisition and nucleic acid extraction

A male *Pammene aurita* specimen (ilPamAuri1) was collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.77, longitude –1.34) on 24 July 2021. The specimen was taken from woodland habitat by Douglas Boyes (University of Oxford) using a light trap. The specimen was identified by the collector and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute (WSI). The ilPamAuri1 sample was weighed

Figure 1. Photograph of the *Pammene aurita* (ilPamAuri1) specimen used for genome sequencing.

Table 1. Genome data for *Pammene aurita*, ilPamAuri1.1.

Project accession data		
Assembly identifier	ilPamAuri1.1	
Species	*Pammene aurita*	
Specimen	ilPamAuri1	
NCBI taxonomy ID	1870148	
BioProject	PRJEB55029	
BioSample ID	SAMEA10979080	
Isolate information	ilPamAuri1, male, whole organism (genome sequencing and Hi-C scaffolding)	
Assembly metrics*		***Benchmark***
Consensus quality (QV)	62.5	*≥ 50*
k-mer completeness	100%	*≥ 95%*
BUSCO**	C:98.1%[S:97.3%,D:0.8%], F:0.5%,M:1.5%,n:5,286	*C ≥ 95%*
Percentage of assembly mapped to chromosomes	99.81%	*≥ 95%*
Sex chromosomes	Z chromosome	*localised homologous pairs*
Organelles	Mitochondrial genome assembled	*complete single alleles*
Raw data accessions		
PacificBiosciences SEQUEL II	ERR10008907	
Hi-C Illumina	ERR10015064	
Genome assembly		
Assembly accession	GCA_947086415.1	
Accession of alternate haplotype	GCA_947086445.1	
Span (Mb)	1,041.8	
Number of contigs	245	
Contig N50 length (Mb)	9.2	
Number of scaffolds	51	
Scaffold N50 length (Mb)	37.5	
Longest scaffold (Mb)	72.7	

* 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/ilPamAuri1.1/dataset/CAMTYY01/busco.

and dissected on dry ice with tissue set aside for Hi-C sequencing. Whole organism tissue was disrupted using a Nippi Powermasher fitted with a BioMasher pestle. 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

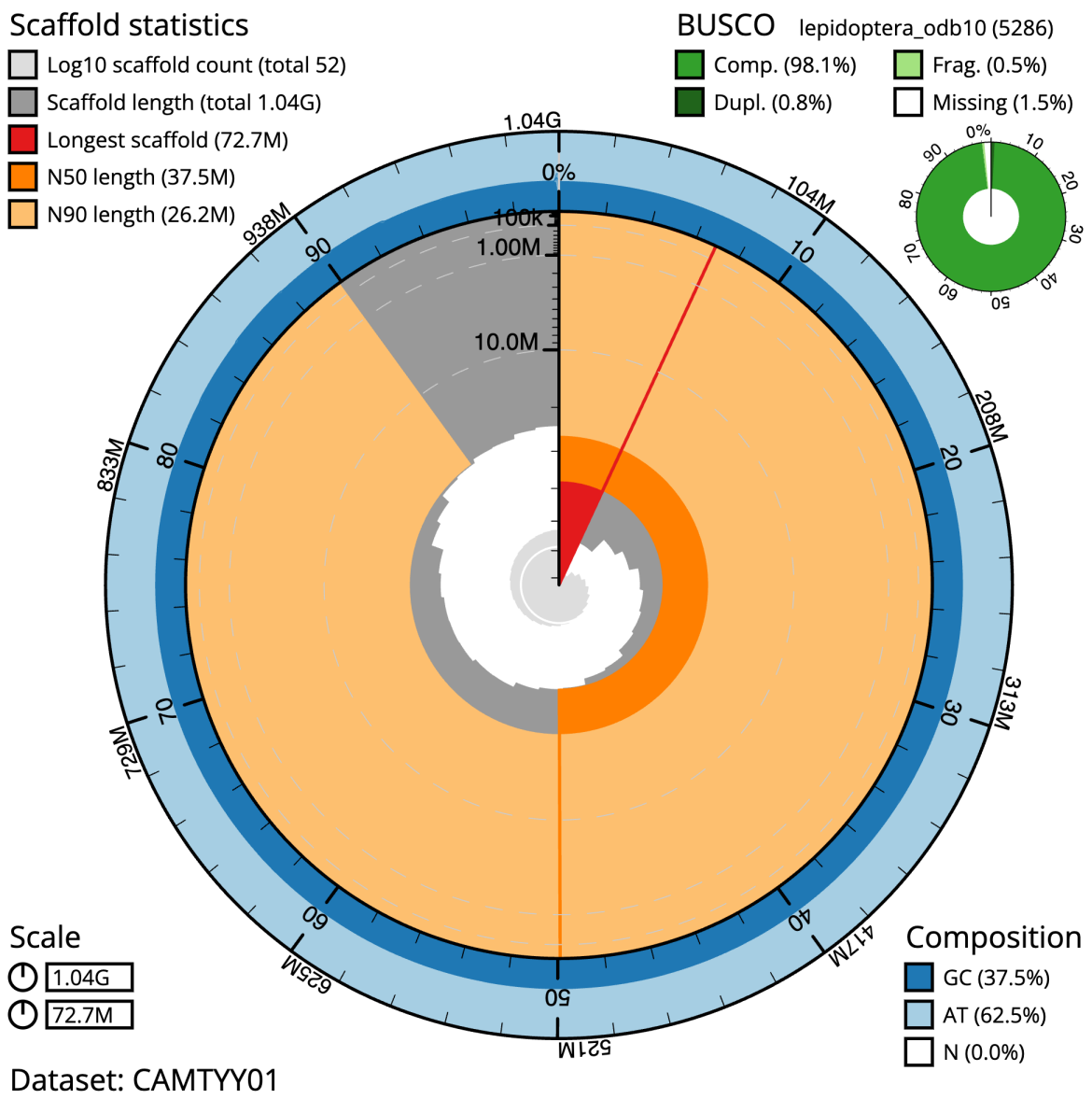

Figure 2. Genome assembly of *Pammene aurita*, ilPamAuri1.1: metrics. The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 1,041,812,917 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (72,712,871 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (37,501,620 and 26,227,413 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/ilPamAuri1.1/dataset/CAMTYY01/snail.

Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

Sequencing

Pacific Biosciences HiFi circular consensus DNA sequencing libraries were constructed according to the manufacturers' instructions. DNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi) instrument. Hi-C data were also generated from tissue of ilPamAuri1 using the Arima v2 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

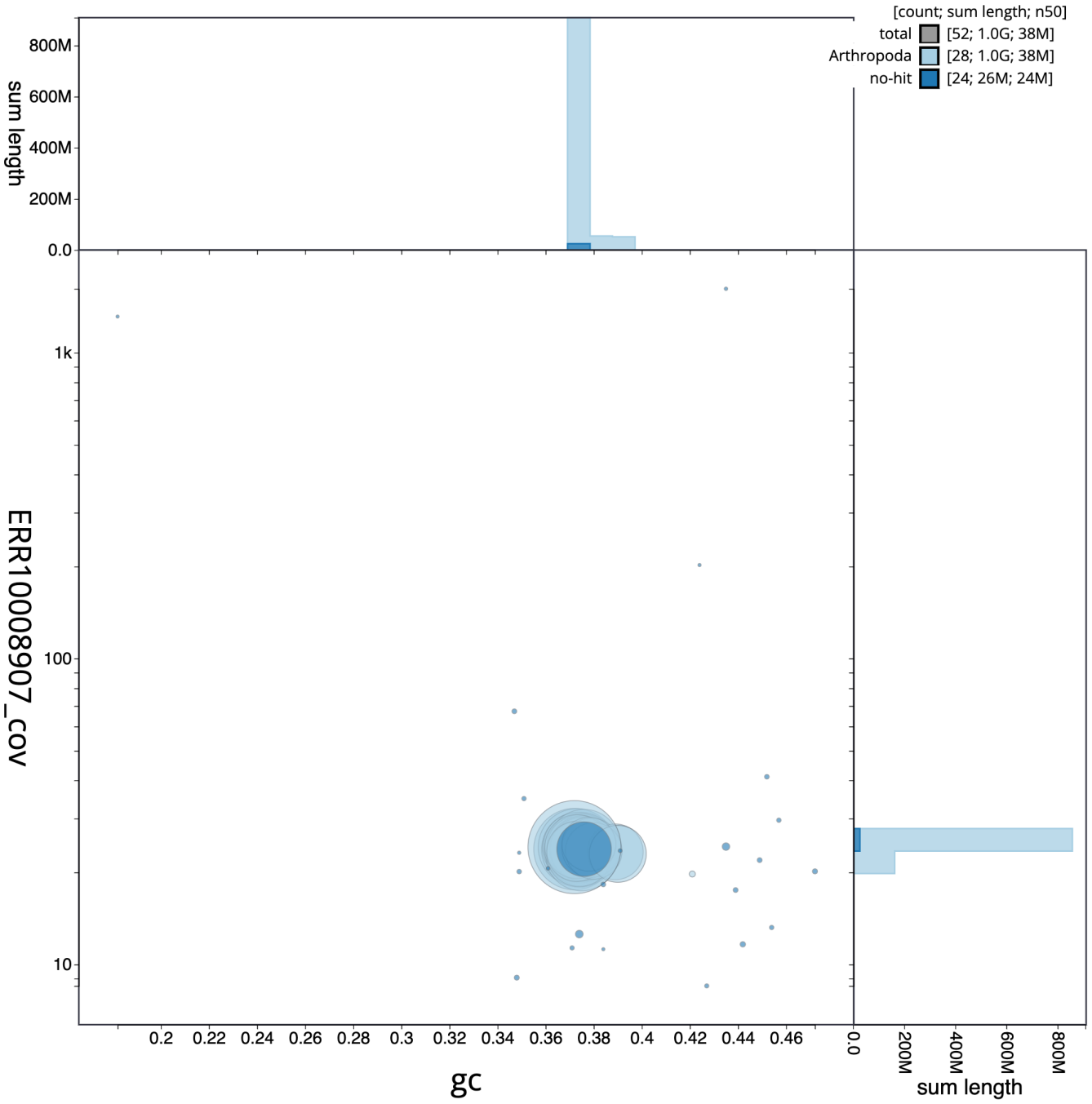

Figure 3. Genome assembly of *Pammene aurita*, ilPamAuri1.1: GC coverage. BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilPamAuri1.1/dataset/CAMTYY01/blob.

purge_dups (Guan *et al.*, 2020). The assembly was then scaffolded with Hi-C data (Rao *et al.*, 2014) using YaHS (Zhou *et al.*, 2023). 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.*, 2022), which performed annotation using MitoFinder (Allio *et al.*, 2020). To evaluate the assembly, MerquryFK was used to estimate consensus quality (QV) scores and *k*-mer completeness (Rhie *et al.*, 2020). The genome was analysed, and BUSCO scores (Manni *et al.*, 2021; Simão *et al.*, 2015) were generated within

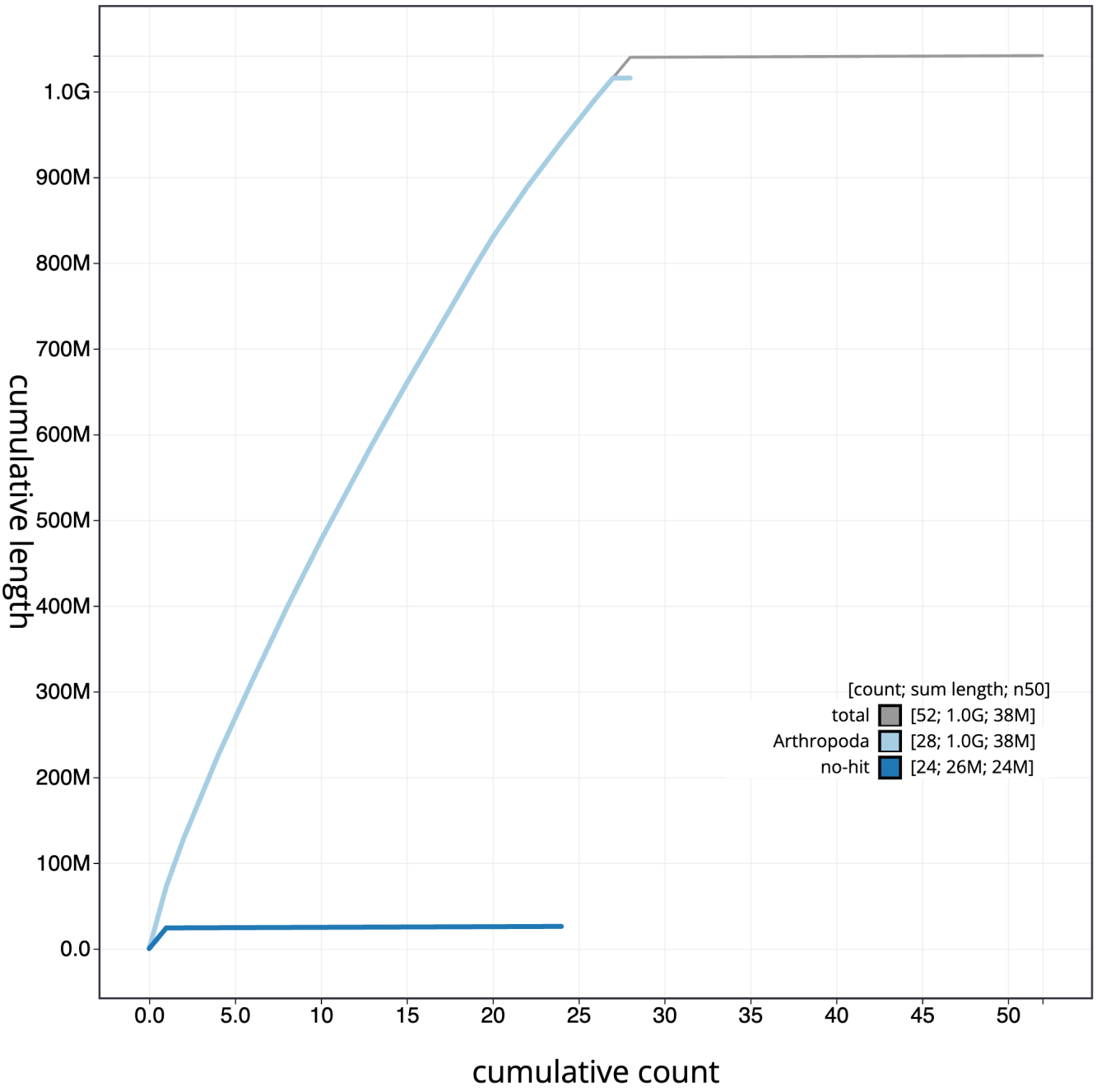

Figure 4. Genome assembly of *Pammene aurita*, ilPamAuri1.1: cumulative sequence. BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilPamAuri1.1/dataset/CAMTYY01/cumulative.

the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of software tool versions and sources.

Ethics and 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, 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. All efforts are undertaken to minimise the suffering of animals

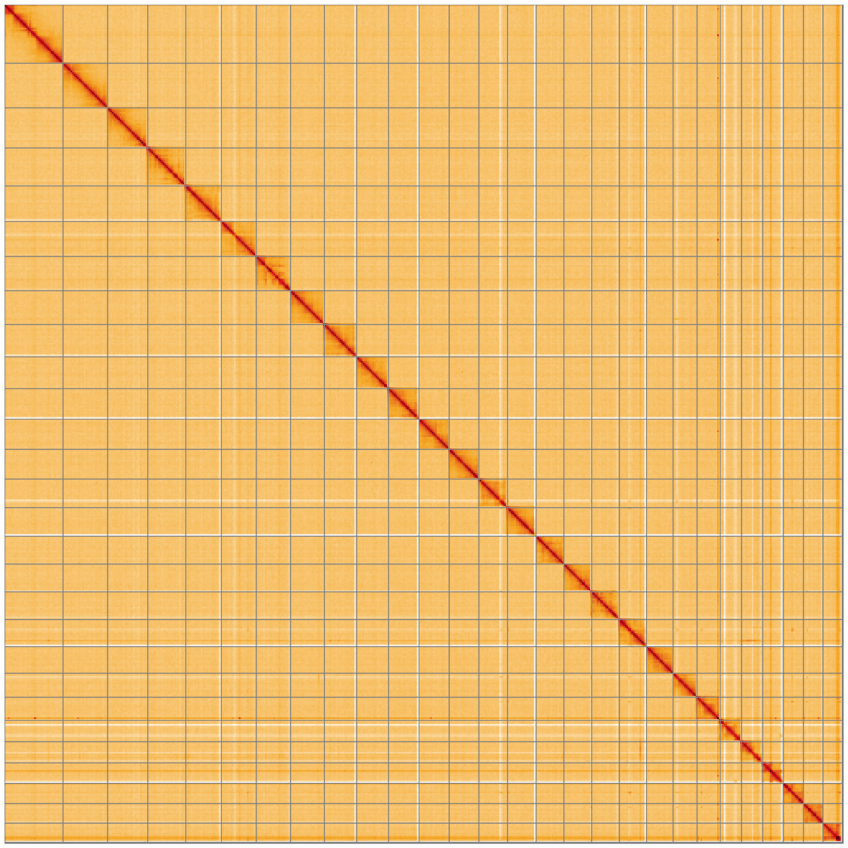

Figure 5. Genome assembly of *Pammene aurita*, ilPamAuri1.1: Hi-C contact map. Hi-C contact map of the ilPamAuri1.1 assembly, visualised using HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this figure may be viewed at https://genome-note-higlass.tol.sanger.ac.uk/l/?d=XW4dzhIyThitRIyDG5Q5JQ

Table 2. Chromosomal pseudomolecules in the genome assembly of *Pammene aurita*, ilPamAuri1.

INSDC accession	Chromosome	Size (Mb)	GC%
OX352290.1	1	55.38	37.2
OX352291.1	2	49.73	37.3
OX352292.1	3	47.15	37.4
OX352296.1	4	41.96	37.4
OX352293.1	5	44.29	37.5
OX352299.1	6	37.83	37.4
OX352297.1	7	40.1	37.4
OX352298.1	8	39.56	37.3
OX352294.1	9	43.25	37.5
OX352300.1	10	37.5	37.5
OX352314.1	11	25.18	37.2
OX352301.1	12	36.88	37.3
OX352303.1	13	35.41	37.5
OX352295.1	14	42.62	37.6
OX352304.1	15	34.59	37.3
OX352305.1	16	34.48	37.4
OX352306.1	17	34.37	37.5
OX352308.1	18	32.99	37.5
OX352302.1	19	35.48	37.4
OX352307.1	20	33.65	37.3
OX352309.1	21	29.8	37.3
OX352310.1	22	28.75	37.9
OX352315.1	23	24.24	37.6
OX352313.1	24	25.35	38.9
OX352312.1	25	26.23	38
OX352316.1	26	23.78	37.8
OX352311.1	27	26.6	39
OX352289.1	Z	72.71	37.2
OX352317.1	MT	0.02	18.3

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.0.7	https://github.com/blobtoolkit/blobtoolkit
BUSCO	5.3.2	https://gitlab.com/ezlab/busco
Hifiasm	0.16.1-r375	https://github.com/chhylp123/hifiasm
HiGlass	1.11.6	https://github.com/higlass/higlass
Merqury	MerquryFK	https://github.com/thegenemyers/MERQURY.FK
MitoHiFi	2	https://github.com/marcelauliano/MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/PretextView
purge_dups	1.2.3	https://github.com/dfguan/purge_dups
YaHS	yahs-1.1.91eebc2	https://github.com/c-zhou/yahs

used for sequencing. 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: *Pammene aurita* (sycamore piercer). Accession number PRJEB55029; https://identifiers.org/ena.embl/PRJEB55029. (Wellcome Sanger Institute, 2022)

The genome sequence is released openly for reuse. The *Pammene aurita* 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 available 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.4789928.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.4893703.

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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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Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Version 1

Reviewer Report 16 May 2024

https://doi.org/10.21956/wellcomeopenres.21326.r78464

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✔ **Yash Sondhi**
University of Florida, Florida, USA

Overview:
The report sequences the genome of the SYcaoTortricidae moth *Pammene aurita* (Sycamore piercer) using long read sequencing. They use Hi-C data to build the chromosome scaffold map is high quality and clearly identifies 28 chromosomes. They also assemble a mitochondrial genome and provide some future plans for an ensemble based gene prediction and annotation. As expected from Pac-Bio data, the assembly has a high N5- of 37.5 M and is 1041.8 Mb. There is the primary and secondary assembly representing some version of the phased assemblies are deposited.
The authors do a good job describing the life history and biogeography of the moth and report the methods and tools they use to generate and assess the genome well.

Minor comments
1)Could the authors briefly discuss how the results of 1000 MB chromosome and 28 chromosomes match with any other previous reports of closely related species?

2)The authors mention Ensemble prediction will be done from the remaining RNA-seq data. Can details of the RNA-sequencing be mentioned, are they whole animal or specific tissues and are the raw RNA reads accessible? Having access to this data will be valuable for confirming the gene prediction models if this data is available.

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: Lepdiotera molecular evlution, sensory biology, sytematics and taxonomy

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 13 May 2024

https://doi.org/10.21956/wellcomeopenres.21326.r70859

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Niklas Wahlberg
Department of Biology, Lund university, Lund, Sweden

This report on the genome of Pammene aurita (Lepidoptera: Tortricidae) follows the standard format of genome reports, and as far as I can see, all looks good. It is interesting that the genome size is about double that of the average lepidopteran genome, and eventually it will be interesting to do a comparison of tortricid genomes to see how genome size evolves over time.

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: I am a founding member of the Psyche project, aiming to sequence the genomes of all Lepidoptera species in Europe.

Reviewer Expertise: Phylogenomics, systematics, 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 April 2024

https://doi.org/10.21956/wellcomeopenres.21326.r78465

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Panagiotis Ioannidis
Foundation for Research & Technology Hellas, Crete, Greece

This genome report describes the sequencing and assembly of a tortricid moth (Lepidoptera). The authors provide sufficient background in the Introduction, in relation to the life cycle and geographical range of the animal. Additionally, the methods describe sufficiently the experimental procedures so that others can replicate their analysis.

The resulting assembly is excellent (as expected for HiFi reads + HiC), both in terms of genome contiguity, as well as in gene content (BUSCO). Thus, I believe that it is suitable for use in downstream analyses (e.g. gene prediction, comparative genomics and phylogenomics).

I have some comments of minor importance for the authors:

1) Even though the authors have provided enough details about the life cycle and geographical range, I would expect to see a few more things about this lepidopteran's ecology (e.g. is it a pest?). Additionally, a bit more background about the genomic resources (genome/transcriptome data) that have already been generated by others, would also be a nice thing to report in the Introduction. I believe that both points (ecology and already available genomic data) will emphasize the importance of the herein published genome assembly.

2) I saw the statement about gene prediction in the "Data availability" section and was wondering if the RNAseq data are from the same species/population/individuals that were used for genome sequencing. In general, it would probably be better if the authors also provided a predicted gene set for anyone interested in quickly performing any comparative analyses (because I'm guessing that gene prediction via Ensembl will take some time). But I also understand that such a task is not trivial and maybe it's not even within the scope of these genome reports.

3) Also, regarding the assembly size (~1.0 Gbp), do the authors know if it is close to the expected genome size for this animal? If there are no clues about its genome size from other studies, do the authors know what is the genome size of other, related Lepidoptera?

4) Similarly to the above comment, do the authors know if the total number of chromosomes (27 + 1) identified for this animal makes sense compared to what is known for this species, or for related species.

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 genomics and transcriptomics, bioinformatics

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.