DATA NOTE



The genome sequence of the Green Carpet moth, Colostygia

pectinataria (Knoch, 1781) [version 1; peer review: awaiting

peer review]

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Abstract

We present a genome assembly from an individual female *Colostygia pectinataria* (the Green Carpet; Arthropoda; Insecta; Lepidoptera; Geometridae). The genome sequence is 351.6 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 17.97 kilobases in length.

Keywords

Colostygia pectinataria, Green Carpet moth, genome sequence, chromosomal, Lepidoptera



This article is included in the Tree of Life gateway.

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.

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Species taxonomy

Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Bilateria; Protostomia; Ecdysozoa; Panarthropoda; Arthropoda; Mandibulata; Pancrustacea; Hexapoda; Insecta; Dicondylia; Pterygota; Neoptera; Endopterygota; Amphiesmenoptera; Lepidoptera; Glossata; Neolepidoptera; Heteroneura; Ditrysia; Obtectomera; Geometroidea; Geometridae; Larentiinae; *Colostygia; Colostygia pectinataria* (Knoch, 1781) (NCBI:txid934931).

Background

Green pigments have a scattered phylogenetic distribution across the insects and are seen in several distinct lineages of Lepidoptera. The Green Carpet moth *Colostygia pectinataria*, family Geometridae, is only distantly related to most green geometrid moths. In *C. pectinataria* the green pigment is spread across the forewings, with the colour being noticeably darker close to the wing base and within an irregular black- and white-edged band. The brightness of the green colour fades during the lifetime of the adult insect turning to yellow or white, but the outlines of the wing markings remain distinctive.

The species is widely distributed across the Palaearctic from the west of Ireland and Portugal to Belarus, Ukraine and Russia, and from Scandinavia to Italy (GBIF Secretariat, 2023). In Britain, C. pectinataria has been recorded from almost the entirety of England, Scotland, Wales and Northern Ireland; it can be common in gardens, woodlands, farmland and moorland habitats where the larval food plant Galium spp. is found (NBN Atlas Partnership, 2023). Adults are nocturnal but often disturbed in the daytime and frequently seen flying at dusk; adults are also attracted to light. Analysis of data from a standardised network of light traps showed that C. pectinataria has steadily increased in abundance in Britain over the past 50 years (Boyes et al., 2019). In southern Britain, the species has two generations per year, with first brood adults encountered from May to June, and second brood adults from August to September; in northern Britain there is generally only one generation per year. The boundary between these two phenologies is changing; for example, in Leicestershire in the midlands of England, the species had one annual generation until around 1998, switching to two generations by 2004 (Palmer, 2023). Changes in phenology and abundance may be related to climate change (Boyes et al., 2019; Palmer, 2023).

A genome sequence of the Green Carpet moth *Colostygia pectinataria* was determined as part of the Darwin Tree of Life project. The genome sequence will facilitate research into biological responses to changing climatic conditions, the control of life cycle timing, and the biochemistry of insect pigment production.

Genome sequence report

The genome was sequenced from one female *Colostygia pectinataria* (Figure 1) collected from Marley Fen, Wytham, Oxfordshire, UK (51.77, -1.31). A total of 39-fold coverage in Pacific Biosciences single-molecule HiFi long reads was generated. Primary assembly contigs were scaffolded with



Figure 1. Photograph of the *Colostygia pectinataria* (ilColPect1) specimen used for genome sequencing.

chromosome conformation Hi-C data. Manual assembly curation corrected 9 missing joins or mis-joins and removed 2 haplotypic duplications, reducing the assembly length by 0.31% and the scaffold number by 9.76%.

The final assembly has a total length of 351.6 Mb in 36 sequence scaffolds with a scaffold N50 of 12.7 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 (99.9%) of the assembly sequence was assigned to 31 chromosomal-level scaffolds, representing 30 autosomes and the Z sex chromosome. Chromosome-scale scaffolds confirmed by the Hi-C data are named in order of size (Figure 5; Table 2). Chromosome Z was identified by synteny to Scotopteryx bipunctaria (GCA_ 949320045.1). As there is half coverage of the Z chromosome, this is a ZO individual and was thus designated as female. 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 67.1 with *k*-mer completeness of 100.0%, 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/934931.

Methods

Sample acquisition and nucleic acid extraction

A female *Colostygia pectinataria* (specimen ID Ox000213, ToLID ilColPect1) was collected in a light trap from Marley

Project accession data				
Assembly identifier	ilColPect1.1			
Species	Colostygia pectinataria			
Specimen	ilColPect1			
NCBI taxonomy ID	934931			
BioProject	PRJEB61341			
BioSample ID	SAMEA7520182			
Isolate information	ilColPect1, female: whole organism (DNA sequencing) ilColPect3: thorax (Hi-C and RNA sequencing)			
Assembly metrics*		Benchmark		
Consensus quality (QV)	67.1	≥50		
k-mer completeness	100.0%	≥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	99.9%	≥95%		
Sex chromosomes	Z	localised homologous pairs		
Organelles	Mitochondrial genome: 17.97 kb	complete single alleles		
Raw data accessions				
PacificBiosciences SEQUEL II	ERR11270461			
Hi-C Illumina	ERR11242551			
PolyA RNA-Seq Illumina	ERR11242552			
Genome assembly				
Assembly accession	GCA_951394395.1			
Accession of alternate haplotype	GCA_951394385.1			
Span (Mb)	351.6			
Number of contigs	47			
Contig N50 length (Mb)	12.5			
Number of scaffolds	36			
Scaffold N50 length (Mb)	12.7			
Longest scaffold (Mb)	18.19			

Table 1. Genome data for Colostygia pectinataria, ilColPect1.1.

* 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 version 5.3.2. 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/ilColPect1_1/dataset/ilColPect1_1/busco.

Fen, Wytham, Oxfordshire, England, UK (latitude 51.77, longitude–1.31) on 2019-08-24. The specimen was collected and identified by Douglas Boyes (University of Oxford) and snap-frozen on dry ice.

The specimen used for Hi-C and RNA sequencing (specimen ID SAN00002620, ToLID ilColPect3) was collected from Glen Strathfarrar, Scotland, UK (latitude 57.41, longitude -4.73) on 2022-06-27 using a moth trap. The specimen was collected



Figure 2. Genome assembly of *Colostygia pectinataria*, **ilColPect1.1: 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 351,575,540 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 (18,186,188 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (12,651,202 and 8,504,000 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/ilColPect1_1/dataset/ ilColPect1_1/snail.

by Andy Griffiths (Wellcome Sanger Institute) and identified by Marc Botham (Centre for Ecology and Hydrology) and flash-frozen in liquid nitrogen.

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, fragmentation, and clean-up. In sample preparation, the ilColPect1 sample was weighed and dissected on dry ice (Jay *et al.*, 2023). Tissue from the whole organism was homogenised using a PowerMasher II tissue disruptor (Denton *et al.*, 2023a). HMW DNA was extracted using the Automated MagAttract v1 protocol (Sheerin *et al.*, 2023). DNA was sheared into an average fragment size of 12–20 kb in a Megaruptor 3 system with speed setting 30 (Todorovic *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



Figure 3. Genome assembly of *Colostygia pectinataria*, **ilColPect1.1: 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/ilColPect1_1/dataset/ilColPect1_1/blob.

sample to eliminate shorter fragments and concentrate the DNA. 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.

JA 2023). The RNA concentration was assessed using a Nanodrop 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.

RNA was extracted from thorax tissue of ilColPect3 in the Tree of Life Laboratory at the WSI using the RNA Extraction:

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

Automated MagMaxTM mirVana protocol (do Amaral et al.,



Figure 4. Genome assembly of *Colostygia pectinataria*, **ilColPect1.1: 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/ilColPect1_1/dataset/ilColPect1_1/ cumulative.

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 thorax tissue of ilColPect3 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 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 PretextView (Harry, 2022). The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2023), which runs MitoFinder (Allio *et al.*, 2020) or MITOS



Figure 5. Genome assembly of *Colostygia pectinataria*, ilColPect1.1: Hi-C contact map of the ilColPect1.1 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=YUBWukDjSFK2wmETC_QARg.

INSDC accession	Chromosome	Length (Mb)	GC%
OX596349.1	1	14.72	37.5
OX596350.1	2	14.65	37.5
OX596351.1	3	14.39	37.5
OX596352.1	4	14.3	37.5
OX596353.1	5	13.9	37.0
OX596354.1	6	13.55	37.0
OX596355.1	7	13.48	37.5
OX596356.1	8	13.25	37.0
OX596357.1	9	13.07	37.0
OX596358.1	10	12.69	37.0
OX596359.1	11	12.69	37.5
OX596360.1	12	12.65	37.5
OX596361.1	13	12.47	37.5
OX596362.1	14	12.2	37.0
OX596363.1	15	11.83	37.5

Table 2. Chromosomal pseudomolecules in thegenome assembly of Colostygia pectinataria,ilColPect1.

INSDC accession	Chromosome	Length (Mb)	GC%
OX596364.1	16	11.6	37.5
OX596365.1	17	11.52	38.0
OX596366.1	18	11.33	37.5
OX596367.1	19	10.79	38.0
OX596368.1	20	10.79	37.5
OX596369.1	21	10.72	38.5
OX596370.1	22	8.91	38.5
OX596371.1	23	8.85	37.5
OX596372.1	24	8.59	38.0
OX596373.1	25	8.5	37.5
OX596374.1	26	7.5	38.0
OX596375.1	27	6.41	38.5
OX596376.1	28	6.09	39.5
OX596377.1	29	6.04	39.0
OX596378.1	30	5.61	38.5
OX596348.1	Z	18.19	37.5
OX596379.1	MT	0.02	18.5

(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.

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.

Table 3. Software tools: versions and sources.

Software tool	Version	Source
BlobToolKit	4.2.1	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	3	https://github.com/marcelauliano/ MitoHiFi
PretextView	0.2	https://github.com/wtsi-hpag/ PretextView
purge_dups	1.2.5	https://github.com/dfguan/purge_ dups
sanger-tol/ genomenote	v1.0	https://github.com/sanger-tol/ genomenote
sanger-tol/ readmapping	1.1.0	https://github.com/sanger-tol/ readmapping/tree/1.1.0
YaHS	1.2a.2	https://github.com/c-zhou/yahs

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.

Data availability

European Nucleotide Archive: *Colostygia pectinataria* (green carpet). Accession number PRJEB61341; https://identifiers.org/ena.embl/PRJEB61341 (Wellcome Sanger Institute, 2023). The genome sequence is released openly for reuse. The *Colostygia pectinataria* 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.

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