DATA NOTE

The genome sequence of the hazel leaf-roller, *Apoderus coryli* (Linnaeus, 1758) [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

We present a genome assembly from an individual male *Apoderus coryli* (the hazel leaf roller; Arthropoda; Insecta; Coleoptera; Attelabidae). The genome sequence is 428 megabases in span. The majority (98.90%) of the assembly is scaffolded into 20 chromosomal pseudomolecules, with the X and Y sex chromosomes assembled.

Keywords

Apoderus coryli, hazel leaf roller, genome sequence, chromosomal, Coleoptera

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

Open Peer Review

Approval Status

	1	2
version 1		
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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: Crowley LM: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing;

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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; Coleoptera; Polyphaga; Cucujiformia; Attelabidae; Apoderinae; Apoderus; *Apoderus coryli* (Linnaeus, 1758) (NCBI:txid201766).

Background

The hazel leaf-roller, *Apoderus coryli*, is a distinctive leaf-rolling weevil which is widespread and locally common across Europe and western Asia. In the UK, it is common in deciduous woodland across England and Wales, with a few records from southern Scotland. The main host plant is common hazel, *Corylus avellana*, although it is also known to infrequently feed on alder, birch, hornbeam and beech. It may be considered a minor pest of hazelnut cultivation (Gantner, 2000). Adults are 6–8 mm with red elytra, pronotum and bases of the femora. The head, underside and remainder of the legs are black. It has a distinctive elongate head and ‘neck’. Adults are active from May, feeding on hazel leaves in which they leave characteristic round holes. Mating and oviposition occurs in early summer. Females lay a single bright orange egg around 1 mm in size on a leaf before cutting a line across the leaf with their mandibles and rolling it into a cylinder around the egg. The leaf rolling process may take 60–90 minutes and a single female may repeat this for up to 30 eggs across several days (Urban, 2014). Larvae develop and pupate within these leaf rolls. Second generation adults are active in late summer, the larvae of which overwinter within leaf rolls that drop to the ground and pupate in the spring. A previous study has shown that this species has 13 autosomal bivalents, that sex chromosomes exhibit a parachute association and that this species possesses a small amount of heterochromatin (Rozek et al., 2004).

Genome sequence report

The genome was sequenced from one male *A. coryli* (Figure 1) collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.770, longitude -1.339). A total of 59-fold coverage in Pacific Biosciences single-molecule long reads and 93-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 57 missing/misjoins and removed 7 haplotypic duplications, reducing the assembly length by 0.41% and the scaffold number by 24.71%, and increasing the scaffold N50 by 24.75%.

The final assembly has a total length of 428 Mb in 128 sequence scaffolds with a scaffold N50 of 23.7 Mb (Table 1). The majority, 98.90%, of the assembly sequence was assigned to 20 chromosomal-level scaffolds, representing 18 autosomes (numbered by sequence length), and the X and Y sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.1.2 (Simão et al., 2015) completeness of 99.3% (single 97.7%, duplicated 1.6%) using the endopterygota_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Figure 1. Images of the *Apoderus coryli* specimen, icApoCory1, used for genome sequencing. Top, the specimen prior to collection; bottom, the specimen during preservation and processing, shown with a 43.9 mm FluidX sample tube.

Methods

Sample acquisition and DNA extraction

A single male *A. coryli* was collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.770, longitude -1.339) by Liam Crowley, University of Oxford, using a pooter. The sample was snap-frozen on dry ice and stored using a CoolRack.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The icApoCory1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Tissue from the whole organism 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

Table 1. Genome data for *Apoderus coryli*, icApoCory1.1.

Project accession data	
Assembly identifier	icApoCory1.1
Species	<i>Apoderus coryli</i>
Specimen	icApoCory1
NCBI taxonomy ID	NCBI:txid201766
BioProject	PRJEB45119
BioSample ID	SAMEA7520690
Isolate information	Male, whole organism
Raw data accessions	
PacificBiosciences SEQUEL II	ERR6412370
10X Genomics Illumina	ERR6054772-ERR6054775
Hi-C Illumina	ERR6054771
Genome assembly	
Assembly accession	GCA_911728435.1
Accession of alternate haplotype	GCA_911728465.1
Span (Mb)	428
Number of contigs	203
Contig N50 length (Mb)	15.0
Number of scaffolds	128
Scaffold N50 length (Mb)	23.7
Longest scaffold (Mb)	46.0
BUSCO* genome score	C:99.3%[S:97.7%,D:1.6%], F:0.4%,M:0.3%,n:2124

*BUSCO scores based on the endopterygota_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/icApoCory1.1/dataset/CAJVRQ01/busco>.

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.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries were constructed according to the manufacturers' instructions. Sequencing was

performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II and Illumina HiSeq X instruments. Hi-C data were generated from further whole organism tissue using the Arima v2 Hi-C kit and sequenced on a HiSeq X instrument.

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) with the -e flag. One round of polishing was performed by aligning 10X Genomics read data to the assembly with longranger align, calling variants with

Scaffold statistics

- Log10 scaffold count (total 130)
- Scaffold length (total 430M)
- Longest scaffold (46M)
- N50 length (24M)
- N90 length (13M)

BUSCO

endopterygota_odb10 (2124)

- Complete (99.3%)
- Fragmented (0.4%)
- Duplicated (1.6%)
- Missing (0.3%)

Figure 2. Genome assembly of *Apoderus coryli*, icApoCory1.1: 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 428,077,793 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 (45,983,548 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (23,694,900 and 12,555,284 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 endopterygota_odb10 set is shown in the top right. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/icApoCory1.1/dataset/CAJVRQ01/snail>.

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)

Figure 3. Genome assembly of *Apoderus coryli*, icApoCory1.1: 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/icApoCory1.1/dataset/CAJVRQ01/blob>.

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) and annotated with

MitoFinder (Allio *et al.*, 2020). 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 4. Genome assembly of *Apoderus coryli*, icApoCory1.1: 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/icRhaFulv1.1/dataset/CAJPIC01.1/cumulative>.

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, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this

Figure 5. Genome assembly of *Apoderus coryli*, icApoCory1.1: Hi-C contact map. Hi-C contact map of the icApoCory1.1 assembly, visualised in HiGlass.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Rhagonycha fulva*, icRhaFulv1.1.

INSDC accession	Chromosome	Size (Mb)	GC%
OU452198.1	1	45.98	33.1
OU452199.1	2	45.50	32.8
OU452200.1	3	42.38	32.6
OU452201.1	4	30.93	32.1
OU452202.1	5	27.11	32.5
OU452203.1	6	23.69	32.7
OU452204.1	7	21.42	32.8
OU452205.1	8	20.46	32.7
OU452206.1	9	20.22	32.6
OU452207.1	10	18.89	32.9

INSDC accession	Chromosome	Size (Mb)	GC%
OU452208.1	11	17.89	33.2
OU452209.1	12	17.13	33.6
OU452211.1	13	14.69	33.1
OU452212.1	14	13.58	33.5
OU452213.1	15	12.56	33.7
OU452214.1	16	11.87	33
OU452215.1	17	10.51	32.6
OU452216.1	18	10.30	32.9
OU452210.1	X	16.03	31.4
OU452217.1	Y	1.26	24.7
OU452218.1	MT	0.02	27.3
-	Unplaced	5.65	25.5

Table 3. Software tools used.

Software tool	Version	Source
Hifiasm	0.12	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	2.1	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

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: *Apoderus coryli* (hazel leaf-roller). Accession number [PRJEB45119](#); <https://identifiers.org/ena.embl/PRJEB45119>.

The genome sequence is released openly for reuse. The *A. coryli* 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 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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Open Peer Review

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Ardha Apriyanto

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In this report, the authors present a genome sequence of *Apoderus coryli* (Linnaeus, 1758), the hazel leaf-roller. The sequence reported is novel and original. Furthermore, the dataset will provide a useful resource for the scientific community. This reported genome produced a high-quality genome assembly that utilizes various sequencing technologies. However, this report does not address the genome sequence annotation.

Regarding the manuscript, I have some minor comments:

1. There is a duplicate sentence regarding sample collection in the Genome sequence report and Methods section. "collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.770, longitude -1.339)." Please rewrite or omit one of them since it is redundant.
2. Please include a bar scale in Figure 1.
3. The link in Figure 4 is wrong. It belongs to the *R.fulva* CAJPICO1. Please revise this.

Overall, I would endorse it for indexing with minor revisions.

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: Genome assembly, Omics (Genomics, Transcriptomics, Proteomics, Metabolomics), 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, however I have significant reservations, as outlined above.

Reviewer Report 02 December 2021

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Khaled Amiri

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The Genome assembly of the hazel leaf-roller, *Apoderus coryli* is completely descriptive but provides a well-assembled genome with 20 chromosomal pseudomolecules. The author has arrived to this assembly using several genomic platforms. The figures present the cumulative sequence and other parameters. I would, however, briefly compare the sequence presented here with other beetles.

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: Genome science

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.