

## The genome sequence of the hawthorn shieldbug, *Acanthosoma haemorrhoidale* (Linnaeus, 1758)

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DATA NOTE

# The genome sequence of the hawthorn shieldbug, *Acanthosoma haemorrhoidale* (Linnaeus, 1758) [version 1; peer review: 1 approved]

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## Abstract

We present a genome assembly from an individual male *Acanthosoma haemorrhoidale* (hawthorn shieldbug; Arthropoda; Insecta; Hemiptera; Acanthosomatidae). The genome sequence is 866 megabases in span. The majority of the assembly (99.98%) is scaffolded into 7 chromosomal pseudomolecules with the X and Y sex chromosomes assembled. The complete mitochondrial genome was also assembled and is 18.9 kilobases in length.

## Keywords

*Acanthosoma haemorrhoidale*, hawthorn shieldbug, genome sequence, chromosomal, Arthropoda



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

## Open Peer Review


Approval Status 

1

version 1

05 Jul 2022



1. **Andrew J. Mongue** , The University of  
Edinburgh, Edinburgh, UK

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; **Mulley J:** Writing – Original Draft Preparation;

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## Species taxonomy

Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Paraneoptera; Hemiptera; Heteroptera; Panheteroptera; Pentatomomorpha; Pentatomoidea; Acanthosomatidae; Acanthosoma; Acanthosoma haemorrhoidale (Linnaeus, 1758) (NCBI:txid483950).

## Background

The hawthorn shield bug, *Acanthosoma haemorrhoidale*, is a large Pentatomid shield bug, easily recognisable by their size (typically 13mm or more in length) and bright green and red coloration. The species is common on hawthorn (*Crataegus monogyna*), where the berries comprise their principal food source, but are also found in mixed woodland and will feed on leaves of oak, hazel, and other deciduous trees and shrubs. Adults overwinter in leaf litter or under bark, and sometimes in buildings, and emerge in spring. Eggs are laid in several batches in late spring to early summer, and females exhibit no maternal care, unlike other members of the *Acanthosomatidae* (Hanelová & Vilímová 2013; Tsai & Rédei, 2015). First ecdysis to adult emergence takes around 35 days (Hori *et al.*, 1993).

Originally classified as *Cimex haemorrhoidalis* by Linnaeus in 1758, the genus *Acanthosoma* (acantho- = spiny, -soma = body) was raised by Curtis in 1824 for the spined keel on the ventral surface (Curtis, 1824). The species name references the blood red coloration and appearance of discharging blood, particularly from the tip of the abdomen. The species has a trans-palaearctic distribution and comprises at least three currently-recognised subspecies: *A. h. haemorrhoidale*, Linnaeus 1758; *A. h. angulatum*, Jakovlev 1880; *A. h. ouchii*, Ishihara 1950 (Tsai & Rédei, 2015).

The classic work by Southwood and Leston on British land and water bugs (Southwood & Leston, 1959) describes a distribution across much of England and Wales, with only recent colonisation of Northern England. Whilst sporadic records for Scotland are found from the mid-20th century, it appears that a gradual northward range expansion has been underway from at least the mid-1990's, and that the species is now well-established across northern England and is reasonably common up to the central belt of Scotland, with more scattered reports from further north (Ramsay, 2014). A similar northern expansion appears to have occurred in Finland in the mid-20th century (Ramsay, 2014; Southwood & Leston, 1959), and it may be interesting to investigate parallel behavioural or physiological changes in these northward-bound populations. Development is temperature sensitive, with high mortality at 30°C (Hori *et al.*, 1993), and more southern parts of the species range may therefore become unsuitable in the future.

In contrast to groups like the Lepidoptera, where females produce pheromones to attract males, in the Pentatomoidea it seems to be males that produce pheromones, most likely to avoid parasitoids utilising female pheromones to find hosts, and male *A. haemorrhoidale* possess extensive abdominal sternal glands (Staddon, 1990). The genome sequence will facilitate identification of biosynthetic pathways underlying

pheromone production and reception in this species. Similarly, genomic data will shed light on host-symbiont relationships, including not only characterisation of bacterial symbionts themselves, but also the anatomical and behavioural mechanisms for storing and transmitting them to the next generation, such as the midgut crypts and the lubricating organs of females (Kikuchi *et al.*, 2009).

Southwood and Leston report the diploid (2N) karyotype of *A. haemorrhoidale* to be 12, comprising ten autosomes and two sex chromosomes (XX or XY), and this accords with reported modal numbers for other members of the *Acanthosomatidae* (Kaur & Patial, 2017; Rebagliati *et al.*, 2005; Southwood & Leston, 1959).

## Genome sequence report

The genome was sequenced from a single male *A. haemorrhoidale* collected from Wytham woods, Berkshire, UK (Figure 1). A total of 26-fold coverage in Pacific Biosciences single-molecule HiFi long reads and 223-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 321 missing/misjoins and removed 4 haplotypic duplications, reducing the assembly size by 0.08% and the scaffold number by 65.5%, and increasing the scaffold N50 by 112.02%.

The final assembly has a total length of 866 Mb in 72 sequence scaffolds with a scaffold N50 of 33.6 Mb (Table 1). The majority, 99.98%, of the assembly sequence was assigned to 7 chromosomal-level scaffolds, representing 5 autosomes (numbered by sequence length) and the X and Y sex chromosomes (Figure 2–Figure 5; Table 2).

The assembly has a BUSCO v5.2.2 (Manni *et al.*, 2021) completeness of 99.2% (single 97.4%, duplicated 1.8%) using the hemiptera\_odb10 reference set (n=954). While not fully



**Figure 1.** Image of the *Acanthosoma haemorrhoidale* specimen taken prior to preservation and processing.

**Table 1. Genome data for *Acanthosoma haemorrhoidale*, ihAcaHaem1.1.**

<b>Project accession data</b>	
Assembly identifier	ihAcaHaem1.1
Species	<i>Acanthosoma haemorrhoidale</i>
Specimen	ihAcaHaem1 (genome assembly; Hi-C)
NCBI taxonomy ID	483950
BioProject	PRJEB47321
BioSample ID	SAMEA8563710
Isolate information	Male, abdomen (genome sequencing); head/thorax (Hi-C)
<b>Raw data accessions</b>	
PacificBiosciences SEQUEL II	ERR6808043-ERR6808045
10X Genomics Illumina	ERR6688746-ERR6688753
Hi-C Illumina	ERR6688405
<b>Genome assembly</b>	
Assembly accession	GCA_930367205.1
Accession of alternate haplotype	GCA_930374635.1
Span (Mb)	866
Number of contigs	545
Contig N50 length (Mb)	3.36
Number of scaffolds	72
Scaffold N50 length (Mb)	129.2
Longest scaffold (Mb)	193.5
BUSCO* genome score	C:99.2%[S:97.4%,D:1.8%],F:0.1%,M:0.7%,n:2510

\*BUSCO scores based on the hemiptera\_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/ihAcaHaem1.1/dataset/CAKNEZ01/busco#Filters>.

phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

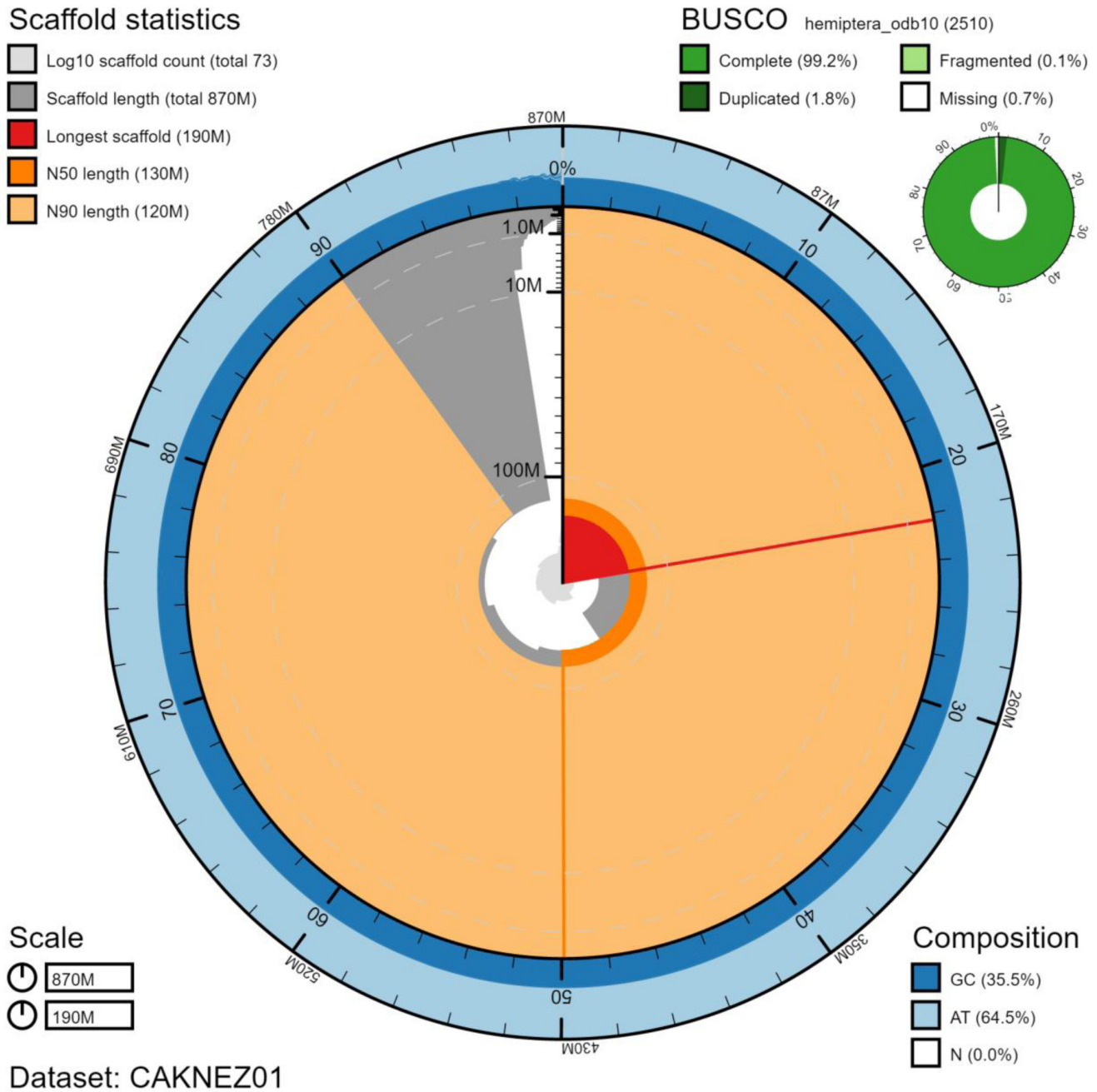
## Methods

### Sample acquisition and nucleic acid extraction

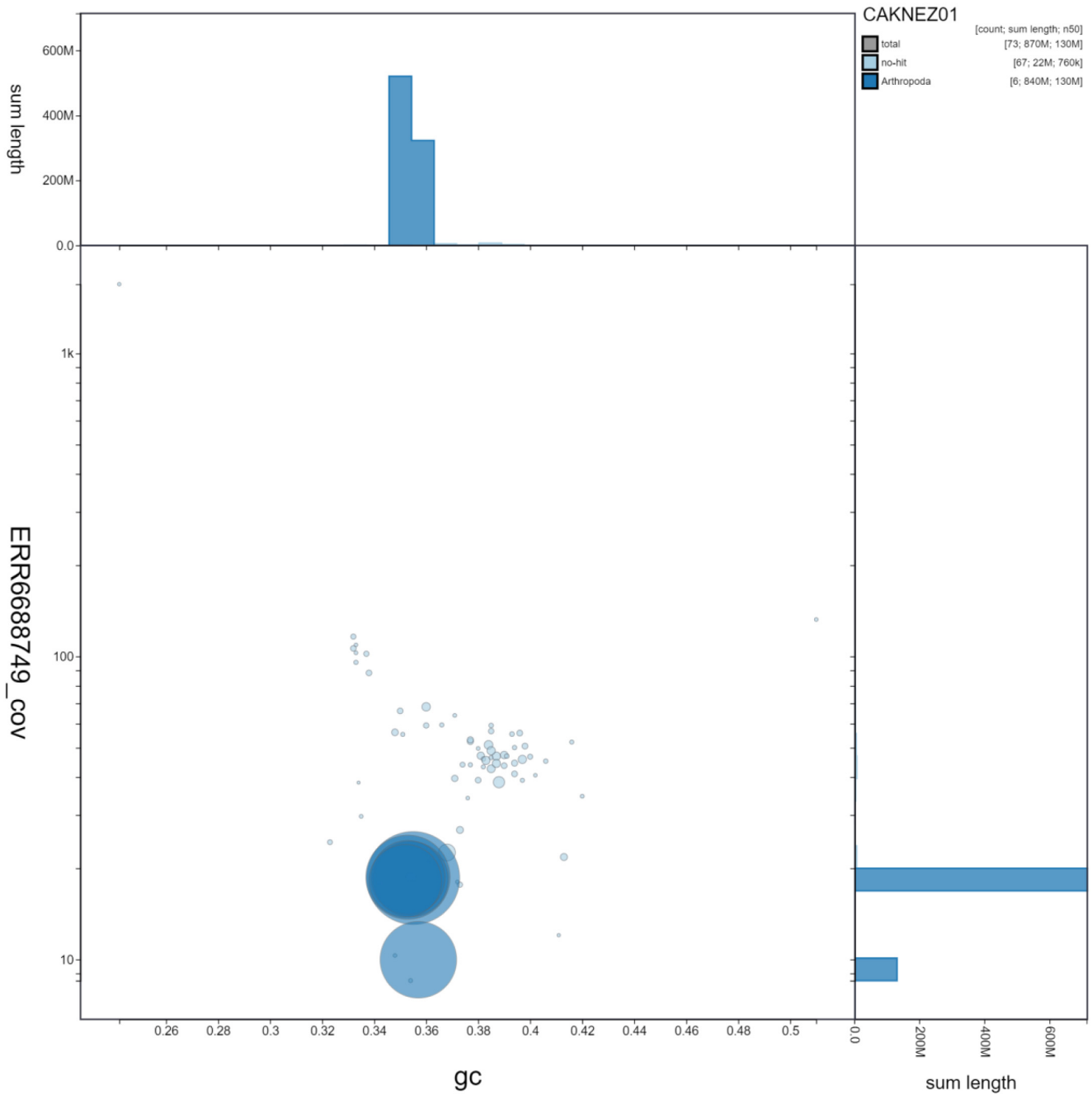
A single *A. haemorrhoidale* specimen (ihAcaHaem1) was collected using a pot from Wytham wood, Berkshire, UK (latitude 51.772, longitude -1.336) by Liam Crowley (University of Oxford). The specimen was identified by Liam Crowley and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The ihAcaHaem1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Abdomen 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.



**Figure 2. Genome assembly of *Acanthosoma haemorrhoidale*, ihAcaHaem1.1: 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 865,622,309 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 (193,544,673 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (129,246,741 and 116,641,292 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 hemiptera\_odb10 set is shown in the top right. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ihAcaHaem1.1/dataset/CAKNEZ01/snail#Filters>.

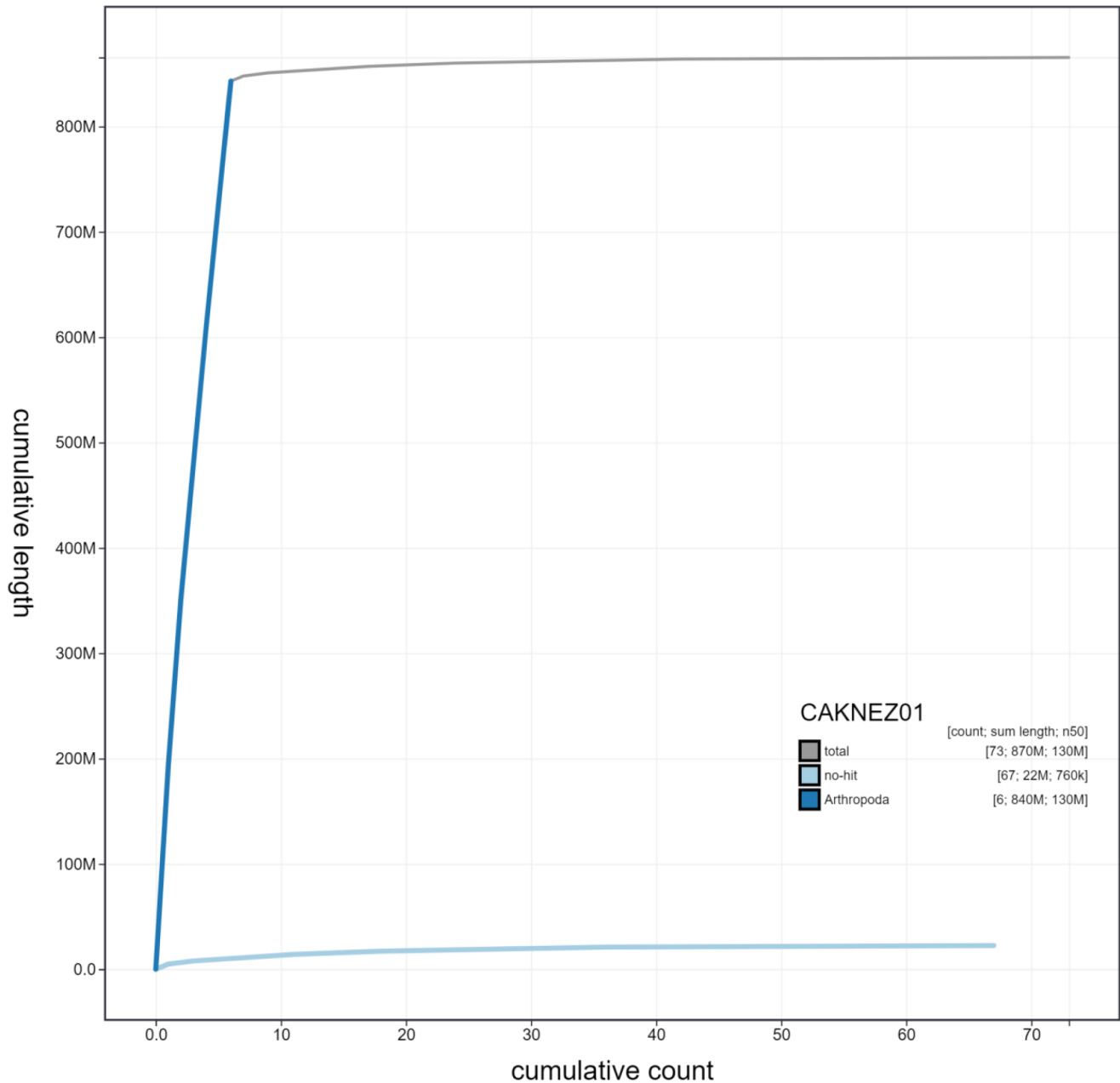


**Figure 3. Genome assembly of *Acanthosoma haemorrhoidale*, ihAcaHaem1.1: GC coverage.** BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length. Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ihAcaHaem1.1/dataset/CAKNEZ01/blob#Filters>.

**Sequencing**

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud 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 (HiFi) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were generated in the Tree of Life laboratory from head/thorax tissue of ihAcaHaem1 using the Arima v2 kit and sequenced on a NovaSeq 6000 instrument.



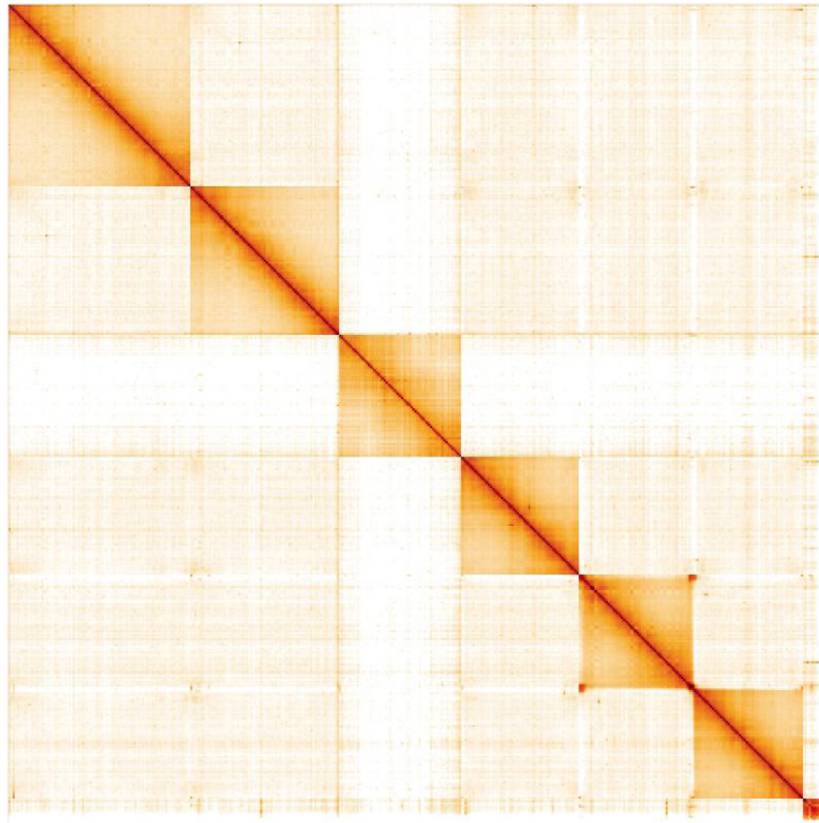
**Figure 4. Genome assembly of *Acanthosoma haemorrhoidale*, ihAcaHaem1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at <https://blobtoolkit.genomehubs.org/view/ihAcaHaem1.1/dataset/CAKNEZ01/cumulative#Filters>.

#### 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`





**Figure 5. Genome assembly of *Acanthosoma haemorrhoidale*, ihAcaHaem1.1: Hi-C contact map.** Hi-C contact map of the ihAcaHaem1.1 assembly, visualised in HiGlass. Chromosomes are arranged in size order from left to right and top to bottom. The interactive Hi-C map can be viewed at <https://genome-note-higlass.tol.sanger.ac.uk/l/?d=SITVSHcSTwOqoICwz4HaZA>.

**Table 2. Chromosomal pseudomolecules in the genome assembly of *Acanthosoma haemorrhoidale*, ihAcaHaem1.1.**

INSDC accession	Chromosome	Size (Mb)	GC%
OV884011.1	1	193.55	35.5
OV884012.1	2	157.4	35.3
OV884013.1	3	125.66	35.4
OV884014.1	4	120.69	35.3
OV884015.1	5	116.64	35.2
OV884009.1	X	129.25	35.7
OV884010.1	Y	4.79	36.8
OV884016.1	MT	0.02	24.2
-	Unplaced	17.64	37.8

(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 (Kerpedjiev *et al.*, 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021), which performs annotation using 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.

#### 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

**Table 3. Software tools used.**

Software tool	Version	Source
Hifiasm	0.15.3-r339	<a href="#">Cheng et al., 2021</a>
purge_dups	1.2.3	<a href="#">Guan et al., 2020</a>
SALSA2	2.2	<a href="#">Ghurye et al., 2019</a>
longranger align	2.2.2	<a href="https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines">https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines</a>
freebayes	1.3.1-17-gaa2ace8	<a href="#">Garrison &amp; Marth, 2012</a>
MitoHiFi	2.0	<a href="#">Uliano-Silva et al., 2021</a>
HiGlass	1.11.6	<a href="#">Kerpedjiev et al., 2018</a>
PretextView	0.2.x	<a href="https://github.com/wtsi-hpag/PretextView">https://github.com/wtsi-hpag/PretextView</a>
BlobToolKit	3.0.5	<a href="#">Challis et al., 2020</a>

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: *Acanthosoma haemorrhoidale* (hawthorn shieldbug). Accession number [PRJEB47321](#); <https://identifiers.org/ena.embl/PRJEB47321>.

The genome sequence is released openly for reuse. The *A. haemorrhoidale* 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.6418202>.

Members of the Darwin Tree of Life Barcoding collective are listed here: <https://doi.org/10.5281/zenodo.6418156>.

Members of the Wellcome Sanger Institute Tree of Life programme are listed here: <https://doi.org/10.5281/zenodo.6418327>

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.6125046>.

Members of the Darwin Tree of Life Consortium are listed here: <https://doi.org/10.5281/zenodo.6418363>.

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# Open Peer Review

Current Peer Review Status: 

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## Version 1

Reviewer Report 05 August 2022

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**Andrew J. Mongue** 

Institute of Evolutionary Biology, The University of Edinburgh, Edinburgh, UK

The authors report the genome assembly of the hawthorn shield bug with brief background and detailed methodology of sequencing and assembly. I understand this reporting format is designed to be concise yet informative and I commend the authors on the level of rigor in describing the process from DNA extraction through to finished assembly. As this and other recent genome assembly papers have demonstrated, the combination of PacBio HiFi and HiC sequencing is an almost guaranteed success for generating highly contiguous insect genomes. Given that this approach seems to be so powerful, it is all the more important that researchers document the methodology in detail so that others can replicate this success. I appreciated the table listing tools and their versions as an example of how to effectively highlight methodological details. Likewise, the accession numbers appropriately link to data that are now available, so these resources have immediate value to the community as well.

I have only two comments for clarification and these focus on the species background context.

Firstly, the authors contrast shield bug pheromone production to that of Lepidoptera, stating that female Lepidoptera produce pheromones to attract males. While this is true of some groups within the order, many species behave much like the bugs described here: males produce pheromones to entice females (see milkweed butterflies as a specific example and generally species in which males have a hair-pencil organ). Please clarify the text on this point.

Secondly, the authors mention the potential for sequencing data to reveal symbiont relationships that the hawthorn shield bug has with bacteria. Host-microbe interactions are particularly well-studied in true bugs like aphids and mealybugs, so I can see the value in this new datapoint for comparison. As such, I was surprised that symbiont screening is never directly mentioned again in the methods or results. Perhaps this is a structural choice from the Tree of Life initiative and these data will be reported elsewhere. Given the interest in hemipteran symbionts from the research community, however, I feel it would be good to include at least a follow-up sentence directing the interested reader to the appropriate resources.

I also noticed two small grammatical errors to correct:

- "The hawthorn shield bug...is a large Pentamoid shield bug, easily recognisable by their size" should be "easily recognisable by its size".
- "The species is common on hawthorn...but are also found in mixed woodland" should be "but is also found in mixed woodland".

**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, population genetics, Lepidopteran and Hemipteran biology

**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.**

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## Comments on this article

### Version 1

Reader Comment 17 Aug 2022

**Jeremy Lanfear**, ELIXIR, Cambridge, UK

A minor point, the location data for the collection of the specimen is listed twice in the article, both times given as "Wytham Wood, Berkshire". I think that should be "Wytham Wood, Oxfordshire"?

**Competing Interests:** No competing interests were disclosed.

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