The genome sequence of the devil’s coach horse beetle, *Ocypus olens* (Müller, 1764) [version 2; peer review: 2 approved]

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Abstract

We present a genome assembly from an individual female *Ocypus olens* (the devil's coach horse; Arthropoda; Insecta; Coleoptera; Staphylinidae). The genome sequence is 1,084 megabases in span. The majority (98.81%) of the assembly is scaffolded into 20 chromosomal pseudomolecules, with the X sex chromosome assembled.

Keywords

*Ocypus olens*, devil's coach horse, genome sequence, chromosomal
Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Coleoptera; Polyphaga; Staphyliniformia; Staphylinidae; Staphylininae group; Staphylininae; Staphylinini; Ocypus; Ocypus olens (Müller, 1764) (NCBI:txid662956).

Background
The devil’s coach horse, *Ocypus olens*, is a large, all-black rove beetle. Reaching up to 32 mm, it is the largest beetle in the family Staphylinidae in the UK, and one of the largest worldwide. It is widespread and generally common across the Palaeartic including North Africa, including throughout mainland UK. It has been introduced to North America and Australasia. It can be found across a range of different habitats, especially damp woodland, grassland, brownfield sites and gardens. The devil’s coach horse is largely nocturnal, sheltering under leaf litter, logs and stones during the day. It is a generalist predator as both a larva and adult, feeding on a wide range of invertebrate species and carrion (Bonacci et al., 2006). Adults can be found all year and overwintering occurs in this stage. Mating occurs in late summer/autumn and eggs are laid 2 to 3 weeks later (Nield, 1976). Adults can be relatively long-lived, living up to 2 years in this stage (Nield, 1976). When agitated, the abdomen is reared and the mandibles opened in a threat-posture. The devil’s coach horse is capable of inflicting a painful bite to humans and readily produces defensive secretions from the mouth and tip of the abdomen. This species has been associated with evil and the devil in folklore since the Middle Ages.

Genome sequence report
The genome was sequenced from one female *O. olens* (Figure 1) collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.775, longitude -1.326) by Liam Crowley, University of Oxford, using a pooter. The sample was identified by the Tree of Life Laboratory at the WSI using TRIzol (Invitrogen), according to the manufacturer’s instructions. Following this, further DNA was extracted for a PacBio top-up. 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 again extracted using the Qiagen MagAttract HMW DNA kit, according to the manufacturer’s instructions. Following this, further DNA was extracted for a PacBio top-up. 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 again extracted using the Qiagen MagAttract HMW DNA extraction kit. 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.

DNA was extracted from the whole organism of icOcyOlen1 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. Following this, further DNA was extracted for a PacBio top-up. 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 again extracted using the Qiagen MagAttract HMW DNA extraction kit. 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.

RNA was extracted from the whole organism in the Tree of Life Laboratory at the WSI using TRIZol (Invitrogen), according to the manufacturer’s instructions. RNA was then eluted in
Table 1. Genome data for *Ocypus olens*, icOcyOlen1.1.

| Project accession data                  |
|----------------------------------------|
| Assembly identifier                    | icOcyOlen1.1                          |
| Species                                | *Ocypus olens*                        |
| Specimen                               | icOcyOlen1                             |
| NCBI taxonomy ID                       | NCBI:txid662956                       |
| BioProject                             | PRJEB45196                             |
| BioSample ID                           | SAMEA7520211                           |
| Isolate information                    | Female, whole organism                 |

| Raw data accessions                    |
|----------------------------------------|
| Pacific Biosciences SEQUEL II          | ERR6412375, ERR6590589                 |
| 10X Genomics Illumina                  | ERR6054955-ERR6054958                 |
| Hi-C Illumina                          | ERR6054776                             |
| Illumina polyA RNA-Seq                 | ERR6286735                             |

| Genome assembly                        |
|----------------------------------------|
| Assembly accession                     | GCA_910593695.1                       |
| Accession of alternate haplotype       | GCA_910593855.1                       |
| Span (Mb)                              | 1,084                                 |
| Number of contigs                      | 733                                   |
| Contig N50 length (Mb)                 | 4.6                                   |
| Number of scaffolds                    | 188                                   |
| Scaffold N50 length (Mb)               | 57.3                                  |
| Longest scaffold (Mb)                 | 69.7                                  |
| BUSCO* genome score                    | C:99.3%[S:98.2%,D:1.1%],F:0.2%,M:0.5%,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/icOcyOlen1.1/dataset/CAJVAY01/busc.

50 μl RNAse-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit 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 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 Arima v2 Hi-C kit and sequenced on HiSeq X.

**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...
Figure 2. Genome assembly of *Ocypus olens*, icOcyOlen1.1: 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 1,083,870,412 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 (69,741,075 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (57,303,393 and 48,121,331 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/icOcyOlen1.1/dataset/CAJVAY01/snail.

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 (Howe et al., 2021) was performed using gEVAL, HiGlass (Kerpedjiev et al., 2018) and Pretex. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva et al., 2021).
Figure 3. Genome assembly of *Ocypus olens*, icOcyOlen1.1: 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/icOcyOlen1.1/dataset/CAJVAY01/blob.

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
Figure 4. Genome assembly of *Ocyopus olen*, icOcyOlen1.1: 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/icOcyOlen1.1/dataset/CAJVAY01/cumulative.

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. Each transfer
Figure 5. Genome assembly of *Ocyopus olens*, icOcyOlen1.1: Hi-C contact map. Hi-C contact map of the icOcyOlen1.1 assembly, visualised in HiGlass.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Ocyopus olens*, icOcyOlen1.1.

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|-----------------|------------|-----------|------|
| OU343047.1      | 1          | 69.74     | 32.9 |
| OU343048.1      | 2          | 67.56     | 32.3 |
| OU343049.1      | 3          | 62.12     | 32.6 |
| OU343050.1      | 4          | 61.53     | 32.3 |
| OU343051.1      | 5          | 61.09     | 32.7 |
| OU343052.1      | 6          | 60.89     | 32.7 |
| OU343053.1      | 7          | 59.74     | 32.7 |
| OU343054.1      | 8          | 57.84     | 32.2 |
| OU343055.1      | 9          | 57.30     | 32.7 |
| OU343056.1      | 10         | 56.54     | 32.3 |
| OU343057.1      | X          | 56.49     | 32.9 |
| OU343058.1      | 12         | 55.64     | 32.3 |
| OU343059.1      | 13         | 54.63     | 32.5 |
| OU343060.1      | 14         | 54.47     | 32.1 |
| OU343061.1      | 15         | 52.48     | 32.9 |
| OU343062.1      | 16         | 50.26     | 32.8 |
| OU343063.1      | 17         | 48.12     | 32.4 |
| OU343064.1      | 18         | 42.33     | 32.4 |
| OU343065.1      | 19         | 41.70     | 33.2 |
| OU343057.1      | X          | 56.49     | 32.9 |
| OU343066.1      | MT         | 0.02      | 25.2 |
| -               | Unplaced   | 13.37     | 29.9 |
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                     |
| SALS2             | 2.2     | Ghurye et al., 2019                   |
| longranger align  | 2.2.2   | https://support.10xgenomics.com/gene...
| freebayes         | 1.3.1-17-gaa2ace8 | Garrison & Marth, 2012 |
| MitoHiFi          | 1.0     | 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/PretextV...
| BlobToolKit       | 2.6.2   | Challis et al., 2020                  |

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: Ocypus olens (Devil’s coach horse). Accession number PRJEB45196; https://identifiers.org/ena.embl/PRJEB45196.

The genome sequence is released openly for reuse. The O. olens 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 the 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.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

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 04 May 2022

https://doi.org/10.21956/wellcomeopenres.19172.r50199

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The genome Data Note for the devil’s coach horse rove beetle presents a clear and comprehensive description of all the steps taken to generate the chromosomal-level assembly spanning just over 1 Gbp with almost 99% assigned to chromosomes including the X chromosome. As a species of ecological interest and as member of the largest order of insects, the rationale for generating these genomic resources is clear. The described data collection and analysis methods follow the best practices in the field and have delivered a high-quality complete and accurate chromosome-level reference genome, with data made available through the European Nucleotide Archive.

Additional points:
- “representing 9 autosomes” – should this not be 19 autosomes?
- In the Hi-C contact map, is it only showing the 19 autosomes?
- Could there be an error in the total scaffold count, because the counts start at zero and go to 187, this means there are 188 scaffolds not 187 – please check.
- “The genome will be annotated using the RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute.” – it seems that this has now been done, so this statement could be updated in the revision.
- If it were made clear that ‘icOcyOlen1’ is the sample ID then the ‘extracted from the whole organism of icOcyOlen1’ sentence would make more sense.

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:** arthropod comparative and evolutionary genomics

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 10 November 2021

https://doi.org/10.21956/wellcomeopenres.19172.r46916

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This manuscript entitled 'The genome sequence of the devil's coach horse, *Ocypus olens* (Müller, 1764)' reports a new chromosomal-scale genome assembly of a rove beetle (family Staphylinidae). The latest methods for genome sequencing, assembly, annotation, and characterization were used in this study, and they are adequately documented in the paper. The sequencing results and assembly are clearly reported and interpreted, and appropriately illustrated, and the data is available as described. Only a few minor edits to the text (detailed below) are suggested before publication.

**Title:**
- There is no indication in the title that this is a beetle, or even an insect. Please consider revising the title to include the word “beetle” after the common name, and/or add
“(Coleoptera: Staphylinidae)” to the title in reference to the order and family of beetle sequenced.

**Species Taxonomy:**
- Since you indicate both the author and year of publication for the name *Ocypus olens*, you should really include a citation to the original description. Alternatively, you could remove the year and only indicate the author last name.

**Background:**
- Line 4: Replace “It's” with “It is”.
- Line 5: Delete “and North Africa”. By definition, North Africa is part of the Palaearctic.
- Line 6: A citation is needed when referencing the introduced range.

**Genome Sequence Report:**
- Lines 2-3: The collection data appears in both this section and the Methods. This redundancy seems unnecessary.

**Methods:**
- Line 1 in the second paragraph of the Methods: Delete “from the whole organism of *icOcyOlen1*.” This is redundant with mention of extraction “from the whole organism” later in the same sentence.
- Was RNA extracted from the same “whole organism” as DNA? Please clarify. The same issue needs clarifying where “head tissue” is mentioned in reference to Hi-C data at the bottom of page 6.
- Figure 2 legend: 3rd to last sentence: There is an excess period at the end of the sentence.
- Figure 3 legend: A period is missing from the end of the sentence ending in “length”.

**Data availability:**
- The scientific name “Ocypus olens” should be italicized.

**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 systematics and evolution (primarily beetles), insect-plant interactions, gene and genome evolution, chemosensation, digestive physiology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.