DATA NOTE

REVISED ERGA-BGE genome of *Dailognatha quadricollis*, an East Mediterranean darkling beetle

[version 2; peer review: 2 approved]

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Abstract

Dailognatha quadricollis (Coleoptera: Tenebrionidae) is a darkling beetle native to the Balkans and Eastern Mediterranean, with a range extending from Croatia to Lebanon. It is a morphologically diverse species, comprising numerous subspecies, particularly concentrated in the Aegean region. The reference genome of *Dailognatha quadricollis* will enable phylogenetic, population and evolutionary research. The entirety of the genome sequence was assembled into 11 contiguous chromosomal pseudomolecules and the X sex chromosome. This chromosome-level assembly encompasses 0.52 Gb, composed of 393 contigs and 322 scaffolds, with contig and scaffold N50 values of 4.3 Mb and 23.6 Mb, respectively.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 29 May 2026		✓ view
version 1 06 Aug 2025	✓ view	? view

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Keywords
Dailognatha quadricollis, genome assembly, European Reference Genome Atlas, Biodiversity Genomic Europe, Earth Biogenome Project, Tenebrionidae

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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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This article is included in the Genome Reports from the Biodiversity Genomics Europe Project collection.

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Author roles: Bolanakis G: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Karakasi D**: Investigation, Resources, Writing – Review & Editing; **Bitzilekis E**: Investigation, Resources, Writing – Review & Editing; **Stratakis M**: Investigation, Resources, Writing – Review & Editing; **Trichas A**: Investigation, Resources, Writing – Review & Editing; **Lymberakis P**: Investigation, Resources, Writing – Review & Editing; **Poulakakis N**: Investigation, Resources, Writing – Review & Editing; **Böhne A**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Monteiro R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Fernández R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Escudero N**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Moussy A**: Investigation, Supervision, Writing – Review & Editing; **Cruaud C**: Investigation, Supervision, Writing – Review & Editing; **Labadie K**: Investigation, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Demirdjian L**: Data Curation, Formal Analysis, Writing – Review & Editing; **Téodori E**: Data Curation, Formal Analysis, Writing – Review & Editing; **Wincker P**: Investigation, Supervision, Writing – Review & Editing; **H. Oliveira P**: Investigation, Supervision, Writing – Review & Editing; **Aury JM**: Data Curation, Formal Analysis, Supervision, Writing – Review & Editing; **Bortoluzzi C**: Visualization, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

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REVISED **Amendments from Version 1**

In this revised version of the genome report we addressed all reviewers comments. In particular, we provide more context on the sex determination mechanism of *Dailognatha quadricollis*, since we were able to sequence the X chromosome. We also removed the part of the text on the ONT sequencing reads, since this genome was produced with HiFi data.

Any further responses from the reviewers can be found at the end of the article

Introduction

Dailognatha quadricollis (Coleoptera: Tenebrionidae) is a darkling beetle found in the Balkans and the East Mediterranean area, with a distribution ranging from Croatia to Lebanon (Iwan *et al.*, 2020). *Dailognatha quadricollis* is a highly diverse species characterised by numerous subspecies, most of which are in the Aegean region. The species is quite widespread and abundant and is mostly found in the Mediterranean shrublands, such as phrygana and maquis. *Dailognatha quadricollis* is classified by the Red Data Book of Greece (NECCA, 2024) as Least Concern. The species feeds on seeds, roots and plant debris, and contributes to plant matter decomposition.

The reference genome of *Dailognatha quadricollis* will aid in understanding the species' phylogeny, taxonomic structure, and evolutionary adaptations, as well as those of its close relatives. The generation of this reference resource was coordinated by the European Reference Genome Atlas (ERGA) initiative's Biodiversity Genomics Europe (BGE) project, supporting ERGA's aims of promoting transnational cooperation to promote advances in the application of genomics technologies to protect and restore biodiversity (Mazzoni *et al.*, 2023).

Materials & Methods

ERGA's sequencing strategy includes Pacific Biosciences (PacBio) for long-read sequencing, along with Hi-C sequencing for chromosomal architecture.

Sample and sampling information

On 04 June 2023, Giannis Bolanakis sampled 7 adult specimens (sex unknown) of *Dailognatha quadricollis*, which were identified by the expert Apostolos Trichas (Figure 1). The specimens were sampled in Psiloritis, Lochria, Akolita, Heraklion, Crete. Sampling was performed under Presidential Decree 67/81 issued by the Greek government. Specimens were hand-picked, euthanized in liquid nitrogen and, until DNA extraction, were preserved at −80°C.

Vouchering information

Physical reference materials have been deposited in the Arthropods Collections of the Natural History Museum of Crete (NHMC) of the University of Crete https://www.nhmc.uoc.gr/en/departments/arthropods/ under proxy voucher ID NHMC.85.2.19030.

Frozen reference tissue material is available from a proxy voucher at the Genomics and Genetic Resources Division of the NHMC https://www.nhmc.uoc.gr/en/departments/genomics under proxy voucher IDs NHMC.85.2.19030.1 and NHMC.85.2.19030.2.

Genetic information

The estimated genome size, based on ancestral taxa, is 0.26 Gb. This corresponds to a diploid genome with a haploid number of 10 chromosomes (2n = 20). The standard mechanism of sex determination in the species is Xyp (Holecová *et al.*, 2002), which is typical for members of the Tenebrionidae family (Şendoğan & Alpagut-Keskin 2016). All information for this species was retrieved from Genomes on a Tree (Challis *et al.*, 2023) and it was used mainly to estimate the amount of sequencing required for genome assembly.

Figure 1. A male specimen of the species *Dailognatha quadricollis.* The individual in the picture is from the island of Crete and is a different specimen from the one sampled and sequenced for this study. Photo credit: Dr Apostolos Trichas.

DNA/RNA processing

DNA was extracted from a whole individual (100 mg) using a conventional CTAB extraction followed by a commercial purification using Qiagen Genomic tips (Qiagen). A detailed protocol is available on protocols.io (https://www.protocols.io/view/hmw-dna-extraction-for-long-read-sequencing-using-bp2l694yzlqe/v1). DNA fragment size selection was performed using Short Read Eliminator (PacBio). Quantification was performed using a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific) and integrity was assessed in a FemtoPulse system (Agilent). DNA was stored at 4°C until usage.

RNA was extracted from a whole individual (70 mg) using a RNeasy Plus Universal Kit (Qiagen) following manufacturer instructions. Residual genomic DNA was removed with 6U of TURBO DNase (2 U/µl) (Thermo Fisher Scientific). Quantification was performed using a Qubit RNA HS Assay and integrity was assessed in a Bioanalyzer system (Agilent). RNA was stored at −80°C.

Library preparation and sequencing

Long-read DNA libraries were prepared with SMRTbell prep kit 3.0 following manufacturers' instructions and sequenced on a Revio system (PacBio). Hi-C libraries were generated from 2 different individuals using the Arima High Coverage HiC kit (following the Animal Tissues low input protocol v01) and sequenced on a NovaSeq 6000

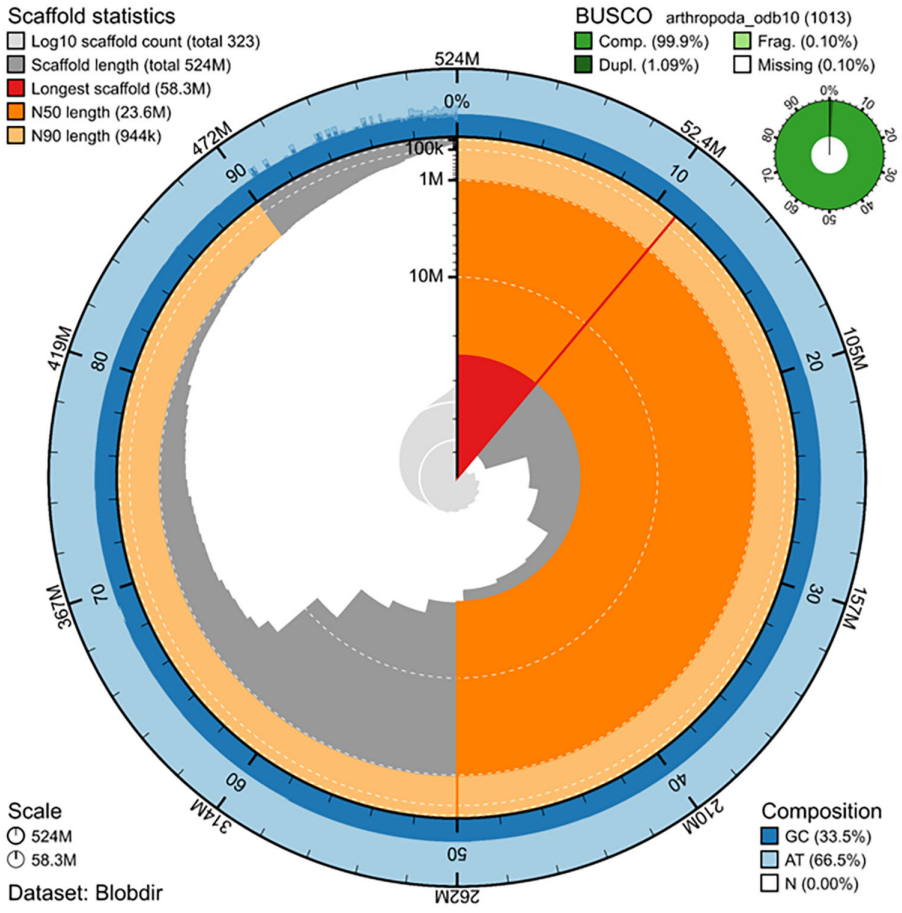

Figure 2. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 524,088,546 bp assembly including the mitochondrial genome. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (58.3 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (23,571,702 and 943,584 bp), respectively. The pale grey spiral shows the cumulative sequence 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 found in the assembled genome from the Arthropoda database (odb10) is shown in the top right.

instrument (Illumina) with 2x150 bp read length. Poly(A) RNA-Seq libraries were constructed using the Illumina Stranded mRNA Prep, Ligation Prep kit (Illumina) and sequenced on an Illumina NovaSeq 6000 instrument (Illumina).

In total 42x HiFi and 177x HiC data were sequenced to generate the assembly.

Genome assembly methods

The genome of *Dailognatha quadricollis* was assembled using the Genoscope GALOP pipeline (https://workflowhub.eu/workflows/1200). Briefly, raw PacBio HiFi reads and Hi-C data were assembled using Hifiasm v0.19.5-r593 (Cheng *et al.*, 2021). The phased assembly was scaffolded using YaHS v1.2 (Zhou *et al.*, 2023) and the resulting scaffolds were curated through manual inspection using PretextView v0.2.5 to remove false joins and to incorporate sequences not automatically scaffolded into their respective locations within the chromosomal pseudomolecules. Chromosome-scale scaffolds confirmed by Hi-C data were named in order of size and, for each chromosome, the longest haplotype was retained as the main haplotype. Additionally, the X chromosome was identified by comparison with the reference genome of *Tenebrio molitor* (icTenMoli1.1). The mitochondrial genome was assembled using Oatk v1.0 (Zhou *et al.*, 2024) and included in the released assembly. Summary analysis of the released assembly was performed using the ERGA-BGE Genome Report ASM Galaxy workflow (https://doi.org/10.48546/workflowhub.workflow.1104.1).

Results

Genome assembly

The genome assembly has a total length of 524,088,546 bp in 322 scaffolds including the mitogenome (Figure 2 & Figure 3), with a GC content of 33.5%. The assembly has a contig N50 of 4,327,000 bp and L50 of 27 and a scaffold N50 of 23,571,702 bp and L50 of 8. The assembly has a total of 71 gaps, totalling 13.8 kb in cumulative size. The single-copy gene content analysis using the Arthropoda database with BUSCO (Manni *et al.*, 2021) resulted in 99.9% completeness (98.8% single and 1.1% duplicated). 80.4% of reads k-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 65.7 as calculated by Merqury (Rhie *et al.*, 2020).

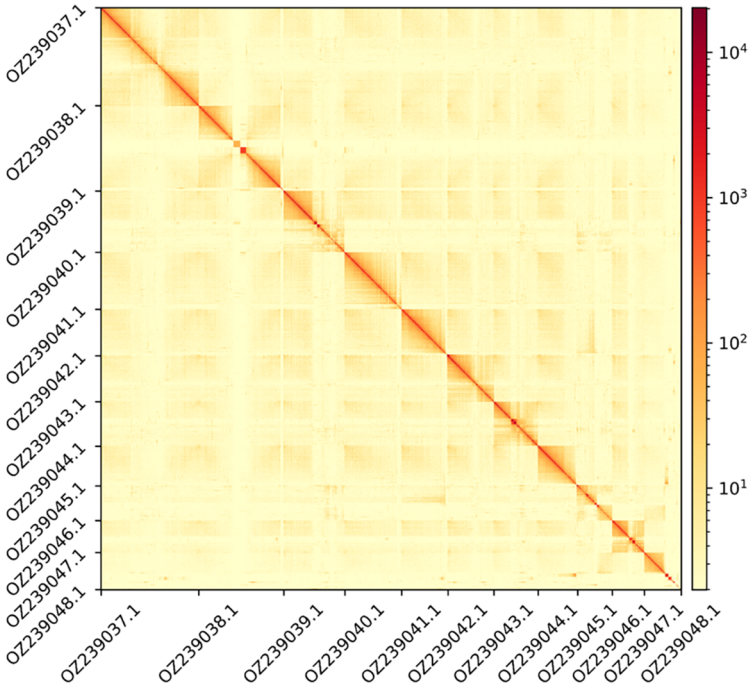

Figure 3. Hi-C contact map showing spatial interactions between regions of the genome. The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. The frequency of contacts is shown on a logarithmic heatmap scale. Hi-C matrix bins were merged into a 100 kb bin size for plotting. The Hi-C map shows the autosomes, the X chromosome (GenBank: OZ239047.1), and the mitochondrial genome (OZ239048.1).

Data availability

D. quadricollis and the related genomic study were assigned to Tree of Life ID (ToLID) 'icDaiQuad1' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB77131. The sample information is available at the following BioSample accessions: SAMEA114349676, SAMEA114349677, and SAMEA114349689. The genome assembly is accessible from ENA under accession number GCA_965183915.1. The genome will be annotated using the generated RNA-Seq data and will be made available through the Ensembl website (https://projects.ensembl.org/erga-bge/). Sequencing data produced as part of this project are available from ENA at the following accessions: ERX12722160, ERX12722161, ERX12725796, ERX12722095, ERX12733446, and ERX14096383. Documentation related to the genome assembly and curation can be found in the ERGA Assembly Report (EAR) document available at https://github.com/ERGA-consortium/EARs/tree/main/Assembly_Reports/Dailognatha_quadricollis/icDaiQuad1. Further details and data about the project are hosted on the ERGA portal at https://portal.erga-biodiversity.eu/data_portal/575809.

Author contributions

DK, PL, and NP coordinated the project; GB collected the species; AT identified the species; DK, GB, EB, and MS sampled and preserved biological material and provided metadata; AsB, RM, RF, and NE provided support in sampling, shipping of biological material, metadata collection, and management; GST extracted DNA, prepared libraries, and performed sequencing under the supervision of AM, CC, KL, PHO, and PW; LD and ET performed genome assembly and curation under the supervision of JMA; CB generated the analysis and report. All authors contributed to the writing, review, and editing of this genome note and read and approved the final version. This work is part of the species assigned to Genoscope, which was instrumental in the wet lab, sequencing, and assembly processes, and represents a key contribution to BGE's outputs.

Author information

Members of the Genoscope Sequencing Team are listed here: https://zenodo.org/records/14611490.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 08 June 2026

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✔ **Yingao Cai**
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I would like to thank the authors for their detailed and thorough responses to my previous comments. The authors have addressed all of my concerns adequately, and the manuscript has been significantly improved. I have no further comments to make and am pleased to recommend the article for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genome assembly, transposable elements evolution, mutation process

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.

Version 1

Reviewer Report 13 November 2025

https://doi.org/10.21956/openreseurope.22548.r62930

? **Yingao Cai**
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The article successfully sequencing and assembled the genome of the beetle *Dailognatha quadricollis*, achieving the high continuous and high accuracy (N50 of contigs: 4.3M and QV 65.7). The authors used both third-generation (TGS) and next-generation sequencing (NGS) technologies, along with chromosome-level sequencing methods, to generate the whole-genome sequncing data. They then applied a well-established genome assembly pipeline to construct contigs and scaffolds. While this research provides a valuable genomic resource for a non-model insect, its conclusions remain limited by several methodological and biological constraints.

Major Concerns:

1. Traditional sex chromosome identification is based on the sequencing depth which the sequencing reads mapping to the contig or chromosomes difference between the male samples and female samples[1, 2]. However, the specimens used in this study do not contain any information about the sex of the individuals. If the sequencing data had included male samples, the authors could have conducted additional analyses to identify and characterize sex chromosomes.
2. The mitochondrial genome and other cytoplasmic DNA like symbiotic bacteria genome can often be assembled as separate contigs using hifiasm. Did the authors find any contigs in the raw hifiasm output correspond to the results of Oatk.
3. The genome size estimated from ancestral taxa in GoaT is 0.26 Gb, while the expected haploid genome size reported on the ERGA assembly GitHub page is 415,868,280 bp. These values are inconsistent with each other. To determine the real genome size, author need to remove the assembly contaminations. Tools such as BlobTools2 can be used to identify and filter out sequences originating from other organisms.

Minor Concerns:

1. ONT and RNA sequencing data seem not applicated in the genome assembly, modified the Materials & Methods parts.

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?

Partly

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genome assembly, transposable elements evolution, mutation process

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, however we have significant reservations, as outlined above.

Author Response 18 May 2026

Chiara Bortoluzzi

We thank the reviewer for their comments. We have addressed them as follow:

1. As specified in the abstract, we were able to assemble only the X chromosome. We provided additional information about the sex mechanisms of the species in the Genetic information section.
2. Yes, we were able to assemble the mitochondrial genome, which has been assigned GenBank accession ID OZ239048.1.
3. We understand the misunderstanding. The genome size estimated from ancestral taxa in GoaT was mainly used to have an estimate of the amount of sequencing required for genome assembly. It is not meant to represent the actual genome size, which is indeed better estimated with other methods.
4. We removed the ONT and RNA reference from the main text.

Competing Interests: No competing interests were disclosed.

Reviewer Report 06 November 2025

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Arun Arumugaperumal
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The data note describes the whole genome sequence of a beetle, *Dailognatha quadricollis*. There is not much literature present regarding the beetle. This is the first high-quality genome sequence of the beetle to be reported. The genome sequence reported herewith will be a valuable resource to study its phylogeny and explain the nature of the beetle, on analysis. The authors have used long-read sequencing and Hi-C data to arrive at a chromosome-level assembly.

The assembly reported here is of size 0.52 Gb spread among 11 chromosomes. However, the authors are not sure about the sex of the sample. They had identified an X chromosome on comparison with the genome of a related beetle. The contig N50 of 4.3 Mb and scaffold N50 of 23.6 Mb indicate the good quality of the genome in terms of truncated sequences. It will be further evident once the annotation has been released in Ensembl. BUSCO analysis also showed that the genome has 99.9% completeness with respect to odb10 database.

The mitochondrial genome has also been separated from the chromosomal sequences. However, not much information about it has been reported in the manuscript. The links provided for the datasets and the accession IDs are active. The data note can be indexed.

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: Bioinformatics; 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.

Author Response 06 Nov 2025

Chiara Bortoluzzi

We thank the reviewer for their positive feedback. We would like to higlight that the gene annotation for *Dailognatha quadricollis* is now available in the Ensembl website: https://projects.ensembl.org/erga-bge/.

Competing Interests: No competing interests were disclosed.