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DATA NOTE

REVISED ERGA-BGE genome of *Dendarus foraminosus*: an IUCN Least Concern darkling beetle endemic to Crete (Greece)

[version 3; peer review: 4 approved]

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Abstract

Dendarus foraminosus Mulsant and Rey, 1855 is a darkling beetle in the family Tenebrionidae and one of the many *Dendarus* species endemic to the island of Crete. *Dendarus foraminosus* is a commonly found species and is widespread in the lowland and montane phrygana and maquis of central Crete. The species is classified as Least Concern (LC) by the IUCN Red List. The reference genome of *Dendarus foraminosus* will enable phylogenetic, population, and evolutionary research regarding this endemic species and its close relatives. A total of 11 contiguous chromosomal pseudomolecules (sex chromosomes

Open Peer Review

Approval Status ✓ ✓ ✓ ✓

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version 3 (revision) 15 Jan 2026	✓ view		✓ view	
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version 1 30 Jun 2025	? view	? view		

included) were assembled from the genome sequence. This chromosome-level assembly encompasses 0.59 Gb, composed of 430 contigs and 415 scaffolds, with contig and scaffold N50 values of 24.4 Mb and 51.9 Mb, respectively.

Keywords
Dendarus foraminosus, genome assembly, European Reference Genome Atlas, Biodiversity Genomics Europe, Earth Biogenome Project, Tenebrionidae family, Crete

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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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REVISED **Amendments from Version 2**

In this current version, we addressed the reviewer comments and changed the caption of Figure 3, specifying that of the 11 chromosomes, 9 are autosomes and 2 are sex chromosomes.

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

Introduction

Dendarus foraminosus is a darkling beetle in the family Tenebrionidae. The species is endemic to the island of Crete (Trichas, 2008) and is mostly found in the central parts of the island, from lowland phrygana and maquis to montane shrublands. In the west- and the east-side of the island, the species is replaced by its congeneric species *D. opacus* and *D. puncticollis* respectively, while in the higher altitudes of Psiloritis and Dikti mountains by *D. politus*. All these species together form the *Dendarus* Cretan lineage (Trichas *et al.*, 2020). Like all the other *Dendarus* species that form the Cretan clade, *D. foraminosus* is characterized by a "vase-neck"shaped humeral bump (Trichas *et al.*, 2020). From its close relatives, *D. foraminosus* is easily distinguished by the large, irregular longitudinal grooves on the elytra, that are the result of the fusion of two or more elytral punctures (Trichas, 2008). These punctures are less harsh and deep in the other Cretan species of this clade. In fact, they are almost missing in the montane species *D. politus* (Trichas, 2008). It is noteworthy that the punctuation of *D. foraminosus* differs between populations, producing various gradients between elevations. *Dendarus foraminosus* displays the typical sexual dimorphism among Tenebrinoidae, that is, the tarsal segments of the male are larger and wider than the ones of the female. According to the IUCN Red List (Red List Necca 2025, unpublished assessment), the species is not threatened by extinction, as it occurs in great abundance in many localities and various habitats in Crete.

The species larvae are saprophytophagous, participating in the decomposition of the rotting plant matter.

The reference genome of *D. foraminosus* will help in understanding the phylogenetic relationships of the species of the genus *Dendarus* in Crete, the species delimitation of the Cretan lineage, the recovery of its population structure, and the species adaptation in the Cretan environment in the face of climate change.

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 advance in the application of genomics technologies to protect and restore biodiversity (Mazzoni *et al.*, 2023).

Materials & methods

ERGA's sequencing strategy includes Oxford Nanopore Technology (ONT) and/or Pacific Biosciences (PacBio) for long-read sequencing, along with Hi-C sequencing for chromosomal architecture, Illumina Paired-End (PE) for polishing (i.e. recommended for ONT-only assemblies), and RNA sequencing for transcriptome profiling, to facilitate genome assembly and annotation.

Sample and sampling information

On April 9th, 2022, 11 male adults of *Dendarus foraminosus* were sampled by Giannis Bolanakis (Figure 1). The species was identified by the expert entomologist Dr. Apostolos Trichas. The specimens were hand-picked in Mount Kedros, Rethymno, Crete (Greece). Sampling was performed under the Presidential Decree 67/81 of the Greek Government. The specimens were euthanized in liquid nitrogen and were preserved at -80°C until DNA extraction.

Vouchering information

Physical reference material for the here sequenced specimen has been deposited in the Arthropods Collections of the Natural History Museum of Crete of the University of Crete (NHMC) https://www.nhmc.uoc.gr/en/departments/arthropods/ under voucher ID NHMC.85.2.26267.

Frozen reference tissue material of abdomen, head and pronotum of *D. foraminosus* have been deposited in the Genomics and Genetic Resources Division of the NHMC https://www.nhmc.uoc.gr/ under voucher ID NHMC.85.2.26267.

Genetic information

The estimated genome size, estimated by Genomes on a Tree (GoaT) (Challis *et al.*, 2023) by ancestral state reconstruction, is 0.41 Gb. This is a diploid genome with a haploid number of 10 chromosomes (2n = 20). All information for this species was retrieved from GoaT.

DNA/RNA processing

DNA for Illumina and PacBio sequencing was extracted from a whole male individual (80 mg) using a conventional CTAB extraction followed by a commercial purification using Qiagen Genomic tips (QIAGEN, MD, USA). 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, CA, USA). Quantification was performed using a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific)



Figure 1. A darkling beetle (*Dendarus foraminosus*) from a different specimen from the one sampled for this study. The specimen was photographed on the island of Crete. Photo credit: Dr. Apostolos Trichas.

and integrity was assessed in a FemtoPulse system (Agilent). DNA was stored at 4 °C until usage. RNA was extracted from a second male individual using an RNeasy Plus Universal Kit (Qiagen) following manufacturer instructions. RNA was extracted from the whole individual (80 mg) and extracted RNA was then treated 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 the SMRTbell prep kit 3.0 following manufacturers' instructions and sequenced on a Revio system (PacBio). Hi-C libraries were generated from a whole female individual using the Dovetail Omni-C Kit (following the Insects & marine invertebrates' protocol v1.2) and sequenced on a NovaSeq 6000 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.

Genome assembly methods

The genome of *Dendarus foraminosus* was assembled using the Genoscope GALOP pipeline (https://workflowhub.eu/workflows/1200). Briefly, raw PacBio HiFi reads were assembled using Hifiasm v0.19.5-r593. Retained haplotigs were removed using purge_dups v1.2.5 with default parameters and the proposed cutoffs. We did not observe any over-purging and loss of valid gene content, as confirmed by BUSCO before (99.3% completeness, 98.1% single and 1.2% duplicate) and after (99.3% completeness, 98.1% single and 1.2% duplicate) purging. The purged assembly was scaffolded using YaHS v1.2 and assembled scaffolds were then curated through manual inspection using PretextView v0.2.5 to remove false joins and incorporate sequences not automatically scaffolded into their respective locations within the chromosomal pseudomolecules. Two scaffolds with low coverage, and homology with *Tenebrio molitor* (X, Y) chromosomes, were renamed as X and Y respectively. The telomeric repeat pattern found with TelFinder is TCGGG (Sun *et al.*, 2023). Chromosome-scale scaffolds confirmed by Hi-C data were named in order of size. The mitochondrial genome was assembled as two circular forms using Oatk v1.0 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).

Genome annotation methods

A gene set was generated using the Ensembl Gene Annotation system (Aken *et al.*, 2016), primarily by aligning publicly available short-read RNA-seq data from BioSample SAMEA112751342 to the genome. Gaps in the annotation were filled via protein-to-genome alignments of a select set of clade-specific proteins from UniProt (UniProt Consortium, 2019), which had experimental evidence at the protein or transcript level. At each locus, data were aggregated and consolidated, prioritising models derived from RNA-seq data, resulting in a final set of gene models and associated non-redundant transcript sets. To distinguish true isoforms from fragments, the likelihood of each open reading frame (ORF) was evaluated against known metazoan proteins. Low-quality transcript models, such as those showing evidence of fragmented ORFs, were removed. In cases where RNA-seq data were fragmented or absent, homology data were prioritised, favouring longer transcripts with strong intron support from short-read data. The resulting gene models were classified into two categories: protein-coding, and long non-coding. Models that did not overlap protein-coding genes and were constructed from transcriptomic data were considered potential lncRNAs. Potential lncRNAs were further filtered to remove single-exon loci due to their unreliability. Putative miRNAs were predicted by performing a BLAST search of miRBase (Kozomara *et al.*, 2019) against the genome, followed by RNAfold analysis (Gruber *et al.*, 2008). Other small non-coding loci were identified by scanning the genome with Rfam (Kalvari *et al.*, 2018) and passing the results through Infernal (Nawrocki & Eddy, 2013).

Results

Genome assembly

The Pacbio HiFi library generated 32.4 Gb of data, whereas Illumina libraries generated 37.5, 44.9, and 22.2 Gb of data respectively for DNAseq, Hi-C, and RNA-seq. The corresponding values of coverage are 55x, 63x, 76x, and 37x. The genome assembly has a total length of 593.991 Mb in 415 scaffolds including two circular forms of the mitogenome (Figure 2 and Figure 3), with a GC content of 35.9%. It features a contig N50 of 24.404 Mb (L50 = 9) and a scaffold N50 of 51.873 Mb (L50 = 5). There are 15 gaps, totalling 2.0 kb in cumulative size. The single-copy gene content analysis using the Arthropoda database with BUSCO (Manni *et al.*, 2021) resulted in 100% completeness (98.9% single and 1.1% duplicated). The BUSCO completeness score did not change significantly when using a taxonomically appropriate single-copy ortholog dataset, such as the Endopterygota database (99.2% completeness, 98.4% single and 0.8% duplicated). 71.3% of reads k-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 63.4 calculated by Merqury (Rhie *et al.*, 2020).

Genome annotation

The genome annotation consists of 15,992 protein-coding genes with associated 23,634 transcripts, in addition to 2,335 non-coding genes (Table 1). Using the longest isoform per transcript, the single-copy gene content analysis using the Arthropoda odb10 database with BUSCO resulted in 96.9% completeness. Using the OMAmer Metazoa-v2.0.0.h5 database for OMArk (Nevers *et al.*, 2025) resulted in 93.7% completeness and 79.2% consistency (Table 2).

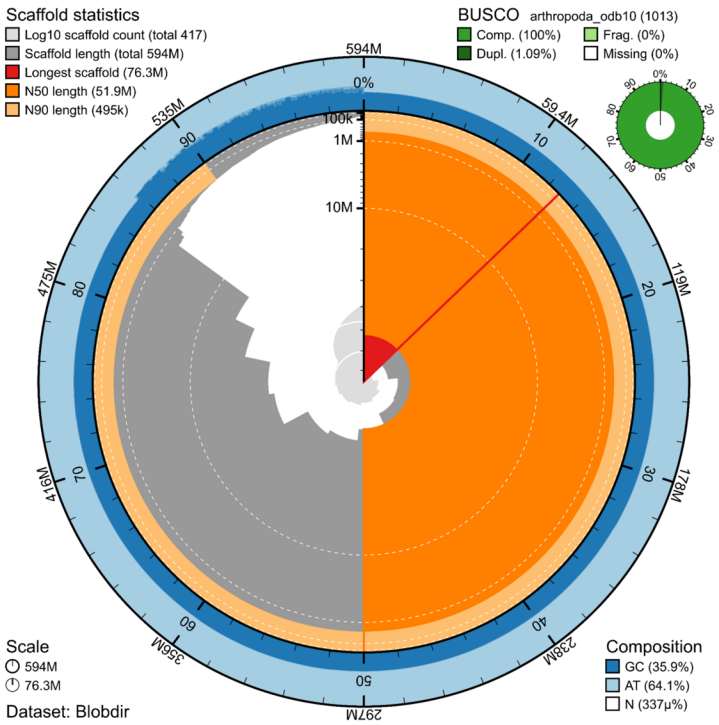

Figure 2. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 593.991 Mb assembly including the mitochondrial genome. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (76.3 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (51.873 Mb and 0.494 Mb), 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 on the top right.

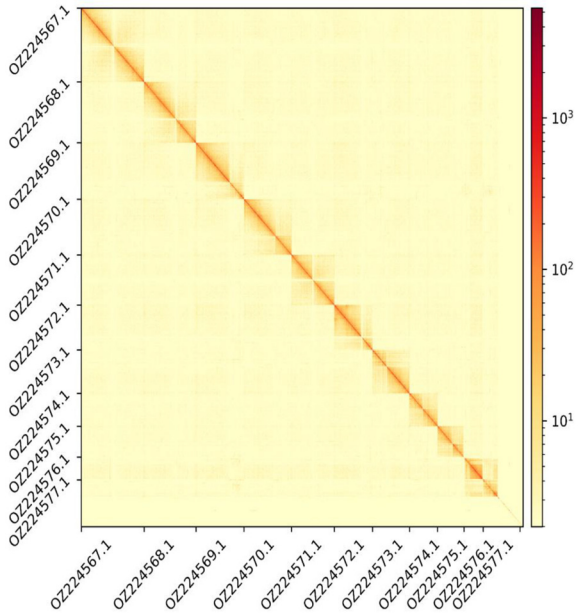

Figure 3. Hi-C contact map showing spatial interactions between regions of the genome. The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. The frequency of contacts is shown on a logarithmic heatmap scale. Hi-C matrix bins were merged into a 100 kb bin size for plotting. On both axes the GenBank names of the 9th largest autosomes are shown along with the X chromosome (GenBank accession: OZ224576.1) and Y chromosome (GenBank accession: OZ224577.1).

Table 1. Statistics from assembled gene models.

	No. genes	No. transcripts	Mean gene length (bp)	No. single-exon genes	Mean exons per transcript
mRNA	15,992	23,634	10,478	554	5.3
pseudogene	0.0	0.0	0.0	0.0	0.0
snoRNA	22	22	116	22	1.0
lncRNA	1,864	1,978	4,892	3	2.2
miRNA	0.0	0.0	0.0	0.0	0.0
snRNA	71	17	136	71	1.0
rRNA	77	77	692	77	1.0
scRNA	1	1	128	1	1.0
tRNA	300	300	74	300	1.0
Other ncRNA	3	3	298	3	1.0

Table 2. Annotation completeness and consistency scores calculated by BUSCO run in protein mode (arthropoda_odb10) and OMArk (Metazoa-v2.0.0.h5).

	Complete	Single copy	Duplicated	Fragmented	Missing
BUSCO	981 (96.9%)	969 (95.7%)	12 (1.2%)	8 (0.8%)	24 (2.3%)
OMArk	4,465 (93.7%)	4,175 (87.6%)	290 (6.1%)	-	298 (6.2%)
	Consistent	**Inconsistent**	**Contaminants**	**Unknown**	
OMArk	12,671 (79.2%)	339 (2.1%)	0.0 (0.0%)	2,982 (18.6%)	

Data availability

Dendarus foraminosus and the related genomic study were assigned to Tree of Life ID (ToLID) 'icDenFora10' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB77350. The sample information is available at the following BioSample accessions: SAMEA112751340, SAMEA112751341, and SAMEA112751342. The genome assembly is accessible from ENA under accession number GCA_965152765.1 and the annotated genome is 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: ERX12116622, ERX12732899, ERX12732900, ERX12116621, and ERX12733285. ERR13362399, ERR13362013, and ERR13362014. 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/blob/main/Assembly_Reports/Dendarus_foraminosus/icDenFora10/. Further details and data about the project are hosted on the ERGA portal at https://www.ebi.ac.uk/biodiversity/data_portal/2712997.

Author contributions

DK coordinated the project; GB collected the species; AT identified the species; GB, MS, EB, NP, PL and DK 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; the GST extracted DNA, prepared libraries, and performed sequencing under the supervision of AM, CC, KL, PHO and PW; SD, ET, LD and JMA performed genome assembly and curation under the supervision of JMA; LH, SS, and FM performed genome annotation; 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

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✔ **Arun Arumugaperumal**
Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai - 602105, Tamil Nadu, India

Figure 3 caption has been corrected. The article can be indexed.

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.

Reviewer Report 16 January 2026

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✔ **Terrence Sylvester**
University of Memphis, Tennessee, USA

The authors have done a fantastic job of addressing my previous comments. I do not have further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: 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.

Version 2

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Valentina Peona
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The authors addressed the comments fully. I do not have further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Evolutionary genomics, sex chromosome evolution, centromere evolution, transposable elements

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 07 January 2026

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Christopher B Cunningham
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The project produced quality resources for the insect genomics community. The methods are sound and relatively well documented. The article has enough details to be reasonably reproducible with the inclusion of the pipeline documentation. The data supporting the article are

publicly available.

Abstract. Should include at least one sentence about the quality of the annotation. It would be optimal to comment on if there was anything unusual observed.

Keywords. These are repeated form the title and abstract so add no value. Google indexes off these too so they should reflect some broader value.

Introduction. Background. The stated motivation for this study in the introduction is fine – part of a very large survey. However, it would be nice for the reader to understand if this is the first of its genus, is of particular interest for ecological or genetics studies, or something unusual about its biology. Helping the reader understand what this genome might help with investigation in. Most of the Intro is dedicated to a morphological description of the genus

Methods. An empirical estimate of the genome size is needed if the author want to be confident – KAT or GenomeScope or Flow Cytometry.

What version of OrthoDB was used? BUSCO completeness needs to be assessed with the updated OrthoDB version 12. Difference from 10 to 12 have made a big difference for several insects I work with and colleagues have reported the same.

Is the rationale for creating the dataset(s) clearly described?
Partly

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: Insects, Genomics, Epigenetics, Behavior, Reproduction

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 31 December 2025

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The data note describes the whole genome sequencing and analysis of a darkling beetle, *Dendarus foraminosus*. This is the first report of the whole genome sequence of this beetle species. The assembly reported here is of size 593.991 Mb distributed among 11 chromosomal pseudomolecules. The authors have identified the X and Y chromosomes since a male specimen had been used for sequencing. The authors have used a combination of long-read sequencing and short-read sequencing for obtaining a high quality genome assembly. The assembly contigs have been scaffolded using Hi-C data. The annotation is available as GFF3 file from Ensemble. BUSCO analysis has shown 100% completeness with respect to the arthropoda database.

In Figure 3 caption, it was mentioned that "On both axes the GenBank names of the 11th largest autosomes are shown". But out of the 11, two were sex chromosomes. Kindly correct it.

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.

Version 1

Reviewer Report 26 September 2025

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Valentina Peona
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In this genome report, the authors present the genome assembly of the darkling beetle *Dendarus foraminosus*. The methods implemented are solid and the resulting genome assembly is of good quality.

Minor comments

- I think it would be nice for the reader to have available a longer introduction to the species about ecological role, morphology and about why this species was chosen to be sequenced in the first place. I think the authors already introduce it but if possible it would be good to have a slightly longer introduction.
- At the end of the introduction there is a repetition of “promote”/”promoting”. I suggest to make the sentence less redundant.
- In the Genome assembly methods section, the authors mention the discovery of a telomeric repeat. I suggest to add a reference to the tool used or describe the method implemented.
- In the same section, the authors also state that the mtDNA was assembled in two circular forms. I think it would be useful to clarify if these two forms correspond to two separate haplotypes.
- Not sure but maybe the word “Degree” in the sentence “Sampling was performed under the Presidential Degree 67/81 of the Greek Government.” should be “Decree”

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: Evolutionary genomics, sex chromosome evolution, centromere evolution, transposable elements

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.

Author Response 10 Nov 2025

Chiara Bortoluzzi

In this revised version of the Genome Report, we have addressed all reviewer's comments as follow:

1. As also highlighted by the other reviewer, we have now expanded on the introduction.
2. We have rephrased the last part of the introduction to avoid repetitions.
3. The telomeric motif was detected using TelFinder (https://journals.asm.org/doi/10.1128/spectrum.03928-22). The genome assembly lacks the canonical TTAGGG motif, 'TCGGG' is a putative motif. No experimental methods were used.
4. We used Oatk to identify the mtDNA. The two forms correspond to two separate haplotypes.
5. We changed 'Degree' to 'Decree'.

Competing Interests: No competing interests were disclosed.

Reviewer Report 15 August 2025

https://doi.org/10.21956/openreseurope.22173.r56309

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Terrence Sylvester
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In this genome report, the authors present the genome assembly of a darkling beetle, *Dendarus foraminosus*. The manuscript provides some contextual information about the species, including its distribution on the island of Crete and larval feeding patterns. However, to improve accessibility and utility—particularly for novice taxonomists—additional details on the species' morphology, life history, diagnostic features for distinguishing it from closely related species, and sexual dimorphism (e.g., how males and females can be identified) would be highly beneficial.

In the Methods section, the authors mention that genome size was estimated based on ancestral taxa. This approach requires further clarification. Genome sizes can vary substantially even among

closely related taxa, and reliance on ancestral estimates may not be sufficiently accurate for planning sequencing depth or evaluating assembly completeness. I recommend using a k-mer–based approach (e.g., using GenomeScope or similar tools) with the raw reads or including flow cytometry estimates, if available, for more accurate genome size inference.

The manuscript states that whole bodies were used for DNA, RNA, and Hi-C library preparation. Please specify the sex of the individuals used for each of these sequencing types, as this can impact genome representation, especially with respect to sex chromosomes.

I also suggest reporting BUSCO results before and after the removal of duplicated contigs. This would help assess whether the deduplication process led to any potential over-purging and loss of valid gene content.

In the Results section, consider including the amount of sequencing data generated for each library type, along with the expected read depth based on the estimated genome size. These metrics are important for evaluating whether the genome assembly was sufficiently covered.

The reported genome size of 593,991,149 bp should be expressed as 593.991 Mb for clarity and consistency with standard genome reporting practices. Similarly, assembly statistics like N50 should be reported in megabases (e.g., “13.2 Mb” rather than “13,200,000 bp”) for readability and alignment with other genome publications.

For BUSCO, the authors should consider using a more taxonomically appropriate single-copy ortholog dataset. Using a set that is too distantly related can artificially inflate completeness scores. For this work, the Endopterygota ortholog set would be more appropriate than broader sets like Insecta or Metazoa.

The manuscript currently lacks a clear description of assembly quality assessment methods. If such evaluations (e.g., misassembly detection, consensus accuracy, structural integrity checks) were part of the assembly pipeline, please indicate this in the Methods section. If external tools were used, list them along with their versions and relevant parameters.

Lastly, I suggest evaluating the OZ224577.1 contig more closely to confirm that it is correctly scaffolded. In addition, the authors should label sex chromosomes (i.e., identify which scaffolds correspond to X and Y, if applicable), especially if sex-specific material was used.

Overall, the genome of *Dendarus foraminosus* is a valuable addition to coleopteran genomics, and the assembly appears to be of good quality. Addressing the points above will greatly enhance the clarity, rigor, and reproducibility of the manuscript.

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: 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, however I have significant reservations, as outlined above.

Author Response 10 Nov 2025

Chiara Bortoluzzi

In this revised version of the Genome Report, we have addressed all reviewer's comments in the following way.

1. We have now expanded the introduction providing additional details on the species morphology, life history, diagnostic features, and sexual dimorphism.
2. We rephrased the section about the ancestral genome size to avoid any further confusion. There are two important aspects to consider here. First, it is impractical for large collaborative initiatives, such as the European Reference Genome Atlas (ERGA), to measure genome size from sampled specimens, as these are production-based, with collaborators and collectors across Europe. Furthermore, the diversity of collector contributors, locality, budget, lack of manpower, size of specimens, and the preservation method (very often specimens are flash frozen right after field identification), makes flow cytometry impractical for all samples. The second point to consider is that estimates are obtained only for taxa and lineages with no genome size available: the estimate of genome size from GoaT is used prior to sequencing to help calculate sequencing effort (e.g. library prep reagents and number of Revio flow cells) and it is known that the value might not be exact, but it is an educated guess. The assumption that a species might have its genome size within the range described for its taxonomic group is the basis for GoaT estimates: the median of genome sizes are computed from taxa with direct measurements and propagated to the closest common ancestor, then back to fill the missing data. More information on how GoaT calculates ancestral values to propagate can be found in https://wellcomeopenresearch.org/articles/8-24/v1. K-mer genome sizes are part of our pipelines and are used routinely to assess the need for top-up sequencing during the process of producing a genome. The final assessment of a sequence-based genome size is its assembly span, which, together with K-mer inferred genome size measurements, are not the definitive amount of DNA in cells, but a reference to the size of chromosome assemblies. We have now changed the main text accordingly to clarify this aspect of the estimated genome size.
3. We specified that Illumina and Pacbio sequencing were performed on DNA obtained from a male individual, Hi-C from a female one, and RNAseq from a second male individual. This information has now been included in the manuscript.

4. We ran BUSCO before and after the removal of duplicated contigs and results were identical in both cases. BUSCO score before purging: C:99.3%[S:98.1%,D:1.2%],F:0.1%,M:0.6%,n:3754,E:12.7%. BUSCO score after purging: C:99.3%[S:98.1%,D:1.2%],F:0.1%,M:0.6%,n:3754,E:12.7%. Purge_dups filtered out less than 30 Mb, mainly composed of small contigs. For completeness, we reported these results in the main text.
5. We have now included in the Results section the following sentences: "The Pacbio HiFi library generated 32.4 Gb of data, whereas Illumina libraries generated 37.5, 44.9, and 22.2 Gb of data respectively for DNAseq, Hi-C, and RNA-seq. The corresponding values of coverage are 55x, 63x, 76x, and 37x".
6. The reported genome size and assembly statistics have been changed to megabases in the main text as requested.
7. The BUSCO completeness score when using the Endopterygota lineage was 99.2% (C:99.2%[S:98.4%,D:0.8%],F:0.5%,M:0.3%,n:2124,E:3.8%), which is not very different from the BUSCO score reported in the main text. For completeness purposes, we reported both results in the Results section.
8. All assembly quality assessment methods are reported in the main manuscript via the ERGA Assembly Report (EAR) document available at https://github.com/ERGA-consortium/EARs/blob/main/Assembly_Reports/Dendarus_foraminosus/icDenFora10/ . This document reports all relevant information on the pre/post assembly curation process that ERGA internal reviewers will use to confirm that the assembly meets the desired quality metrics, taking into consideration peculiarities of each case. At the end of the document, tools used in the quality assessment are also reported.
9. The internal ERGA reviewer, which is an expert in genome curation and genome assembly, did not identify any issues concerning the scaffolding of OZ224577.1. We are therefore confident that the contig was correctly scaffolded. We did not label the sex chromosomes, because, although present in the final assembly, their presence in Figure 2 would have made the Figure unreadable. That is why we decided to plot only the eleven largest autosomes.

Competing Interests: No competing interests were disclosed.