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DATA NOTE

REVISED ERGA-BGE reference genome of *Lewinskya acuminata*, a common epiphytic Mediterranean moss with disjunct populations in California and Ethiopia

[version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

The reference genome of *Lewinskya acuminata* (H. Philib.) F. Lara, Garilleti & Goffinet will enable phylogenomic, biogeographic, and evolutionary studies within the *Orthotrichaceae* and related bryophyte lineages at a depth previously inaccessible. This species of moss is among the most representative of the Mediterranean epiphytic communities and can be readily identified by its long-acuminate leaves, fusiform capsules with a vestigial exostome, a well-developed endostome of six broad segments, and a dark, puckered peristome mouth when dry. The entirety of the genome sequence was assembled into 6 contiguous chromosomal pseudomolecules, while the mitochondrial and chloroplastic genomes were assembled,

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Approval Status ✓ ✓ ?

	1	2	3
version 2 (revision) 30 Mar 2026		✓ view	
version 1 24 Nov 2025	✓ view	? view	? view

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respectively, as a single contig of 104,820 bp and two contigs of 123,029 bp and 122,994 bp. This chromosome-level assembly encompasses 0.25 Gb, composed of 51 contigs and 13 scaffolds, with contig and scaffold N50 values of 11.5 Mb and 40.8 Mb, respectively.

Keywords
Lewinskya acuminata, genome assembly, European Reference Genome Atlas, Biodiversity Genomics Europe, Earth Biogenome Project, Bryophyta, moss, Orthotrichaceae

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Author roles: Aguado-Ramsay P: Investigation, Project Administration, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Lara F**: Investigation, Resources, Writing – Review & Editing; **Draper I**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Conejero M**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Böhne A**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Monteiro R**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **Marcussen T**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **H. Struck T**: Methodology, Project Administration, Supervision, Writing – Review & Editing; **A. Oomen R**: 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 – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED **Amendments from Version 1**

'We thank the reviewer for their comments. In this revised version of the Genome Report we have corrected several sections as suggested by the reviewers, to resolve any possible confusion. We also corrected Figure 1 and all typos pointed out by the reviewers. The only two major changes are a new Table with information on the chromosomes and their length and a new Figure displaying the blob plot'.

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

Introduction

Lewinskya acuminata was originally described as *Orthotrichum acuminatum* H. Philib. (Philibert, 1881) and was subsequently transferred to the newly segregated genus *Lewinskya* after the recent division of *Orthotrichum*, which separated the monoicous species with superficial stomata (Lara *et al.*, 2016). It is an epiphytic moss of the family Orthotrichaceae, readily distinguished by its long-acuminate leaves and characteristic fusiform capsules with a vestigial exostome, a developed endostome of six broad segments, and a dark, puckered mouth when dry. This configuration of the peristome and capsules allows for the hygrocastic release of spores (in conditions of high atmospheric humidity and moss hydration), while most of its congeners do so in dry conditions, xerocastically (Lara *et al.*, 1999). The species is widespread across the Mediterranean Basin, occurring on most major islands and mainland territories of both Europe and North Africa, from Anatolia (Turkey) to the Iberian Peninsula, Morocco, and Macaronesia (Calleja *et al.*, 2020). In recent decades, it has also been recorded in scattered localities across northwestern and central Europe (Müller, 2019). Furthermore, disjunct populations have been discovered in California and Ethiopia (Calleja *et al.*, 2020; Vigalondo *et al.*, 2016). This moss is one of the most representative species within Mediterranean epiphytic communities (Lara & Garilleti, 2014), contributing to local biodiversity in a significant part of the Western Palearctic region. This species is autoicous and karyotype analyses of most species within the genus have revealed a haploid chromosome number of six (Rice *et al.*, 2015).

Lewinskya acuminata has been assessed for the IUCN Red List of Threatened Species (Europe assessment) in 2018 and is listed as *Least Concern*. Other conservation categories have been proposed for some European territories: Vulnerable (VU) in Great Britain; Data Deficient (DD) in Madeira, Montenegro, and Slovenia; and Near Threatened (NT) in Sicily (Hodgetts & Lockhart, 2020).

The generation of a reference genome for *Lewinskya acuminata* will allow for in-depth studies of evolutionary processes, genetic diversity, and speciation patterns within Orthotrichaceae, and will contribute to the conservation and taxonomic assessment of both described and yet-undescribed species (Aguado-Ramsay *et al.*, 2024; Matanov *et al.*, 2025). *Lewinskya* is a cosmopolitan genus with approximately 80 recognized species, several of which have emerged from recent taxonomic studies (e.g., Lara *et al.*, 2024; Lara *et al.*, 2025; Vigalondo *et al.*, 2020). Several other complexes from different continents are being re-evaluated through integrative taxonomy, for which the reference genome is likely to be extremely valuable.

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 aim 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 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 transcriptomic profiling, to facilitate genome assembly and annotation.

Sample and sampling information

On 31 January 2024, Pablo Aguado-Ramsay and Francisco Lara sampled one specimen of *Lewinskya acuminata* (hermaphrodite monoecious). The specimen was determined based on morphology using Lara & Garilleti (2014) and it was identified by Pablo Aguado-Ramsay and Francisco Lara in Hoyo de Manzanares, Madrid, Spain. The biological material collected in Spain, and used to generate digital sequences, was retrieved from wildlife taxa regulated by the Spanish Royal Decree 124/2017 (https://www.boe.es/eli/es/rd/2017/02/24/124). No ABS permits were granted as utilization of the material falls under exemption in Article 3(2) of the Spanish Royal Decree 124/2017 and was confirmed by the national authorities. The whole dry individual was harvested from the tree and was taken alive directly to the laboratory. Dry apical parts of the gametophores with double checked taxonomic identification were individually selected, eliminating sporophytes and reducing contamination risks, carefully manipulating the plant to avoid modifying gene expression. Shots were then weighed and preserved at –80 °C until DNA extraction.

Vouchering information

The colony was deposited in the herbarium of the Universidad Autónoma de Madrid https://sweetgum.nybg.org/science/ih/herbarium-details/?irn=172673 under voucher ID MAUAM-Brio 5489.

Frozen reference tissue material is available from the same individual at the Museo Nacional de Ciencias Naturales https://www.mncn.csic.es/es under voucher ID MNCN-ADN 151724.

Genetic information

The estimated genome size, based on ancestral taxa, is 0.48 Gb and this corresponds to a diploid genome with a haploid number of 6 chromosomes (2n=12). The genome that was here sequenced and assembled is the haploid genome (n=6) whose genome size of 0.25 Gn well aligns with the haploid expectation. All information for this species was retrieved from Genomes on a Tree (Challis *et al.*, 2023).

DNA/RNA processing

DNA was extracted from approximately 200 mg of modular colony tissue ground in liquid nitrogen with mortar and pestle. The resulting powder was incubated in 10 mL of extraction buffer (100 mM Tris–HCl, 20 mM EDTA, 1.5 M NaCl, 1% sarkosyl, 2% polyvinylpyrrolidone [PVP], and 0.2% β-mercaptoethanol) at 21 °C for 1 h under agitation (300 rpm). DNA purification was subsequently performed following the general procedure described by Ramakrishnan *et al.*, 2017. DNA fragment size selection was performed using Short Read Eliminator (PacBio). DNA quantification was performed using a Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific), and DNA integrity was assessed using a Genomic DNA 165 Kb Kit (Agilent) on the Femto Pulse system (Agilent). The DNA was stored at +4°C until used.

RNA was extracted from a modular colony (50 mg) using RNeasy Powerplant 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 until used.

Library preparation and sequencing

Long-read DNA library was prepared with SMRTbell prep kit 3.0 following manufacturers' instructions and sequenced on a Revio system (PacBio). Hi-C library was generated from the same colony used for DNA extraction using the Arima High Coverage HiC Kit (following the Animal Tissues low input protocol v01) and sequenced on NovaSeq X Plus instrument (Illumina) with 2x 150 read length. In total 39x HiFi and 150x HiC data were sequenced to generate the assembly.

Genome assembly methods

The genome was assembled using the Genoscope GALOP pipeline (https://workflowhub.eu/workflows/1200). Briefly, raw PacBio HiFi reads were assembled using Nextdenovo v2.5.2 (Hu *et al.*, 2024). Remaining allelic duplications were removed using purge_dups v1.2.5 (Guan *et al.*, 2020) with default parameters and the proposed cutoffs. This assembly was scaffolded using YaHS v1.2.2 (Zhou *et al.*, 2023) and assembled scaffolds were then curated through manual inspection using PretextView v0.2.5 (Harry, 2022) to remove false joins and 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. Finally, the mitochondrial and chloroplastic genomes were assembled respectively as a single contig of 104,820 bp and two contigs of 123,029 bp and 122,994 bp using the Oatk v1.0 (Zhou *et al.*, 2025) and included in the released assembly (GCA_965213355.2) (Table 1). 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 256,705,263 bp in 13 scaffolds including the mitogenome and chloroplastic genomes (Figure 1, Figure 2, Figure 3), with a GC content of 37.5%. The assembly has a contig N50 of 11,461,491 bp and L50 of 7 and a scaffold N50 of 40,771,022 bp and L50 of 3. The assembly has a total of 38 gaps, totalling 4.7 kb in cumulative size. The single-copy gene content analysis using the Embryophyta database (odb10) with BUSCO (Manni *et al.*, 2021) resulted in 84.8% completeness (77.7% single and 7.1% duplicated). The relatively high proportion of missing BUSCO genes (13.4%) reflects a bias in the Embryophyta database towards vascular plants; such values are therefore common when bryophytes are assessed using this lineage. 97.5% of reads k-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 54.4 as calculated by Merqury (Rhie *et al.*, 2020).

Table 1. Chromosomal pseudomolecules in the genome assembly of *Lewinskya acuminata*.

NCBI Accession	Chromosome	Size (Mb)
OZ243776.1	1	65.7
OZ243777.1	2	43.2
OZ243778.1	3	40.7
OZ243779.1	4	38.4
OZ243780.1	5	35.0
OZ243781.1	6	31.4
OZ263618.1	MIT	0.10
OZ263619.1	PLTD_1	0.12
OZ263620.1	PLTD_2	0.12

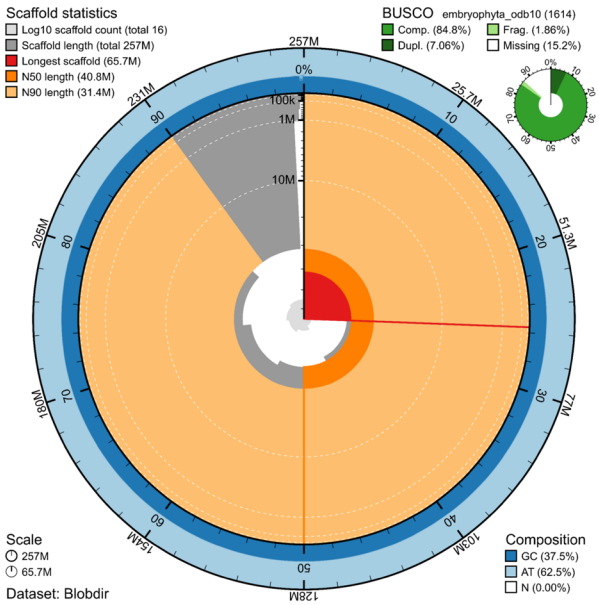

Figure 1. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 256,705,263 bp assembly including the mitochondrial genome, the chloroplastic genomes, and unlocalised contigs. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (65.7 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (40,771,022 and 31,400,860 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 Embryophyta database (odb10) is shown in the top right.

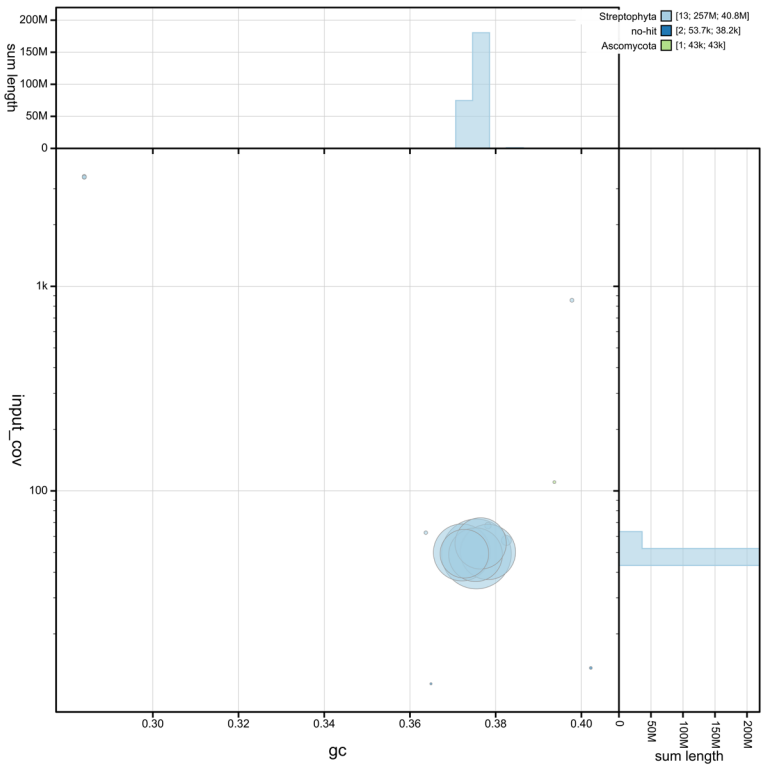

Figure 2. GC coverage plot generated for the *Lewinskya acuminata* assembly using blobtoolkit. Individual chromosomes and scaffolds are represented by each circle. The circles are sized in proportion to chromosome/scaffold length. Histograms show the sum length of chromosome/scaffold size along each axis. Color of circles indicate taxonomic hits of each Phylum represented in the assembly.

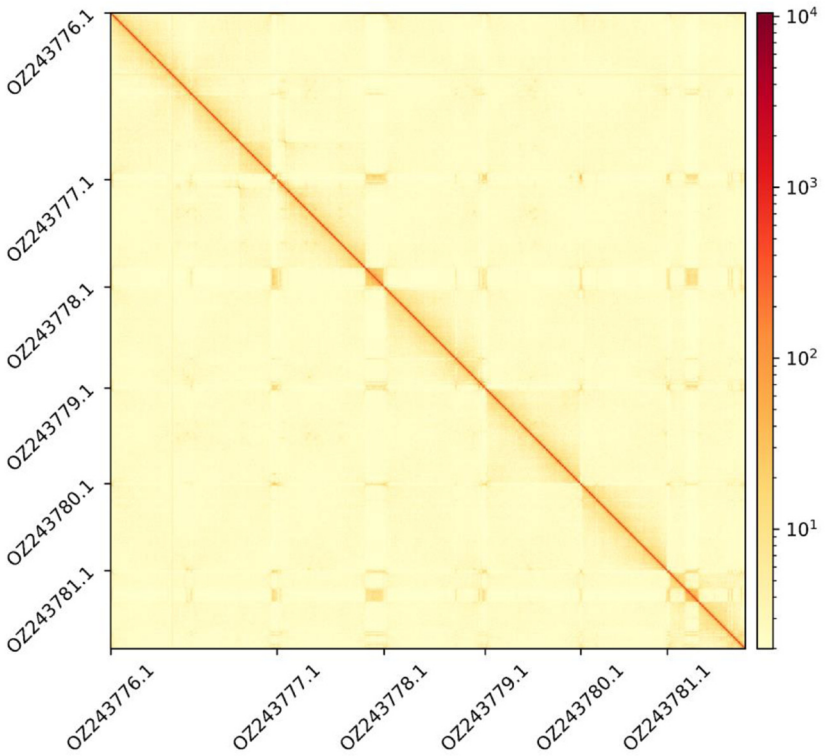

Figure 3. Hi-C contact map showing spatial interactions between regions of the genome. The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. The frequency of contacts is shown on a logarithmic heatmap scale. Hi-C matrix bins were merged into a 150 kb bin size for plotting.

Data availability

Lewinskya acuminata and the related genomic study were assigned to Tree of Life ID (ToLID) 'cbLewAcum8' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB79984. The sample information is available at the following BioSample accessions: SAMEA115283941 and SAMEA115283947. The genome assembly is accessible from ENA under accession number GCA_965213355.2 and the annotated genome will be available through the Ensembl webpage (https://projects.ensembl.org/erga-bge/). Sequencing data produced as part of this project are available from ENA at the following accessions: ERX14096377, ERX14170726, and ERX14514454. 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/Lewinskya_acuminata/cbLewAcum8. Further details and data about the project are hosted on the ERGA portal at https://portal.ergbiodiversity.eu/data_portal/1172132.

Author contributions

PAR coordinated the project; PAR and FL collected and identified the species; PAR sampled and preserved biological material and provided metadata; AsB, RM, TM, THS, RAO, PAR, ID, and MC 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, PW, and PHO; 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 April 2026

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Panagiotis Ioannidis
Foundation for Research & Technology- Hellas, Crete, Greece

The authors have addressed all my concerns and I have no further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: insect genomics; bioinformatics

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

Version 1

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The authors report the assembly of the genome a reference individual of the epiphytic moss *Lewinskya acuminata*. Good coverage of HiFi reads and Illumina HiC data were combined to produce an assembly that could be scaffolded into 6 pseudochromosomes (the expectation given

2n =12) and a small number of unplaced contigs, plus the organelles.

In general, the assembly stats are very good, but the BUSCO reports relatively low completeness (84%) and quite high duplication (7%) which might suggest some underlying complications. It could be that this taxonomic group is not well-catered for by the ODB datasets, but that is not my understanding for bryophytes in general. Some comment on this would be valuable.

The assembly pipeline makes no direct mention of polishing (although I note that polishing options are available in the Galop pipeline). Could this be made more clear? There are some omissions from the reporting that I would like to see included and the template is less comprehensive and more unstructured than the standard DToL reporting. This would be resolved with better templating and a more comprehensive set of metadata in tables (What Embryophyta gene set version is being assessed, for example?).

I note that a voucher specimen was retained but wonder whether verification of species via DNA barcode might also be a useful step in the sample QC.

Personally, I would like to see some information on the properties of the gDNA since the authors have performed extensive QC of the sample. I would also prefer to see some information on the kmer profiling (does genome size/heterozygosity fit with expectations?). RNA has been extracted but an annotation is not provided in this report.

The two reported chloroplast assemblies obtained are colinear and are 99.89% identical. I would like to see additional supporting evidence that this is not an assembly artefact.

I would also query the extent to which it is useful to contain both chloroplast sequences in the reference assembly if this represents genuine heteroplasmy- with this degree of similarity it may be more helpful to map to a shared reference and call variants.

The confusion in total scaffold number (13 in the text vs 16 in the snail plot) I think reflects the inclusion of the organelle genomes in the blobtools analysis- perhaps this could be clarified. Please note that Kb should be kb (e.g. in Genomic DNA 165 Kb Kit).

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

Yes

Are the protocols appropriate and is the work technically sound?

Partly

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: Genomics, Bioinformatics, Evolution

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 05 Feb 2026

Chiara Bortoluzzi

We thank the reviewer for their comment. We addressed all comments as follow:

1. Also pointed out by the other reviewers, we now clarified in the main text why the BUSCO completeness score is relatively low. Briefly, the high missing score is often elevated when using the embryophyta_odb10 lineage set in bryophytes, as the lineage is biased towards vascular plants.
2. We have expanded the presentation of the results, as also suggested by another reviewer. The Report now has an additional Table on the set of chromosomes and their length and an additional figure (blob plot).
3. No verification of species via DNA barcode was performed.
4. Most of the information requested by the reviewer (k-mer, heterozygosity, etc) is already reported in the ERGA Assembly Report or EAR, which is reported in the main text already. RNA was extracted, but the annotation is not provided because is undergoing.
5. As also suggested by another reviewer, we now corrected the confusion associated with the number of chromosomes.

Competing Interests: No competing interests were disclosed.

Reviewer Report 20 January 2026

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Panagiotis Ioannidis
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This manuscript describes the sequencing and assembly of the genome of the plant species Lewinskya acuminata. The Introduction section includes enough general details about this species, mostly regarding its habitat and ecology.

Additionally, the Methods section provides a detailed account of the sequencing and assembling

methodology. Most of the computational tools used for assembling are also widely used in other genome sequencing projects (purge_dups, YaHS, PretextView).

The resulting genome assembly is very contiguous with a relatively low CC ratio (13 scaffolds / 6 chromosomes = 2.16). Moreover, its QV score (54.4) and k-mer completeness (97.5%) are good. However, its BUSCO score (84% complete BUSCOs) denotes that this assembly could possibly be better; most of the HiFi-based assemblies these days have a BUSCO score of >97%. I would strongly recommend that the authors try another genome assembler such as Hifiasm which is known to produce high quality genome assemblies from HiFi reads.

I was also happy to see that a genome annotation will be also available for this assembly (using the herein generated RNAseq data, I'm assuming?). This is very important as it drastically increases the usefulness of a genome assembly; without one, a genome assembly cannot be used for quite a number of comparative studies. Unless, of course, genome annotation is performed which is a resource-demanding technically-challenging process.

Additionally, I believe that there are a few things missing from the Results section, which would greatly help in evaluating the quality of the assembly

1) some sort on QC on the HiFi reads would be necessary; for example, a GenomeScope plot. This is essential for showing the quality of your starting material.

2) a table is needed in which to display all the basic assembly features (contig N50, scaffold N50, L50, BUSCO scores, number of contigs, number of scaffolds etc).

3) another table is needed showing the lengths (and accessions) of the chromosome-level scaffolds so that the reader knows what the exact length of each chromosome.

4) a blob plot is also necessary in order to get a proper overview of the assembly scaffolds. I find this plot especially useful for many reasons; for checking the GC content and sequencing coverage of the assembly scaffolds, as well as for getting a hint about the possible origin of the extra scaffolds.

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; bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 27 Jan 2026

Chiara Bortoluzzi

We thank the reviewer for their comments and suggestions. All comments have been addressed as follow:

1. As also pointed out by Reviewer 1, the 'low' BUSCO completeness score is due to a bias in the embryophyta database used in the BUSCO analysis. We described this bias in the 'Results' section.
2. Yes, the genome annotation will be available through the Ensembl website and it will make use of the RNA-Seq data generated along with the genome assembly.
3. All these additional QC are already reported in the ERGA Assembly Report (or EAR) which accompanies this Genome Report, as reported in the Methods section (https://github.com/ERGA-consortium/EARs/blob/main/Assembly_Reports/Lewinskya_acuminata/cbLewAcum8/cbLewAcum8.1_E).
4. As before, all these additional information are already reported in the EAR (https://github.com/ERGA-consortium/EARs/blob/main/Assembly_Reports/Lewinskya_acuminata/cbLewAcum8/cbLewAcum8.1_E).
5. We added a table showing the lenghts (and accessions) of the chromosome-level assembly.
6. We added a Figure showing the blob plot results.

Competing Interests: No competing interests were disclosed.

Author Response 30 Jan 2026

Chiara Bortoluzzi

We thank the reviewer for their feedback. We have addressed all commens as follow:

1. As also pointed out by Reviewer 1, the ‘low’ BUSCO completeness score is due to a bias in the embryophyta database used in the BUSCO analysis. We described this bias in the Results section.
2. Yes, the genome annotation will be available through the Ensembl website and it will make use of the RNA-Seq data generated along with the genome assembly.
3. All these additional QC are already reported in the ERGA Assembly Report (or EAR) which accompanies this Genome Report, as reported in the Methods section (https://github.com/ERGA-consortium/EARs/blob/main/Assembly_Reports/Lewinskya_acuminata/cbLewAcum8/cbLewAcum8.1_E).

4. We added an additional table to the Report with information on the chromosomes and their length.
5. We now added a new Figure (blobl plot) to address this comment.

Competing Interests: No competing interests were disclosed.

Reviewer Report 02 January 2026

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Qilin Yang
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This Genome Note presents a high-quality reference genome assembly for the epiphytic moss Lewinskya acuminata (Orthotrichaceae). The resulting assembly is highly contiguous, resolving into 6 chromosomal pseudomolecules (consistent with the known karyotype) with a total length of ~257 Mb and high base quality (QV >50). The authors clearly outline the significance of the species for phylogenomic and biogeographical studies within the Mediterranean and disjunct populations.
General Comments

The manuscript is well-written, the methodology is state-of-the-art, and the resulting data appears to be of excellent quality. The Hi-C map (Figure 2) shows very clean chromosome separation, and the Snail plot (Figure 1) indicates high contiguity. The work is technically sound and provides a valuable resource for the bryology and genomics communities.

I have answered "Yes" to all questionnaire items, as the data is accessible and the methods are replicable. However, I have a few specific suggestions for clarification regarding the description of ploidy/genome size and the organelle assembly that would improve the precision of the manuscript before final indexing.

Specific Points for Improvement:

1. Clarification of Ploidy and Life Stage
In the "Genetic information" section, the text states: "The estimated genome size, based on ancestral taxa, is 0.48 Gb. This is a diploid genome with a haploid number of 6 chromosomes (2n=12)."
Later, in "Sample and sampling information," the authors correctly note they sampled "apical parts of the gametophores," which in mosses represents the haploid (n) life stage.
The final assembly size is ~0.25 Gb. This is perfectly consistent (half the size) with the 0.48 Gb

estimate if the estimate refers to the 2C (diploid) content and the assembly represents the 1C (haploid) content.
Suggestion: Please clarify the "Genetic information" section to explicitly state that the target assembly is the haploid genome (n=6). Phrasing it as "This is a diploid genome" is technically true for the sporophyte phase but potentially confusing for a gametophyte assembly. Explicitly stating that the assembled size (0.25 Gb) aligns with the haploid expectation derived from the 0.48 Gb diploid estimate would assist the reader.

2. Plastid Genome Description
In the Abstract, it is stated: "...1 mitochondrial genome, and 2 plastid genomes."
In the Methods: "...chloroplastic genomes were assembled respectively as ... two contigs of 123,029 bp and 122,994 bp..."
Suggestion: It is highly unusual for a moss to have two distinct plastid genomes. It is more likely that the assembly process resolved the plastome into two contigs (perhaps split by the Inverted Repeat regions) or that the software output two isomers.
If these are two contigs representing one plastid genome, please rephrase to "the plastid genome was assembled into 2 contigs."
If these represent two structural isomers of the same genome, please clarify this to avoid the impression that the species possesses two biological plastid genomes.

3. BUSCO Scores
The BUSCO completeness is 84.8% (Missing 15.2%). While this is a good score, "Missing" scores in bryophytes are often elevated when using the standard embryophyta_odb10 lineage set, as the dataset is biased towards vascular plants.
Suggestion: A brief sentence in the Results acknowledging that the missing BUSCO percentage is typical for bryophytes assessed against the Embryophyta dataset would provide better context for non-specialist readers who might otherwise view >10% missing genes as a quality issue.

4. Scaffold Count Discrepancy
Text: "The genome assembly has a total length of 256,705,263 bp in 13 scaffolds..."
Figure 1 Legend: "Log10 scaffold count (total 16)"
Suggestion: Please reconcile these numbers. It is possible the Snail plot includes small unlocalized contigs or the organelles that were not counted in the text summary.

5. Typo
Abstract: "...six contiguous chromosomal pseudomolecules..."
Results: "...contig N50 of 11.5 Mb..."
The text is generally clean, but please ensure the distinction between "contigs" and "scaffolds" is used consistently when discussing the final chromosome-level assembly. (The current usage seems correct, this is just a reminder to double-check).

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: plant_molecular_biology_and_bioinformatics

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

Author Response 27 Jan 2026

Chiara Bortoluzzi

We thank the reviewer for their comments and for their kind words. We have addressed all points as follow:
1. We have now corrected the 'Genetic information' section as suggested.
2. We have changed the abstract to match what reported in the 'Methods' section to avoid any further confusion.
3. We thank the reviewer for point out the issue with the BUSCO lineage. We have now corrected this in the 'Results' section to provide more context.
4. We have now corrected Figure 1.
5. All typos have been corrected.

Competing Interests: No competing interests were disclosed.

Author Response 30 Jan 2026

Chiara Bortoluzzi

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

1. We now corrected the ‘Genetic information’ section as suggested.
2. We changed the abstract to match what reported in the Methods section to avoid any further confusion.
3. We have corrected the BUSCO score in the Results section.
4. We have now corrected Figure 1.
5. We have corrected all typos.

Competing Interests: No competing interests were disclosed.