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DATA NOTE

REVISED ERGA-BGE reference genome of *Leviellus thorelli*, a common orb-weaving spider representing the *Zygiellidae* family

[version 2; peer review: 2 approved]

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Abstract

The *Leviellus thorelli* reference genome provides the first high-quality genomic resource for *Zygiellidae*, a family of orb-weaving spiders with a dynamic systematic history and distinct for constructing webs with a characteristic spiral-free sector. As part of the European Reference Genome Atlas (ERGA), we generated a chromosome-level assembly for *L. thorelli* that is organized into 13 contiguous chromosomal pseudomolecules. This chromosome-level assembly encompasses 2.20 Gb and is composed of 939 contigs and 130 scaffolds, with contig and scaffold N50 values of 5.4 Mb and 167.1 Mb, respectively. This genome represents a valuable addition to the growing collection of spider genomes. With *Zygiellidae* now included among the available genomes of true orb-weavers, this is a key resource for comparative studies into the genomic basis of orb web and silk evolution.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 10 Feb 2026		
version 1 27 Nov 2025	✓ view	✓ view

1. **Zhisheng Zhang**, Southwest University, Chongqing, China

2. **Guilherme Gainett**, Harvard Medical School. Boston Children's Hospital, Boston, USA

Keywords
Leviellus thorelli, genome assembly, European Reference Genome Atlas, Biodiversity Genomics Europe, Earth Biogenome Project, Zygiellidae, free sector orb weaver

Any reports and responses or comments on the article can be found at the end of the article.

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

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Author roles: Gregorič M: Investigation, Resources, Writing – Original Draft Preparation, Writing – Review & Editing; **Bužan E**: Investigation, Project Administration, 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; **Gut M**: Investigation, Writing – Review & Editing; **Aguilera L**: Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Câmara Ferreira F**: Data Curation, Formal Analysis, Writing – Review & Editing; **Cruz F**: Data Curation, Formal Analysis, Writing – Review & Editing; **Gómez-Garrido J**: Data Curation, Formal Analysis, Writing – Review & Editing; **S. Alioto T**: 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. We have addressed all comments as follow:

1. We have updated the reference, which is indeed published and not in press.

2. We updated the 'Genetic information' section to clarify the concept of 'ancestral taxa' and the 12 chromosomes.

3. We did not add any additional information on the contamination analyses, because are very well described in the EAR reported in the Genome Report.

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

Introduction

Leviellus thorelli (Ausserer, 1871) is an orb-weaving spider species, currently classified in the family *Zygiellidae*, a small lineage recently recognised as distinct from other true orb weaving spiders (Kuntner *et al.*, 2023). Members of this family, recognised within the subfamily *Zygiellinae*, were considered to belong to the families *Tetragnathidae* and *Araneidae*, but have since been elevated to family rank to reflect their phylogenetic position and morphological diagnosability (Kuntner *et al.*, 2023; but see Hormiga *et al.*, 2023). The *Leviellus* genus currently includes several species distributed throughout the Palearctic region, with *L. thorelli* being one of the most widespread representatives (World Spider Catalog, 2025). *Leviellus thorelli* occurs across Central, Southern, and South-Eastern Europe, inhabiting open woodland, grassland, and anthropogenic environments, such as gardens and buildings (Nentwig *et al.*, 2025; World Spider Catalog, 2025). This species is a medium-sized orb-weaver that, typical of the *Zygiellidae* family, constructs vertical orb webs with a characteristic spiral-free sector; the web hub is connected to a silken-tube retreat with a signal line, reflecting a behavioural adaptation distinct from most other orb weavers (Gregorič *et al.*, 2015). Like many spider species, *Leviellus thorelli* is a generalist predator that contributes to ecosystem balance by regulating insect populations.

From a systematic and evolutionary perspective, *L. thorelli* and its relatives are of particular interest, because they represent an early-diverging lineage within the clade of true orb weaving spiders, the *Orbipurae* (Kuntner *et al.*, 2023). Comparative genomic data from this group can therefore provide important insights into the diversification of web architecture, web-building behaviours, silk mechanical properties, and silk gene evolution. Despite their abundance, members of the *Zygiellidae* family have previously not been represented by a reference-quality genome, limiting our understanding of genomic evolution across orb-weaving spiders. Thus, the reference genome of *L. thorelli* represents the first high-quality genome from the *Zygiellidae* family, providing a valuable resource for phylogenomic analyses aimed at refining spider systematics and taxonomy and comparative studies on the evolution of webs and silk. It will also enhance our capacity to explore genome structure and evolution both across *Orbipurae* and *Araneoidea*, providing a basis for future work in spider molecular ecology and functional genomics.

Leviellus thorelli is currently not classified as threatened on the IUCN Red List or any other endangered-species lists.

The generation of this reference resource was coordinated by the European Reference Genome Atlas (ERGA, https://www.erga-biodiversity.eu/) initiative's Biodiversity Genomics Europe (BGE, https://biodiversitygenomics.eu/) 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 07 September 2023, Matjaž Gregorič hand collected six specimens of *Leviellus thorelli* (five females, one male), at Kremenica, Slovenia (lat = 45.941472, lon = 14.548028) and Ig, Slovenia (lat = 45.964847, lon = 14.519679), which were determined based on morphology from primary taxonomic literature (Levi, 1974). The specimens were identified by Matjaž Gregorič in Slovenia. No permit was required for the collection of the here used specimens, as communicated and confirmed by the Slovenian Ministry of Natural Resources and Spatial Planning (communicated on 16. October 2023). Specimens were hand collected, euthanized by being put alive at -80 °C, and until DNA extraction, they were preserved at -80 °C.

Vouchering information

Physical reference materials for the here sequenced specimen have been deposited in the Slovenian Museum of Natural History, Ljubljana, Slovenia https://www.pms-lj.si/en/ under the accession number ARA8125-ARA8128.

Tissues from prosoma and from four whole organisms (proxies), as well as residual DNA and RNA, were deposited in the Leibniz Institute for the Analysis of Biodiversity Change (LIB) Biobank, Museum Koenig, Bonn, Germany, under the collection IDs ZFMK-TIS-102871, ZFMK-TIS-102868, ZFMK-TIS-102869, ZFMK-TIS-102870, ZFMK-TIS-102872, ZFMK-DNA-FD19596994, and ZFMK-RNA-FD19597045, respectively. Genitals used for future specimen identification are deposited at the Slovenian Museum of Natural History, Ljubljana, Slovenia https://www.pms-lj.si/en/.

Genetic information

The estimated genome size, estimated by Genomes on a Tree (GoaT) (Challis et al., 2023) by ancestral state reconstruction is 2.18 Gb. This estimated genome size corresponds to a diploid genome with a haploid number of 12 chromosomes (2n=24). All information for this species was retrieved from Genomes on a Tree (Challis *et al.*, 2023).

DNA/RNA processing

DNA was extracted from the prosoma of an adult female (qqLevThor3) using the Blood & Cell Culture DNA Mini Kit (Qiagen) following the manufacturer's instructions. 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 sequenced.

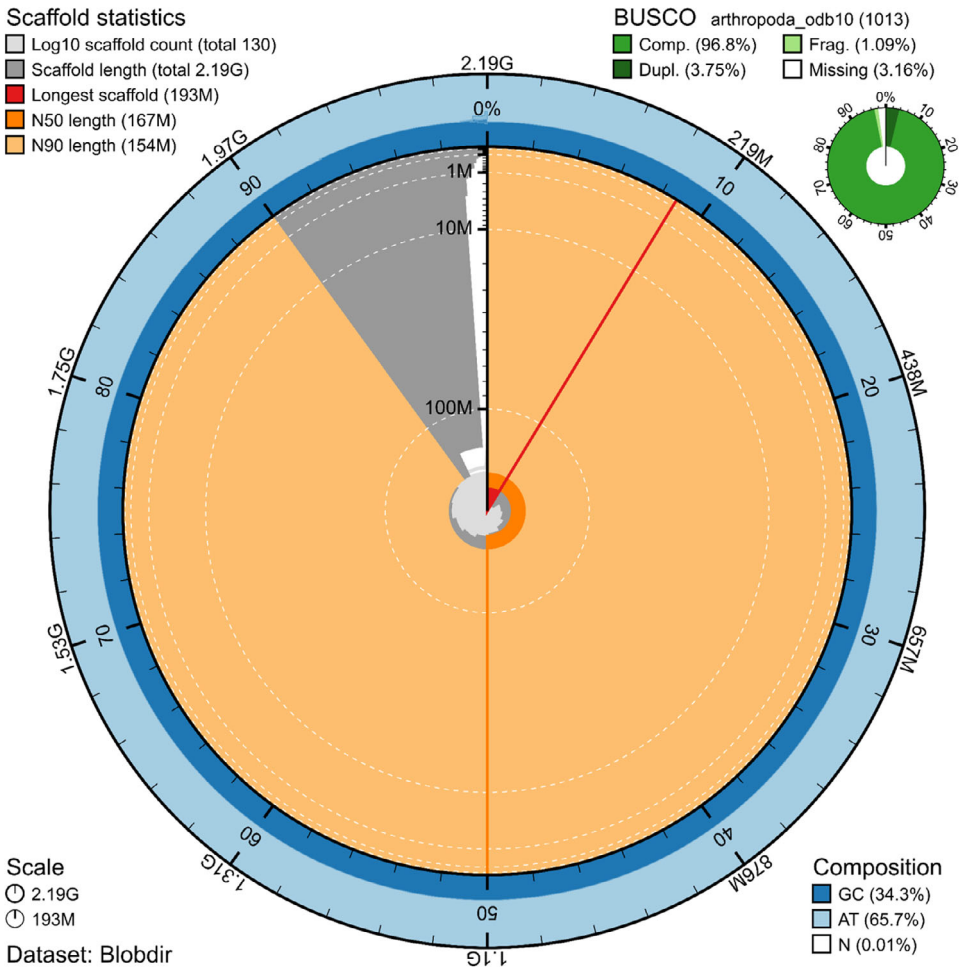


Figure 1. Snail plot summary of assembly statistics.

RNA was extracted from prosoma using a RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA quantification was performed using the Qubit RNA BR kit, and RNA integrity was assessed using a Bioanalyzer 2100 system (Agilent) RNA 6000 Nano Kit (Agilent). The RNA was stored at -80 °C until sequenced.

Library preparation and sequencing

For long-read whole genome sequencing, a library was prepared using the SQK-LSK114 Kit (Oxford Nanopore Technologies, ONT), which was then sequenced across two R10.4.1 flow cells on a PromethION 24 A Series instrument (ONT). A short-read whole-genome sequencing library was prepared using the KAPA Hyper Prep Kit (Roche). A Hi-C library was prepared from the prosoma of a different adult female (qqLevThor2) using the Dovetail Omni-C Kit (Cantata Bio), followed by the KAPA Hyper Prep Kit for Illumina sequencing (Roche). The RNA library was prepared using the KAPA mRNA Hyper prep kit (Roche). All short-read libraries were sequenced on a NovaSeq 6000 instrument (2x150bp, Illumina). In total 40x Oxford Nanopore, 62x Illumina WGS shotgun, and 125x HiC data were sequenced to generate the assembly.

Genome assembly methods

The genome was assembled using the CNAG CLAWS pipeline (Gomez-Garrido, 2024). Briefly, reads were preprocessed for quality and length using Trim Galore v0.6.7 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and Filtlong v0.2.1 (https://github.com/rrwick/Filtlong), and initial contigs were assembled using NextDenovo v2.5.0 (Hu *et al.*, 2024), followed by polishing of the assembled contigs using HyPo v1.0.3 (Kundu *et al.*, 2019), removal of retained haplotigs using purge-dups v1.2.6 (Guan *et al.*, 2020) and scaffolding with YaHS v1.2a (Zhou *et al.*, 2024). Finally, assembled scaffolds were curated via manual inspection using Pretext v0.2.5 with the Rapid Curation Toolkit (https://gitlab.com/wtsi-grit/rapid-curation) to remove any false joins and incorporate any sequences not automatically scaffolded into their respective locations in the chromosomal pseudomolecules (or super-scaffolds). Summary analysis of the released assembly was performed using the ERGA-BGE Genome Report ASM Galaxy workflow (10.48546/workflowhub.workflow.1103.2).

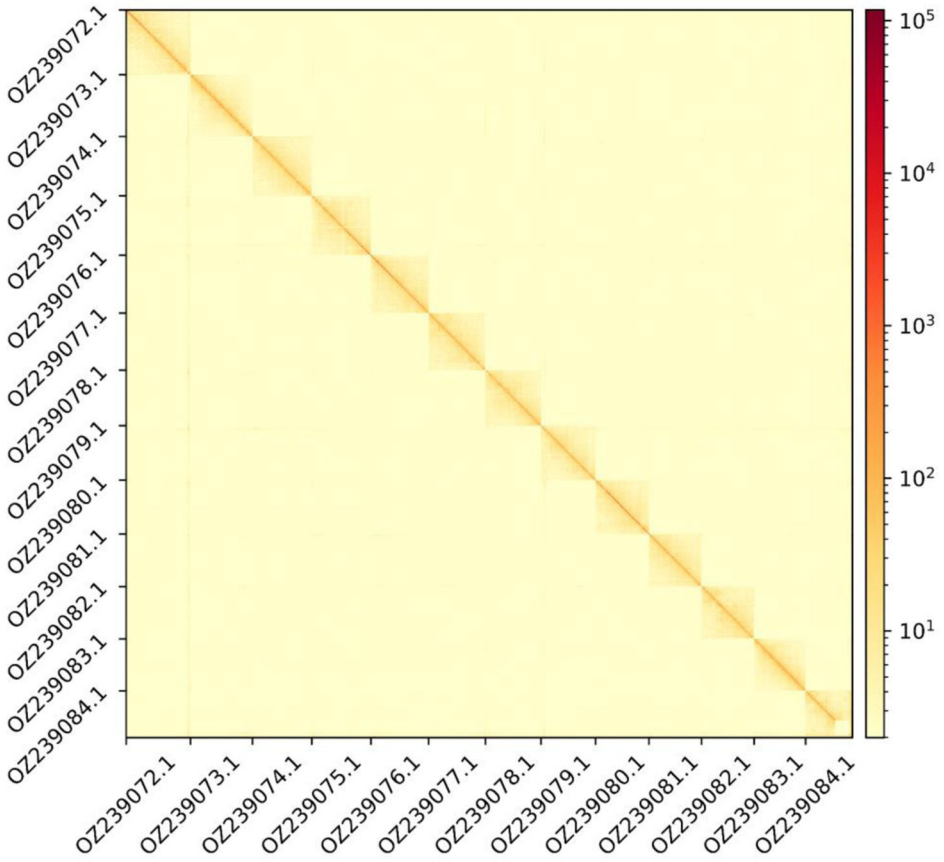


Figure 2. Hi-C contact map showing spatial interactions between regions of the genome.

Results

Genome assembly

The genome assembly has a total length of 2,190,932,837 bp in 13 superscaffolds and 17 additional unlocalized and 100 unplaced scaffolds (Figure 1 & Figure 2), with a GC content of 34.3%. The assembly has a contig N50 of 5,371,768 bp and L50 of 119 and a scaffold N50 of 167,117,025 bp and L50 of 7. The assembly has a total of 809 gaps, totalling 161.8 kb in cumulative size. The single-copy gene content analysis using the Arthropoda database with BUSCO (Manni *et al.*, 2021) resulted in 96.8% completeness (93.1% single and 3.8% duplicated). 93.2% of reads k-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 47.5 as calculated by Merqury (Rhie *et al.*, 2020).

The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 2,190,932,837 bp assembly. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (193 Mb, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (167,117,025 and 154,116,833 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.

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 200 kb bin size for plotting.

Author contributions

EB coordinated the project; MG collected the species; MG identified the species; MG 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; LA and MG extracted DNA, prepared libraries, and performed sequencing; FCF, FC, and JG-G performed genome assembly and curation under the supervision of TSA; 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.

Data availability

Leviellus thorelli and the related genomic study were assigned to Tree of Life ID (ToLID) 'qqLevThor3' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB77917. The sample information is available at the following BioSample accessions: SAMEA115177265 and SAMEA115177266. The genome assembly is accessible from ENA under accession number GCA_965183905.1 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: ERX12756489, ERX13167067, ERX14095502, and ERX14095503. 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/Leviellus_thorelli/qqLevThor3. Further details and data about the project are hosted on the ERGA portal at https://portal.erga-biodiversity.eu/data_portal/1662264.

Acknowledgements

We acknowledge the support of the Freiburg Galaxy Team: Saim Momin and Björn Grüning, Bioinformatics, University of Freiburg (Germany), funded by the German Federal Ministry of Education and Research BMBF grant 031 A538A de. NBI-RBC and the Ministry of Science, Research and the Arts Baden-Württemberg (MWK) within the framework of LIBIS/de. NBI Freiburg. We acknowledge the assembly reviewer, Jo Wood from the Wellcome Sanger Institute.

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Open Peer Review

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Version 1

Reviewer Report 29 January 2026

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✔ **Guilherme Gainett**
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The presented spider genome is a high-quality resource, and I have no major concerns. Great contribution to the growing number of spider genomes.

A few comments and questions:

-page 1 -> (Kuntner et al., 2023; in press) -> As far as I can tell, this is not in press anymore.
-page 3: The estimated genome size, based on ancestral taxa -> could you please clarify ancestral taxa? It seems incorrect to refer to higher-level taxa or sister groups as ancestral if that's the intended meaning.
-page 3: extracted from the thorax -> prosoma is the most used term in arachnid literature.
-page 3: could you please clarify the statement about 12 chromosomes? How was this number estimated? 13 chromosomes are assembled later.

Was there any contamination analysis done? A BlobToolKit GC-coverage plot could be shown to support the cleanness of the assembly. Could the small unassembled bits be due to contamination?

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: Arachnology, Genomics, Evodevo, Zoology

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 30 Jan 2026

Chiara Bortoluzzi

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

1. We have updated the reference, which is indeed published and not in press.
2. We updated the 'Genetic information' section to clarify the concept of 'ancestral taxa' and the 12 chromosomes.
3. We did not add any additional information on the contamination analyses, because are very well described in the EAR reported in the Genome Report.
4. We replaced the word 'thorax' throughout the manuscript as suggested by the reviewer.

Competing Interests: No competing interests were disclosed.

Reviewer Report 02 January 2026

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Zhisheng Zhang
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The *Leviellus thorelli* reference genome provides the first high-quality genomic resource for *Zygiellidae.* This chromosome-level assembly encompasses 2.20 Gb and is composed of 939 contigs and 130 scaffolds, with contig and scaffold N50 values of 5.4 Mb and 167.1 Mb, respectively. And this genome represents a valuable addition to the growing collection of spider genomes.

The rationale for creating the dataset was clearly described. The protocols appropriate and the work were technically sound.

The sufficient details of methods and materials were provided to allow replication by others. The

datasets were clearly presented in a useable and accessible format.

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: spider genome, evolution, adaptation

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 05 Jan 2026

Chiara Bortoluzzi

We thank the reviewer for approving the Genome Report of *Leviellus thorelli*.

Competing Interests: No competing interests were disclosed.