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DATA NOTE

ERGA-BGE genome of *Albinaria teres* (Olivier, 1801): a rock-dwelling land snail endemic to the island of Crete, Greece

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

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Abstract

Albinaria teres is a small rock-dwelling terrestrial gastropod of Greece. It is a polytypic species containing ten subspecies, all endemic to the island of Crete, highlighting the high diversification which characterize the whole genus of *Albinaria*. The reference genome of *Albinaria teres* offers a crucial resource for uncovering phylogenetic relationships within the enigmatic genus of *Albinaria,* as well as for unravelling the genetic basis of its environmental adaptations. The entirety of the genome sequence was assembled into 31 contiguous chromosomal pseudomolecules. This chromosome-level assembly encompasses 1.71 Gb, composed of 418 contigs and 43 scaffolds, with contig and scaffold N50 values of 7.88 Mb and 57.6 Mb, respectively.

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Introduction

Albinaria teres belongs to the genus *Albinaria* Vest, 1867 (Stylommatophora: Clausiliidae) which is characterized by very high morphological and genetic differentiation (Gittenberger, 1991). It is one of the approximately 40 *Albinaria* species endemic to the island of Crete in Greece. It is distributed to the eastern part of Crete and some of its satellite islets where it is found on limestone rocks (Welter-Schultes, 2010). *Albinaria teres* is a polytypic species containing ten subspecies namely *A. t. distans* (L. Pfeiffer, 1865), *A. t. extensa* (L. Pfeiffer, 1865), *A. t. manselli* (O. Boettger, 1883), *A. t. nordsiecki* Zilch, 1977, *A. t. phalanga* (O. Boettger, 1883), *A. t. teres* (Olivier, 1801), *A. t. toplouensis* Welter-Schultes & Wiese, 1991, *A. t. vermiculata* (O. Boettger, 1883) and *A. t. xerocampensis* Wiese, 1990 (MolluscaBase, 2025).

The species has been assessed in the Red List of Greece (NECCA, 2023) and in the IUCN Red List (Triantis & Chelmis, 2023) as Least Concern.

Despite the great effort of researchers over the past 40 years on the systematics of *Albinaria*, the taxonomy and phylogeny of the genus is still not resolved (Bamberger *et al.*, 2022; Becher *et al.*, 2024; De Weerd & Gittenberger, 2005; Dimopoulou *et al.*, 2017; Douris *et al.*, 1995; Douris *et al.*, 1998a; Douris *et al.*, 1998b; Giokas *et al.*, 2000; Kornilios *et al.*, 2015; Páll-Gergely *et al.*, 2012; Schilthuizen *et al.*, 1993; Schilthuizen *et al.*, 1995; Schilthuizen *et al.*, 2004; Schilthuizen & Gittenberger, 1995; Van Moorsel *et al.*, 2000). A high-quality reference genome for *A. teres* will serve as a reference for the entire genus of *Albinaria*, enabling more sophisticated methods of phylogenetic inference. It will help to clarify the phylogenetic relationships between the species of *Albinaria*, refine their taxonomic classifications, and improve our understanding of the evolutionary processes shaping the high diversity observed within the genus.

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

Danae Karakasi and Charalampos Fassoulas sampled 25 specimens of *Albinaria teres* (Figure 1) by hand picking at Karydi, Lasithi, Crete, Greece on 5 July 2023. Determination was based on expert identification of Malacologist Dr. Katerina Vardinoyiannis. Sampling was performed under Presidential Decree 67/1981 issued by Greek State Law. Mollusc foot tissue from the specimens was cut and was immediately flash frozen in liquid nitrogen and preserved in a freezer at -80 ℃ until DNA extraction.



Figure 1. Electronic voucher image of the collected individuals of *Albinaria teres*. Images of collected individuals are available in ERGA's EBI BioImageArchive dataset (www.ebi.ac.uk/biostudies/bioimages/studies/S-BIAD1012?query=ERGA) under accession IDs SAMEA114349646, SAMEA114349647, SAMEA114349658, SAMEA114349659, SAMEA114349660, SAMEA114349661, SAMEA114349662, SAMEA114349663, SAMEA114349664 and SAMEA114349665.

Vouchering information

Physical reference materials for the sequenced specimen have been deposited in the Invertebrates Collections of the Natural History Museum of Crete (NHMC) – University of Crete (https://www.nhmc.uoc.gr/en/departments/invertebrates) under the accession ID NHMC.50.52125.

Frozen reference tissue material of mollusc foot 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 the accession IDs NHMC.50.52125.11-16.

Genetic information

The estimated genome size, based on ancestral taxa, is 2.85 Gb. This is a diploid genome with a haploid number of 31 chromosomes (2n=62) and unknown sex chromosomes. All information for this species was retrieved from Genomes on a Tree (Challis *et al.*, 2023).

DNA/RNA processing

DNA was extracted from 30 mg of foot tissue using the Genomic-tip 20/G kit (Qiagen, MD, USA) following manufacturer instructions 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) and integrity was assessed in a FemtoPulse system (Agilent). DNA was stored at 4 ℃ until usage.

RNA was extracted from foot muscle (20 mg) using the 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 the SMRTbell prep kit 3.0 following manufacturers' instructions and sequenced on a Revio system (PacBio).

Hi-C libraries were generated from foot muscle tissue using the Arima High Coverage HiC kit (following the Animal Tissues low input protocol v01) and sequenced on a NovaSeq6000 instrument (Illumina) with 2x150 bp read length. In total 57.4 Gb PacBio HiFi, 280.4 Gb Illumina WGS shotgun, and 52.3 Gb HiC data were sequenced to generate the assembly.

Genome assembly methods

The genome of *A. teres* 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. 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. The mitochondrial genome was assembled as a single contig 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 (De Panis, 2024b).

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: SAMEA114349672 to the genome. Gaps in the annotation were filled via protein-to-genome alignments of a select set of clade-specific proteins from UniProt (The 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). Summary analysis of the released annotation was performed using the ERGA-BGE Genome Report ANNOT Galaxy workflow (De Panis, 2024a), incorporating tools such as AGAT v1.2, BUSCO v5.5 and OMArk v0.3.

Results

Genome assembly

The genome assembly has a total length of 1,710,616,219 bp in 43 scaffolds including the mitogenome (Figure 2 & Figure 3), with a GC content of 38.99%. The assembly has a contig N50 of 7,880,036 bp and L50 of 74 and a scaffold N50 of 57,558,842 bp and L50 of 11. The assembly has a total of 375 gaps, totaling 43.7 kb in cumulative size. The single-copy gene content analysis using the Metazoa database with BUSCO (Manni *et al.*, 2021) resulted in 97.6% completeness (88.8% single and 8.8% duplicated). 82.1% of reads k-mers were present in the assembly and the assembly has a base accuracy Quality Value (QV) of 58.0 as calculated by Merqury (Rhie *et al.*, 2020).

Genome annotation

The genome annotation consists of 14,748 protein-coding genes with an associated 21,441 transcripts, in addition to 7,088 non-coding RNA genes of various types (Table 1). Using the longest isoform per transcript, the single-copy gene content analysis using the metazoa_odb10 database with BUSCO, run in protein mode, resulted in 91.1% completeness. Using the OMAmer Lophotrochozoa database for OMArk resulted in 93.40% completeness and 70.30% consistency (Table 2).

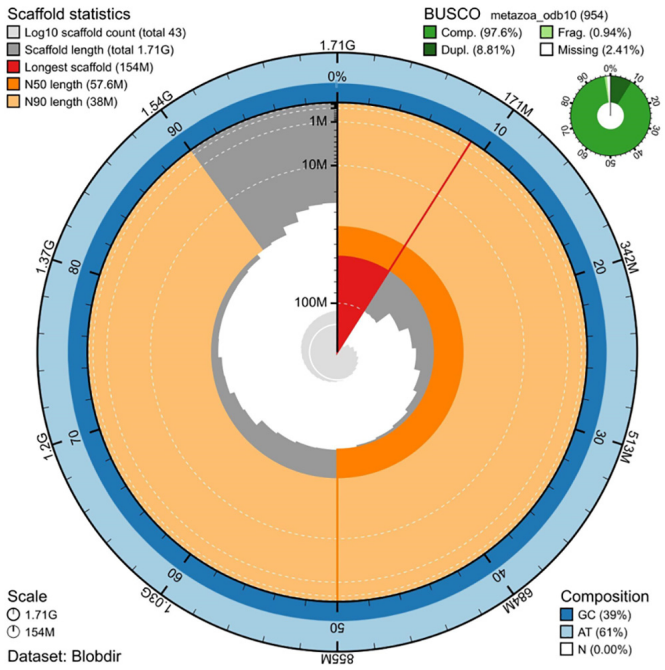

Figure 2. Snail plot summary of assembly statistics. The main plot is divided into 1,000 size-ordered bins around the circumference, with each bin representing 0.1% of the 1,710,616,219 bp assembly including the mitochondrial genome. The distribution of sequence lengths is shown in dark grey, with the plot radius scaled to the longest sequence present in the assembly (154,472,788 bp, shown in red). Orange and pale-orange arcs show the scaffold N50 and N90 sequence lengths (57,558,842 and 38,046,388 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 Metazoa database (odb10) is shown in the top right.

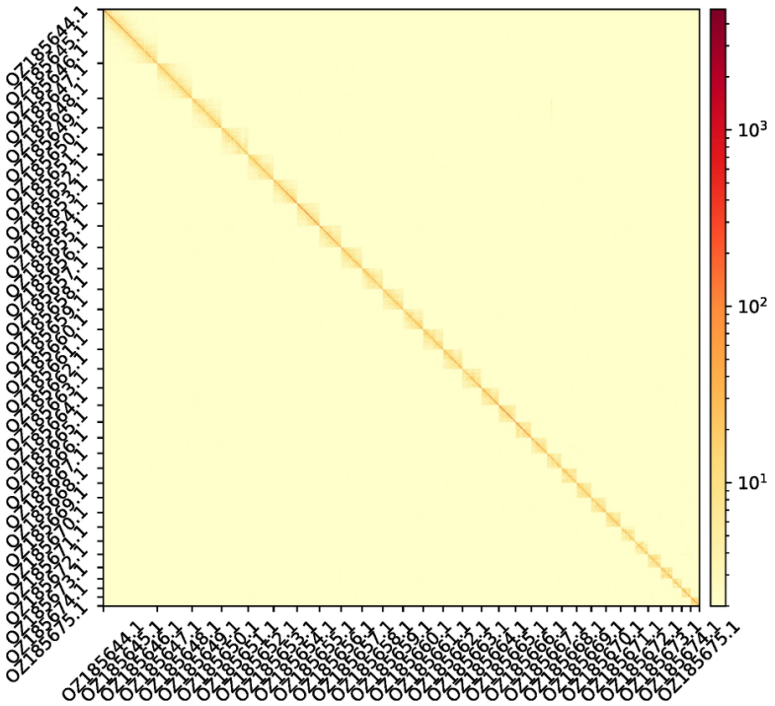

Figure 3. Hi-C contact map showing spatial interactions between regions of the genome. The diagonal corresponds to intra-chromosomal contacts, depicting chromosome boundaries. The frequency of contacts is shown on a logarithmic heatmap scale. Hi-C matrix bins were merged into a 100 kb bin size for plotting.

Table 1. Statistics from assembled gene models.

	No. genes	No. transcripts	Mean gene length (bp)	No. single-exon genes	Mean exons per transcript
Protein-coding	14,748	21,441	28,731	676	8.1
lncRNA	3,442	3,788	8,248	2	2.4
snRNA	312	312	149	312	1.0
snoRNA	616	616	170	616	1.0
rRNA	351	351	826	351	1.0
tRNA	649	649	76	649	1.0
Other non-coding	1,718	1,718	73	1,718	1.0

Table 2. Annotation completeness and consistency scores calculated by BUSCO run in protein (metazo_odb) and OMArk (Lophotrochozoa).

	Complete	Singular	Duplicated	Fragmented	Missing
BUSCO	869 (91.1%)	811 (85.0%)	58 (6.1%)	41 (4.3%)	44 (4.6%)
OMArk	2,01 (93.40%)	1,635 (75.94%)	376 (17.46%)	-	142 (6.60%)
	Consistent	**Inconsistent**	**Contaminants**	**Unknown**	
OMArk	10,368 (70.30%)	1,473 (9.99%)	0 (0.05)	2,907 (19.71%)	

Data availability

Albinaria teres and the related genomic study were assigned to Tree of Life ID (ToLID) 'xgAlbTers5' and all sample, sequence, and assembly information are available under the umbrella BioProject PRJEB77226 (Genoscope Sequencing Team *et al.*, 2024). The sample information is available at the following BioSample accessions: SAMEA114349668 and SAMEA114349670. The genome assembly is accessible from GenBank under accession number GCA_964271275.1. Sequencing data produced as part of this project are available from SRA at the following accessions: ERX12733447, ERX12733458, ERX12737188 and ERX12737189. The genome annotation is available from Ensembl under accession GCA_964271275.2 (Tricomi *et al.*, 2025). All data are published under CC0 licence. 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/Albinaria_teres/xgAlbTers5. Further details and data about the project are hosted on the ERGA portal at https://portal.erga-biodiversity.eu/data_portal/69427.

Author contributions

DK collected the species, KV identified the species, DK sampled and preserved biological material and provided metadata, NE, RM, AB, THS, RF, DK, GB, EB, MS, PL, NP and TM 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; LD, AN, and JMA performed genome assembly and curation, TB 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://doi.org/10.5281/zenodo.14611490

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Publisher Full Text

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Yasuto ISHII
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This study presented a chromosome-level genome assembly of *Albinaria teres*. No other genome assembly is available for clausiliid land snails. Furthermore, very few chromosome-level genome assemblies have been successfully constructed for land snails. Considering these reasons, the chromosome-level assembly provided is outstandingly valuable. This assembly has some advantages compared to previous land snail assemblies: the high completeness and continuity (BUSCO completeness > 97%; contig N50 = 7.8 Mb), and the gene annotation which is publicly accessible. Despite this excellence, there are some concerns listed below.

What kind of "sophisticated" phylogenetic analyses do you think resolve the phylogenetic issue of *Albinaria* snails? Recently, some phylogenetic relationship seems to be resolved using RAD-seq (Bamberger et al., 2022; Becher et al., 2024), and perhaps any reference genome is not required. Show rationales and cite references. I suppose this argument is very important since it clarifies the purpose of this study.

In the introduction, the authors suggested that the assembly will be a reference for entire *Albinaria* snails. Although the Cretan *Albinaria* snails are closely related (even though they should not be monophyletic [Schilthuizen et al., 2004, https://doi.org/10.1111/j.1095-8312.2004.00391.x]), it is unclear why this assembly will be the reference for the entire *Albinaria* snails?

The reasons of the identification should be provided. Metadata of reference assemblies are very important, and species identification may be the most fundamental. As such, the identification should be verified by readers. I suppose that "... based on expert identification" is not enough descriptive. The images are valuable, but the reason for the identification is quite helpful as well.

The generated assembly size is highly deviated from the estimate based on ancestral taxa. It is advisable to estimate genome size using genomescope. Given the sequenced data seems enough and the assembly is highly completed, the ancestral taxa-based estimate sounds weird.

In the abstract, the authors stated that 31 pseudo-chromosomal scaffolds were obtained.

However, the assembly consists of 43 scaffolds. Why were 12 scaffolds discarded? I was not able to find any reasons. I realize that the prior estimate of chromosome numbers is 31, but again, it is unclear whether this estimate is reliable. In fact, the prior estimate for genome size was highly deviated from that of obtained assembly.

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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, evolutionary biology, land snails

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.

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Srivarshini Saravanakumar
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This study presents a draft reference genome for the clausiliid land snail genus *Albinaria*, using *Albinaria teres* as the focal species. The genus is characterized by exceptional morphological variability, genetic differentiation, extreme geographic structuring, and a large number of

described subspecies, many of which are narrowly endemic.

The stated objective is to generate genomic resources that support evolutionary, taxonomic, and conservation research in a group that has so far been studied mainly using morphology and limited molecular markers. However, the manuscript does not clearly situate the sampled individuals within the current taxonomic framework of *Albinaria*, particularly at the subspecies level, which limits assessment of how representative the reference genome is for the genus.

The rationale for producing a reference genome for *Albinaria* requires clearer articulation. Although the lack of genomic resources for clausiliid snails is noted, the manuscript does not specify which biological or taxonomic questions cannot be addressed using existing mitochondrial or reduced marker datasets. Genome-scale data could be directly linked to unresolved issues such as species and subspecies delimitation, rapid diversification, or historical gene flow, but these connections are not explicitly developed.

The manuscript also does not demonstrate how the absence of a reference genome currently constrains conservation planning or biodiversity assessment, nor does it identify concrete applications of genomic data in this context.

The choice of *Albinaria teres* as a representative species for the genus is insufficiently justified. The manuscript does not explain whether this species occupies an especially phylogenetically informative position, is likely to exhibit genomic characteristics that are typical of the genus, or rather, was selected primarily for practical reasons such as availability or DNA quality. Given the high levels of divergence reported among *Albinaria* species and subspecies, it is necessary to justify the assumption that a single genome of *Albinaria teres* can function as a broadly applicable reference. A discussion of expected genomic divergence within the genus and its implications for read mapping, annotation transfer, and comparative analyses is required.

Several methodological gaps further limit the interpretability of the study. First, the sampling strategy is not clearly described in relation to the pronounced taxonomic and geographic structuring of *Albinaria*, which is critical given the fine-scale differentiation known within the genus. This lack of context makes it difficult to evaluate how representative the sampled individuals are for genome assembly and downstream comparative analyses.

In addition, genome size estimation is based on assumptions derived from ancestral or generalized gastropod taxa rather than on empirical measurements. Studies of other gastropods, such as *Potamopyrgus antipodarum*, demonstrate that genome size can deviate substantially from ancestral expectations due to lineage-specific processes, including polyploidy, genome expansion, and structural variation (e.g., McElroy et al. 2021; Fields et al., 2024; Jalinsky et al., 2025; Neiman et al, 2025). These examples illustrate why extrapolating genome size from ancestral taxa is unreliable and underscore the need for direct estimation in *Albinaria*. Given that short-read sequencing data are already available, a k-mer–based genome size estimation would provide an empirical and taxon-specific benchmark (e.g. as outlined in recent methodological work on k-mer approaches; https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2024.1451730/full), rather than relying on indirect assumptions.

Empirical genome size estimation would also be particularly valuable for assessing reference

genome quality. The expected genome size inferred from ancestral taxa is substantially larger than the reported scaffold length, raising the possibility of unresolved haplotypic duplication or incomplete assembly. Without an independent genome size estimate, it is difficult to distinguish between true biological variation and assembly artefacts. Similarly, chromosome number and genome architecture are inferred from ancestral or generalized gastropod patterns without validation for *Albinaria*. Direct estimates of chromosome number would help determine whether the current scaffolds approach chromosome-scale assemblies or represent a more fragmented genome. Finally, clearer reporting of which individuals and tissues were used for long-read, short-read, and RNA sequencing would substantially improve transparency and reproducibility, and would allow readers to better track how different data types contributed to the final assembly and annotation.

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

Partly

Are the protocols appropriate and is the work technically sound?

Partly

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: evolution of sexual reproduction, Potamopyrgus antipodarum

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.