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## GENOME NOTE

# Two draft genomes of enigmatic Solenogastres (Mollusca, Aplacophora) *Epimenia babai* and *Neomenia megatraperazata* [version 1; peer review: 1 approved, 1 approved with reservations]

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## Abstract

### Abstract\*

Many molluscan genomes have been published to date, however only three are from representatives of the subphylum Aculifera (Polyplacophora, Caudofoveata, and Solenogastres), the sister taxon to all other molluscs. Currently, genomic resources are completely lacking for Solenogastres. This gap in knowledge hinders comparative and evolutionary studies. Here, we sequenced the genomes of the solenogaster aplacophorans *Epimenia babai* Salvini-Plawen, 1997 and *Neomenia megatraperazata* Salvini-Plawen & Paar-Gausch, 2004 using a hybrid approach combining Oxford Nanopore and Illumina reads. For *E. babai*, we produced a 628 Mbp haploid assembly (N50 = 413 Kbp, L50 = 370) that is rather complete with a BUSCO completeness score of 90.1% (82.0% single, 8.1% duplicated, 6.0% fragmented, and 3.9% missing). For *N. megatraperazata*, we produced a 412 Mbp haploid assembly (N50 = 132 Kbp, L50 = 881) that is also rather complete with a BUSCO completeness score of 85.1% (81.7% single, 3.4% duplicated, 8.1% fragmented, and 6.8% missing). Our annotation pipeline predicted 25,393 gene models for *E. babai* with a BUSCO score of

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Approval Status ? ✓

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92.4% (80.5% single, 11.9% duplicated, 4.9% fragmented, and 2.7% missing) and 22,463 gene models for *N. megatrapezata* with a BUSCO score of 90.2% (81.0% single, 9.2% duplicated, 4.7% fragmented, and 5.1% missing). Phylogenomic analysis recovered Solenogastres as the sister taxon to Polyplacophora and Aculifera as the sister taxon to all other sampled molluscs with maximal support. These represent the first whole-genome resources for Solenogastres and will be valuable for future studies investigating this understudied group and molluscan evolution as a whole.

## Keywords

Aculifera, Aplacophora, Solenogastres, genome

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## Introduction

With their incredible diversity in morphology, life history and ecology, molluscs are of great interest to diverse fields of biology ranging from ecology to evolutionary developmental biology to biomechanics. To date there are high-quality genomes available for six of the eight molluscan classes publicly available, although sampling is heavily biased toward the economically and ecologically important members of the clade Conchifera (e.g. Gastropoda, Bivalvia and Cephalopoda; reviewed by [Gomes-dos-Santos \*et al.\* 2020](#) and [Sigwart \*et al.\* 2021](#)). As the sister taxon to all other molluscs, the clade Aculifera (Polyplacophora + Aplacophora) is important to our understanding of molluscan evolution and innovation. Despite their importance, only three aculiferan genomes have been published to date, two species of chitons (Polyplacophora; [Varney \*et al.\* 2021](#); [Varney and Yap-Chiongco \*et al.\* 2022](#)) and one species of Caudofoveata ([Wang \*et al.\* 2024](#)). Therefore, we sequenced the genomes of two species of Solenogastres – *Neomenia megatrapezata* [Salvini-Plawen & Paar-Gausch 2004](#) and *Epimenia babai* [Salvini-Plawen, 1997](#) – to facilitate further study of this evolutionarily important taxon.

The two clades of aplacophoran molluscs are united by their distinctive vermiform shape, loss or reduction of the foot, and a chitinous cuticle covered by calcareous integumental spines and scales (sclerites). Solenogastres are distinctive with the presence of a ciliated foot restricted to a ventral groove, an undifferentiated gut, and the lack of true ctenidia, while members of Caudofoveata lack a foot entirely and have the presence of a specialized oral shield used for burrowing in mud ([Todt 2013](#)). The current taxonomy of Solenogastres divides the group into 24 families and four orders (Pholidoskepia, Cavibelonia, Sterrofustia, and Neomeniamorpha) although recent phylogenomic analyses have indicated the need for significant taxonomic revision within this group ([Kocot \*et al.\* 2019](#); [Yap-Chiongco \*et al.\* 2024](#)).

Whereas most solenogasters are just a few millimeters in length ([Todt 2013](#)) both *N. megatrapezata* and *E. babai* are large enough to obtain high-quality genomic and transcriptomic data from a single individual. *E. babai* is a species of Cavibelonia that is relatively easily collected at SCUBA-accessible depths where it feeds on the soft coral *Scleronephthya gracillima* ([Okusu 2002](#)). Because of its relatively large-body size (up to 20 cm in length) and reliable spawning within the laboratory, *E. babai* remains one of very few species of Solenogastres in which development has been described ([Baba 1938, 1940, 1951](#); [Okusu, 2002](#); [Todt and Wanninger 2010](#)). Sequencing of its genome coupled with characterized development and reliability of spawning make *E. babai* a desirable species to serve as a developmental model within Solenogastres. *N. megatrapezata* (up to 18 cm in length) is a species of Neomeniamorpha found in the Southern Ocean off Antarctica ([Salvini-Plawen & Paar-Gausch 2004](#); [Kocot \*et al.\* 2019](#)). *Neomenia* is interesting to study trait evolution within Solenogastres as species within this genus have a complex accessory genital apparatus and lack both ventral foregut glands and a radula ([García-Álvarez & Salvini-Plawen 2007](#)). Together, the sequencing of these two species expands our ability to explore solenogaster evolution and, more broadly, provides an important resource in identifying large-scale patterns in molluscan evolution.

## Methods

### Specimen collection

*Epimenia babai* was collected by Akiko Okusu as described in [Okusu \(2002\)](#). The specimen of *Neomenia megatrapezata* (collector number Ap227) used for genome and transcriptome sequencing was collected by Ken Halanych and Kevin Kocot on 3 February 2013 near the Ross Ice Shelf in Antarctica (NBP12-10 Station 22; 76° 59.8965' S, 175° 05.5920' W, 541 m depth) via Blake trawl and is deposited in the Alabama Museum of Natural History (ALMNH) under catalog number ALMNH:Inv:25736.

Additional solenogasters were used for transcriptome sequencing to provide evidence for genome annotation. A second individual of *N. megatrapezata* (Ap259) was collected by Ken Halanych and Kevin Kocot on 28 November 2013 near Recovitz Island, Antarctica (LMG13-12 Station 2; 64° 24.672' S, 61° 57.790' W, 664 m depth) via Blake trawl. A second individual from the same collection event has been deposited in ALMNH under catalog number ALMNH:Inv:25732 as a voucher. A specimen of *Neomenia* aff. *herwigi* (Ap20; same individual used to generate a 454 transcriptome by [Kocot \*et al.\* 2011](#); NCBI SRA accession number SRR108985) was collected by Ken Halanych on 6 December 2004 off Argentina (63°23' 03.0" S, 60° 03' 24.0" W, 277 m depth) via Blake trawl. A specimen of *Neomenia permagna* (Ap26) was collected on 15 May 2006 by Ken Halanych off Argentina (LMG06-05; 53°47' S, 60° 42' W; 170 m depth) via Blake trawl and is deposited in ALMNH under catalog number ALMNH:Inv:25733. A specimen of an unidentified species of *Epimenia* (possibly also *E. babai*) was collected on 29 June 2015 by Hiroshi Saito off Osezaki, Japan (55 m depth) by SCUBA diving and a tissue sample is deposited in ALMNH under catalog number ALMNH:Inv:25734.

### Extraction, library preparation, and sequencing

To produce short-read genomic data, DNA was extracted using the CTAB-phenol-chloroform method employed by Varney *et al.* (2021), which was based on Doyle & Doyle (1991). For *E. babai*, DNA was extracted from a piece of midbody tissue and sent to Iridium Genomics for Illumina TruSeq sequencing library preparation and 2 X 100 bp paired-end (PE) sequencing on an Illumina HiSeq X. For *N. megatrapezata*, DNA was extracted from hemolymph and sent to the NY Genome Center for Illumina TruSeq sequencing library preparation and sequencing on an Illumina HiSeq X.

To produce long-read data via Oxford Nanopore sequencing, a library for *N. megatrapezata* was produced from DNA extracted from haemolymph using a CTAB-phenol-chloroform method following Varney *et al.* (2021). For the three libraries for *E. babai* and the second library for *N. megatrapezata*, genomic DNA was extracted from cryo-preserved tissue using the EZNA Tissue DNA Kit (Omega Bio-tek) followed by cleaning and size selection for high-molecular-weight fragments with Ampure XP beads. Sequencing libraries were prepared with the LSK-109 ligation-based library preparation kit and sequenced in-house using R9.4.1 RevD flow cells on a GridION. Reads were base called with Guppy 4.0 and trimmed with PoreChop (Wick 2018) with the --discard\_middle flag.

New transcriptome data were generated from the same individual used for WGS for each species and from other individuals of the same or closely related species for genome annotation. For *E. babai*, *Epimania* sp., *Neomenia* aff. *herwigi*, and *Neomenia permagna*, RNA was extracted as described by Kocot *et al.* (2019). RNA concentration was measured using a Qubit 3.0 (Thermo Fisher) fluorometer with the RNA High Sensitivity kit, RNA purity was assessed by measuring the 260/280 nm absorbance ratio using a Nanodrop Lite (Thermo Fisher), and RNA integrity was evaluated using a 1% SB agarose gel. Complementary DNA (cDNA) synthesis was performed using 1 ng of total RNA using the Clontech SMART-Seq HT kit. An Illumina sequencing library was then prepared in house using the Nextera XT DNA Library Preparation Kit with 0.15 ng of cDNA. Final library size and concentration were assessed using an Agilent Fragment Analyzer with the NGS 1-6000 bp kit and sent to Psomagen (Cambridge, MA) for sequencing on an Illumina NovaSeq using a S4 flowcell with 2 X 150 bp PE reads. For *N. megatrapezata*, RNA was extracted from the individual used for genome sequencing (Ap227) and a second individual (Ap259) as described in Kocot *et al.* (2019) and sent to the High-Throughput Genomics Shared Resource of the Huntsman Cancer Institute at the University of Utah for Illumina TruSeq Stranded mRNA Sample Prep Kit with oligo (dT) selection. Sequencing was performed on the Illumina HiSeq 2500 system with 2 X 125 bp PE reads.

### Genome assembly and annotation

Genome size and heterozygosity were estimated based on trimmed reads using GenomeScope2 (Ranallo-Benavidez *et al.* 2020) with a k-mer of 21. Hybrid genome assembly was performed with MaSuRCA 3.3.5 (Zimin *et al.* 2017). Recommended settings for eukaryotes with >20X Illumina coverage were used. Genome quality was assessed following assembly (and after each step involving filtering or polishing the genome assembly; see below) with QUAST 5.0.2 (Mikheenko *et al.* 2018) and completeness with BUSCO 5.2.2 (Manni *et al.* 2021) using the Metazoa odb\_10 dataset and the "--long" flag. We then removed redundant haplotigs with Redundans (Pryszcz & Gabaldón 2016) using the flags --noscaffolding --norearrangements. Finally, the remaining scaffolds were polished with four rounds of Pilon 1.23 using the Illumina paired-end reads, which were first quality- and adapter-trimmed with trimmomatic 1.8.0 (Bolger *et al.* 2014) using "ILLUMINACLIP:adapters.fasta:2:30:10 LEADING 10 TRAILING 10 SLIDINGWINDOW:4:15 MINLEN:50" for *N. megatrapezata* and "ILLUMINACLIP:adapters.fasta:3:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:20 MINLEN:50" for *E. babai*.

For genome annotation, we followed a bioinformatic pipeline that masked repetitive DNA and leveraged both RNA-seq data from that species and protein sequences from diverse aplacophorans as evidence for gene modeling. Repeats in the final assemblies were annotated and softmasked with RepeatMasker using a custom repeat database generated with RepeatModeler (Smit & Hubley 2015). For RepeatModeler, a maximum genome sample size of 1M and the --LTRStruct option were used. For RepeatMasker, the slow and gccalc options were used. The engine used for both programs was rmbblast. Available aplacophoran and select other mollusc proteomes (see data on Figshare for details) were then aligned to the final genome assemblies with ProtHint 2.6 (Brřna *et al.* 2020) with an e-value cutoff of 1e-25. We ran TrimGalore (Krueger *et al.* 2021) on the transcriptome reads with the following settings: "-q 30 --illumina --trim-n --length 50." The trimmed and filtered transcriptome reads were then mapped to the genome using STAR 2.4.0k (Dobin *et al.* 2013) with "--genomeChrBinNbits 15 --chimSegmentMin 50." Annotation of protein-coding genes was performed with BRAKER 2.1.6 (Brřna *et al.* 2021) using the output of ProtHint and STAR with the following settings: "--eptmode --softmasking --crf."

## Phylogenomic analysis

Homologous protein sequences in our newly sequenced solenogaster genomes and the proteomes of 21 other lophotrochozoans, including 16 other molluscs, two annelids, one brachiopod, one phoronid, and one nemertean were used as input for orthology inference using *OrthoFinder* 2.4.0 (Emms & Kelly 2019). We then further refined the output of *OrthoFinder* to construct a matrix of one-to-one orthologs following the pipeline of Varney and Yap-Chiongco *et al.* (2022) except we retained only genes sampled for 18/23 taxa using *PhyloPyPruner* (<https://gitlab.com/fethalen/phylopypruner>). Maximum likelihood phylogenetic analysis was employed on the concatenated supermatrix of amino acid sequences in IQ-Tree 2.1.3 (Minh *et al.* 2020) using the best-fitting model for each partition (-m MFP) with 1000 rapid bootstraps. The tree was arbitrarily rooted with all non-molluscan taxa. Notably, as the genome of *Chaetoderma* sp. (Wang *et al.* 2024) was released following the completion of this analysis, it was not included.

## Results

For *N. megatrapezata*, Illumina genome sequencing yielded 443.02 M reads. Illumina transcriptome sequencing of the same individual of *N. megatrapezata* used for genome sequencing (Ap227) yielded 29.72 M reads and 32.93 M reads for a second individual of *N. megatrapezata* (Ap259). Transcriptomes of other *Neomenia* species resulted in 110.68 M reads for *N. aff. herwigi* and 24.40 M reads for *N. permagna*. GenomeScope analysis estimated the genome size of *N. megatrapezata* to be 294 Mbp and a heterozygosity of 0.142% based on the trimmed PE reads. Following adapter trimming, two flowcells of Oxford Nanopore sequencing yielded 101.31 M reads. Assembly with MaSuRCA resulted in an assembly of 9,870 contigs totaling 347 Mbp (N50 = 119 Kbp, L50 = 1,041) with a BUSCO completeness score of 87.1% (83.9% single, 3.2% duplicated, 8.0% fragmented, and 4.9% missing). After polishing and purging redundant haplotigs, the final *N. megatrapezata* assembly was reduced to 6,168 contigs totaling 412 Mbp (N50 = 132 Kbp, L50 = 881) with a BUSCO completeness score of 85.1% (81.7% single, 3.4% duplicated, 8.1% fragmented, and 6.8% missing). BRAKER predicted 65,328 gene models with 90.6% of BUSCOs detected (80.5% single, 10.1% duplicated, 4.5% fragmented, and 4.9% missing). Removal of gene models not supported by transcriptome or protein evidence resulted in a final gene set for *N. megatrapezata* of 22,463 with a BUSCO completeness score of 90.2% (81.0% single, 9.2% duplicated, 4.7% fragmented, and 5.1% missing).

For *E. babai*, Illumina PE sequencing yielded 693.24 M reads. Illumina transcriptome sequencing of *E. babai* and *Epimenia* sp. yielded 70.06 M and 131.47 M reads, respectively. GenomeScope analysis of PE reads failed to converge for *E. babai* with k-mer sizes of 17 and 21. Therefore, we used the trimmed nanopore data in the program KMC 3 (Kocot *et al.* 2017) with the options -k21 -fm -t16 -m500 -ci1 -cs10000 to obtain k-mer statistics followed by kmc-tools with the transform option to create a histogram for input into GenomeScope2. Oxford Nanopore sequencing of three flowcells data yielded 14.79M reads post adapter trimming with PoreChop. The resulting analysis based on Nanopore data estimated a genome size for *E. babai* of 543 Mbp and heterozygosity of 1.16%. MaSuRCA yielded an assembly of 11,695 contigs totaling 572 Mbp (N50 = 271 Kbp, L50 = 618) with a BUSCO completeness score of 91.3% (77.7% single, 13.6% duplicated, 5.2% fragmented, and 3.5% missing). The final polished and purged *Epimenia* assembly was reduced to 5,965 contigs totaling 628 Mbp (N50 = 413 Kbp, L50 = 370) with a BUSCO completeness score of 90.1% (82.0% single, 8.1% duplicated, 6.0% fragmented, and 3.9% missing). BRAKER predicted 123,904 gene models with a BUSCO completeness score of 93.3% (80.9% single, 12.4% duplicated, 4.7% fragmented, and 2.0% missing). Removal of genes not supported by transcriptome or protein evidence resulted in a final gene set for *E. babai* of 25,393 with a BUSCO completeness score of 92.4% (80.5% single, 11.9% duplicated, 4.9% fragmented, and 2.7% missing).

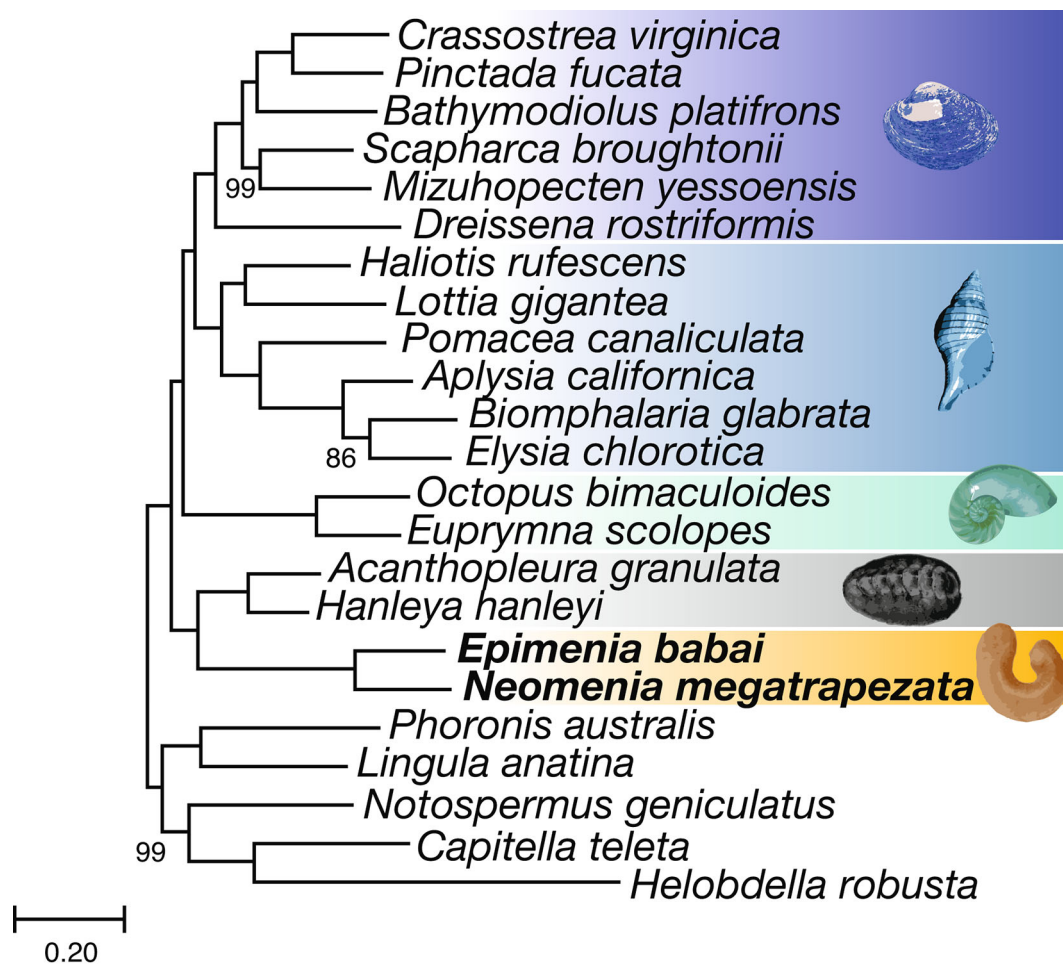
Molluscan genomes sequenced to date range in size from 359 Mbp (Simakov *et al.* 2013) to over 6 Gbp (Song *et al.* 2023) and genome size estimates by Adachi *et al.* (2021) for 141 diverse molluscs (Polyplacophora, Gastropoda, Bivalvia, and Cephalopoda) based on flow cytometry ranged from 469.4 Mbp (measured as a C-value of 0.48) to 5.31 Gbp (C-value of 5.43) with a mean size of 2.61 Gbp (C-value of 2.67) among the sampled molluscan taxa. Kocot *et al.* (2016) utilized Feulgen image analysis densitometry (FAID) to estimate genome sizes in select species of aplacophorans, including the same individual of *Epimenia babai* sequenced here. Genome size of *E. babai* was estimated to be 750 Mbp and that of *Neomenia permagna* was estimated to be 293 Mbp (Kocot *et al.* 2016). Genome size for *E. babai* inferred by GenomeScope based on Oxford Nanopore data is smaller than expected based on FIAD at 543 Mbp. However, we were unable to obtain an estimate based on more accurate PE data as the analysis failed to converge. The estimated genome size for *N. megatrapezata* of 294 Mbp inferred by GenomeScope is comparable to the FIAD estimate of 293 Mbp for the closely related species *N. permagna*. With final assembly sizes of 628 Mbp and 412 Mbp for *E. babai* and *N. megatrapezata*, respectively, solenogaster genomes are fairly small with respect to other molluscs. Based on computational and FAID genome size estimates, genome size within Aculifera is quite variable ranging from as small as 293 Mbp in *N. permagna* to 2.84 Gbp in *Cryptochiton stelleri* (Kocot *et al.* 2016; Hinegardner, 1974). Comparing the aplacophoran classes, the estimated genome size of *Chaetoderma* sp. (Caudofoveata) is quite large (2.45 Gbp) in comparison to both *E. babai* and *N. megatrapezata* (Table 1). Although in some groups it has been shown that genome



**Table 1.** Comparison of genome assembly statistics for available aculiferan genomes.

Species	Class	Size (Gbp)	N50 (Mbp)	BUSCO complete	# Genes	Citation
<i>Epimenia babai</i>	Solenogastres	0.63	0.413	90.1	25,393	This study
<i>Neomenia megatrapezata</i>	Solenogastres	0.41	0.132	85.10%	22,463	This study
<i>Chaetoderma</i> sp.	Caudofoveata	2.45	141.46	89.52%	23,675	Wang <i>et al.</i> 2024
<i>Acanthopleura granulata</i>	Polyplacophora	0.61	23.9	97.40%	81,691	Varney <i>et al.</i> 2021
<i>Hanleya hanleyi</i>	Polyplacophora	2.52	0.065	92.00%	69,284	Varney and Yap-Chiongco <i>et al.</i> 2022
<i>Liolophura japonica</i>	Polyplacophora	0.61	37.34	96.10%	28,010	Hui <i>et al.</i> 2024

size may co-evolve with life-history traits, the observed high level of variation in genome size within Aculifera raises questions about the underlying mechanisms driving this heterogeneity in genome size (Ritchie *et al.* 2017; Beaudreau *et al.* 2021). Further comparisons between solenogaster, caudofoveate, and chiton genomes will aid in our understanding



**Figure 1.** Phylogenetic analysis of Mollusca based on 3,577 nuclear protein-coding genes. Bootstrap support values below 100 are displayed at each node. Image of *Neomenia megatrapezata* was altered from a photo taken by Christoph Held.

of the evolutionary history of Aplacophora and Aculifera and the potential interplay between genome size and, e.g., life history.

Comparison of the full set of *Epimenia* and *Neomenia* gene models to the gene models from 21 other lophotrochozoans in OrthoFinder resulted in 251,468 groups of homologous sequences. Our pipeline selected 7,561 single-copy genes sampled for at least 18 of the 23 taxa. The final PhyloPyPruner output resulted in a supermatrix of 3,577 genes with a total of 1,169,375 amino acids and 20.3% missing data. Of these, *E. babai* was sampled for 2,319 and *N. megatrapezata* was sampled for 2,232. The resulting tree (Figure 1) is strongly supported overall with maximal support for Solenogastres and for Aculifera as the sister taxon to all other sampled molluscs, consistent with recent studies (e.g., Kocot *et al.* 2020; Song *et al.* 2023).

In summary, the genomes of *N. megatrapezata* and *E. babai* are relatively complete and comparable to other published aculiferan genomes (Table 1) with BUSCO completeness scores of 85.1% and 90.1%, respectively. These represent the first sequenced genomes for Solenogastres and are a valuable resource for future studies of molluscan evolution.

### Ethical considerations

Ethics permits were not required to undertake this research.

### Data availability

#### Underlying data

Associated raw data is available under NCBI Bioproject PRJNA1071799: <https://identifiers.org/bioproject:PRJNA1071799>

NCBI Sequence Read Archive (SRA): RNA-Seq of *Epimenia babai*. Accession number [SRX23536357](#)

NCBI SRA: RNA-Seq of *Epimenia* sp. Accession number [SRX23536362](#)

NCBI SRA: RNA-Seq of *Neomenia megatrapezata*. Accession numbers [SRX23536358](#) and [SRX23536359](#)

NCBI SRA: RNA-Seq of *Neomenia* aff. *herwigi*. Accession number [SRX23536361](#)

NCBI SRA: RNA-Seq of *Neomenia permagna*. Accession number [SRX23536360](#)

NCBI SRA: Illumina Sequencing of *Epimenia babai* gDNA. Accession numbers [SRX5511159](#) and [SRX6730477](#).

NCBI SRA: Illumina Sequencing of *Neomenia megatrapezata* gDNA. Accession number [SRX23536356](#)

NCBI SRA: GridION Sequencing of *Epimenia babai* gDNA. Accession number [SRX23536354](#).

NCBI SRA: GridION Sequencing of *Neomenia megatrapezata* gDNA. Accession number [SRX23536355](#)

### Extended data

Figshare: Additional tables with the list of proteomes used for gene annotation and the full BUSCO reports of gene models are available under the file: YapChiongco\_2024\_SupplementaryTables.doc. *Neomenia*\_Nanopore\_GenomeFiles.zip and *Epimenia*\_Nanopore\_GenomeFiles.zip each contain the content below for the respective species, <https://doi.org/10.6084/m9.figshare.25111997.v1> (Yap-Chiongco, 2024).

- 01\_GenomeScope
- 02\_MaSuRCA
- 03\_Redundans
- 04\_Pilon
- 05\_GenomeAssembly\_BUSCOandQuast

- 06\_RepeatModelerandMasker
- 07\_BRAKER
- 08\_AnnotationBUSCO

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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# Open Peer Review

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**Alice Dennis**

University of Namur, Namur, Belgium

The manuscript is very clear and well written. It presents assemblies of two new molluscan genomes, from the highly neglected clade *Aculifera*. These genomes are the first to be sequenced in the class *Solenogastres*. These two genomes are of good quality and are well constructed, using hybrid sequencing methods and assembly. The genomes have been annotated using appropriate models and same-species evidence. I found it interesting to see the comparison of genome size estimates based on Feulgen and kmer methods, next to the final assemblies. After annotation, single copy genes were extracted from both assemblies and well as a set of representative lophotrochozoans species. These were used to construct a phylogeny. This demonstrates the value of this genomic resource for future work in taxonomy and beyond.

I think this work is suitable for indexing as-is. The only place I might ask for a bit more clarification is in the annotation curation. I am curious as to why there might be so many gene models for *Epimania* (123,904). You have reduced this to a more reasonable 25,393 by only retaining genes with direct evidence. This is a good approach, but I worry that a few valid things have been excluded, and that this inflated number of genes perhaps reflects some important aspect of the genome (for example some sort of novel repetitive element).

**Are the rationale for sequencing the genome and the species significance clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a usable and accessible format, and the assembly and**

**annotation available in an appropriate subject-specific repository?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Evolutionary genetics, adaptive physiology, mollusc 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 22 May 2024

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**Gloria Arriagada**

Universidad Andres Bello, Santiago, Santiago Metropolitan Region, Chile

The article entitled “Two draft genomes of enigmatic Solenogastres (Mollusca, Aplacophora) *Epidemia babai* and *Neomenia megatrapezata*” described the genome sequencing of two mollusk form an underrepresented subphylum among the sequenced mollusks. They clearly explain the methodology used for the sequencing and assembling as well as its annotation. Although they provide access to all the information generated, the results are very succinct and only present the statistics and numbers, and do not explain or discuss anything about the annotated genes, as a reader and not been an expert in sequencing I missed that. It was not clear to me how many of the annotated genes are indeed transcribed, since they use transcriptomics also, how many are protein coding genes and where in the physiological or cellular processes are they most represented. Are there repetitive or mobile elements in these genomes? Which fraction of the total genome are they? Are they expressed? I believe a bit of more functional insight of this very relevant genomic information, will make this a more amicable paper for readers interested in both mollusk evolution and functional genomics.

**Are the rationale for sequencing the genome and the species significance clearly described?**

Yes

**Are the protocols appropriate and is the work technically sound?**

Yes

**Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a usable and accessible format, and the assembly and**

**annotation available in an appropriate subject-specific repository?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** virology, host-pathogen interaction, mobile elements, mollusk LTR-retrotransposons

**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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