GENOME NOTE

REVISED The genomes of the aquarium sponges *Tethya wilhelma* and *Tethya minuta* (Porifera: Demospongiae) [version 2; peer review: 2 approved]

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Abstract

Sponges (Phylum Porifera) are aquatic sessile metazoans found worldwide in marine and freshwater environments. They are significant in the animal tree of life as one of the earliest-branching metazoan lineages and as filter feeders play crucial ecological roles, particularly in coral reefs, but are susceptible to the effects of climate change. In the face of the current biodiversity crisis, genomic data is crucial for species conservation efforts and predicting their evolutionary potential in response to environmental changes. However, there is a limited availability of culturable sponge species with annotated high-quality genomes to further comprehensive insights into animal evolution, function, and their response to the ongoing global change. Despite the publication of a few high-quality annotated sponge genomes, there remains a gap in resources for culturable sponge species. To address this gap, we provide high quality draft genomes of the two congeneric aquarium species *Tethya wilhelma* and *Tethya minuta*, small ball-shaped demosponges that are easily maintained long-term in *ex situ* culture. As such, they offer promising opportunities as laboratory models to contribute to advancing our understanding of sponge biology and provide valuable resources for studying animal evolution, function, and responses to environmental challenges.

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Keywords

Tethya wilhelma, Tethya minuta, Porifera, Demospongiae, genome, model organism

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Introduction

Sponges (Phylum Porifera) are sessile aquatic metazoans that occur globally in marine and freshwater habitats. More than 9,600 valid species have been described, the majority of which (7,989 species) belong to Class Demospongiae.[1] Sponges hold a pivotal position in the animal tree of life as one of the earliest metazoan branching lineages, likely originating more than 650 million years ago,[2] but their exact phylogenetic position is still disputed.[3–6] As filter feeders, sponges are ecologically important, especially in coral reefs,[7,8] but are also impacted by climate change, as they bleach due to elevated seawater temperatures.[9,10] In the current biodiversity crisis, genome data is valuable to aid species conservation[11] and genomic data can be used not only to understand evolution and development,[12] but also to predict a species evolutionary potential to adapt to changing environmental conditions due to climate change.[13] To improve the understanding of their response to changing environmental conditions, the availability of culturable sponge species with annotated high-quality genomes is important, but only a few sponge species meet both these criteria yet.

Although the first sponge genome was published in 2010 from the Australian demosponge *Amphimedon queenslandica,*[14] only a handful of annotated high-quality sponge genomes have been published and analysed since then, for example from the freshwater demosponge *Ephydatia muelleri*[15] or the reef-building glass sponge (Class Hexactinellida) *Aphrocallistes vastus.*[16] However, only *A. queenslandica* and *E. muelleri* are culturable yet under controlled conditions. This lack of high-quality genome resources available from culturable sponges hinders a full appreciation of our understanding of animal evolution and function as well as the response of sponges as ecological key players in many aquatic ecosystems to the current climate crisis.

To contribute to filling this gap, we here provide high-quality draft genomes of the two aquarium species *Tethya wilhelma* and *Tethya minuta*. These two congeners are small ball-shaped demosponges that were described in 2001 from public aquaria in Germany.[17] Due to their long-term culturability, they are a laboratory model for many topics, including multicellularity, early-animal evolution, biomineralization, and even cancer (e.g., Refs. 18–25). With the provision of novel high-quality genome data of these two species we aim to enhance the use of these species as valuable sponge model systems.

Methods
Specimens sampled
Specimens of *Tethya wilhelma* and *Tethya minuta* were obtained from the marine research aquaria of the Chair of Paleontology and Geobiology of the Department of Earth and Environmental Sciences at the Ludwig-Maximilians-Universität München (Germany), where they are cultured since about 2010. No permits were needed for the sampling and processing. Voucher specimens are deposited in the Bavarian State Collection for Paleontology and Geology (SNSB-BSPG) under accession numbers SNSB-BSPG.GW33333 (*T. wilhelma*) and SNSB-BSPG.GW41624 (*T. minuta*).

DNA extraction
For both species, genomic DNA was extracted from either fresh or frozen tissue with a modified cetyltrimethylammonium bromide (CTAB; Carl Roth, Germany, Cat. Nr. 9161.1) extraction. The modification concerned the addition of Potassium acetate (KOAc, Sigma-Aldrich, Germany, Cat. Nr. 791733) in step no. 5 of the protocol.[26] Short DNA fragments were removed using AMPure XP (Beckman Coulter, USA, Cat. Nr. A63881) beads to select for long DNA fragments. DNA quantity and quality were controlled on a Nanodrop 1100 and using 1.5% agarose (Biozym, Germany, Cat. Nr. 840004) gels before library preparation as required for the different sequencing platforms used.

Genome assembly: *Tethya wilhelma*
For *Tethya wilhelma*, we took the genome draft (*T. wilhelma*-v1) published by our group in 2017[20] as a starting point and used new sequence data and bioinformatics to further improve it. Additional data was obtained using Hi-C[27] and Chicago (Dovetail Genomics[28]) libraries. For this, four whole sponges were frozen in liquid nitrogen and shipped to Dovetail

Table 1. Sequencing libraries of *Tethya wilhelma* and *Tethya minuta*. All data can be accessed through the European Nucleotide Archive (ENA) https://www.ebi.ac.uk/ena/browser/view/.

Species	Library	Platform	Type	Reads	Total bp	Accession
T. wilhelma	GW33333	MiniSeq	RNA-paired	28.8 M	4.4 Gb	ERR10048047
T. wilhelma	Twa2013	Illumina HiSeq 1500	RNA-paired strand specific	100.7 M	25.2 Gb	SRR4255675
T. wilhelma	Twa03-2014-02-03	Illumina HiSeq 2000	DNA-paired	129.8 M	26 Gb	SRR2163223
T. wilhelma	Tethya_MP	Illumina HiSeq 2000	DNA-paired	140.4 M	35.1 Gb	SRR2296844
T. wilhelma	tetwilh1	Moleculo	DNA long reads	125,150	436.7 Mb	SRR5369934
T. wilhelma	Dovetail	HiC	DNA	421.4 M	126 Gb	ERR12769028
T. wilhelma	Dovetail	Chicago	DNA	430.6 M	129 Gb	ERR12769029
T. wilhelma	GW33333	Nanopore	DNA long reads	131,953	2.8 Gb	ERR12769349
T. wilhelma	GW33333	10X	DNA	388.47 M	5.4 Gb	ERR12771414
T. minuta	GW41624	Nanopore PromethION	DNA long reads	6.39 M	12.73 Gb	ERR12771470
T. minuta	GW41624	Nanopore MinION	DNA long reads	545,492	2.17 Gb	ERR12771471
T. minuta	GW41624	Illumina HiSeq 1500	DNA-paired	42 M	4.2 Gb	ERR12771519
T. minuta	GW41624	Illumina HiSeq 1500	RNA-paired	274 M	13.7 Gb	ERR12771518

Table 2. *Tethya wilhelma* assembly statistics. The final version without bacterial scaffolds (Twi-v4-nb) is highlighted in bold.

Version	# scaffolds	Total size (Mb)	N50 (Mb)	N gaps (kb)
Twi-v1	5936	125.67	0.073	1516
Twi-v2	1353	139.49	5.5	2252.2
Twi-v3	967	138.92	6.1	1069.9
Twi-v4	891	138.93	6.7	1077.5
Twi-v4-no_bacteria	**557**	**126.1**	**6.7**	**1030.7**

Genomics (Scotts Valley, CA) for library preparation and sequencing. The resulting Chicago/Hi-C reads were processed using Dovetail's proprietary software HiRise.[28] After Chicago/Hi-C scaffolding, the assembly (dubbed *T. wilhelma*-v2) had 1,353 scaffolds, totaling 139 Mb, with an N50 of 5.5 Mb.

While some chromosome-sized scaffolds were evident in *T. wilhelma*'s-v2 assembly, many putative chromosomes remained fragmented. Therefore, we tried to improve the assembly's contiguity by adding Moleculo long reads as well as Nanopore long reads, the latter derived from a single run of an Oxford Nanopore MinION (see Table 1). The data was assembled using the programs "SSPACE_Long_Read v1-1"[29] and "GapCloser v.1.12".[30] This assembly version, called Twi-v3, contained 967 scaffolds, totaling 138.92 Mb, with an N50 of 6.1 Mb (see Table 2).

For the assembly of *T. wilhelma*'s genome v4, high molecular weight DNA was extracted, and quality was assessed using a Nanodrop 1100. Fragment size was controlled on a 1.5% agarose gel and an Agilent 2200 TapeStation. Libraries for 10X Genomics (Pleasanton, CA) were generated and sequenced at the University of Potsdam, in collaboration with the group of Prof. M. Hofreiter (Evolutionary Adaptive Genomics, University of Potsdam, Germany), on an Illumina Nextseq500. About 390 M reads were obtained (Table 1) and assembled using the 10X-Genomics software "Supernova 2.1.1".[31] These assembled contigs were then used for scaffolding the *T. wilhelma* v3 assembly using "SSPACE-LongRead v1-1"[29] and then with "P_RNA_scaffolder"[32] using 100.7 M PE (125 bp) and 237 M RNA reads (25.2 Gb). Finally, we used hicstuff 2.3.0[33] and the Hi-C data available to create a contact map of the *T. wilhelma* assembly.

Table 3. *Tethya minuta* **assembly statistics. The final version without bacterial scaffolds (Tmi-v4-bin17) is highlighted in bold.**

Version	# scaffolds	Total size (Mb)	N50 (kb)	N gaps (kb)
Tmi-v3	1043	139.0	788.3	887.0
Tmi-v4-no_bacteria	**244**	**86.07**	**969.3**	**534.5**

This assembly, called Twi-v4, had 891 scaffolds with a total size of 138.9 Mb and an N50 of 6.7 Mb, which also included bacterial scaffolds (see Table 2 and below).

Genome assembly: *Tethya minuta*

For the assembly of *Tethya minuta*, DNA of a single specimen of *Tethya minuta* (sample# GW41624) was extracted with CTAB[26] and sequenced twice, using Oxford Nanopore PromethION (12.73 Gb of long reads) and MinION[34] (2.17 Gb of long reads). Additionally, we Illumina-sequenced 27 Mbp paired-end (100 PE and 150 PE). These data were assembled with wtdbg2,[35] and polished using minimap2.[36] SSPACE_LongRead[29] was used with the available nanopore data to scaffold the assembly. Finally, we used GapCloser 1.12[30] and the available PE reads to close gaps in the assembly which then had a length of 139 Mb and consisted of 1,043 scaffolds (Tmi-v4), but still included bacterial contigs (see Table 3).

Identification of bacterial scaffolds

From earlier versions of the genome, it was clear that *Tethya wilhelma* harbours two associated bacteria, both alphaproteobacteria, with an unknown interaction. With the relatively large scaffolds in the assembly, a clear split was seen in GC content and read mapping coverage (Figure 1). Consequently, we separated all scaffolds with GC content under 47% and defined those as sponge. The remaining scaffolds for the two bacteria were binned using "MetaBAT v2.15-25",[37] with default parameters. For *T. wilhelma*, this yielded 6 bins (see public data repository at Ref. 38 or https://github.com/PalMuc/2Tethya_genomes/tree/main/03-bacteria) with 1 bin corresponding to a single Rhizobiales species (genome appx 7.5 Mb), and the other 5 bins corresponding to a Roseobacter species (genome appx 4.8 Mb). These scaffolds were removed from the final *Tethya wilhelma* genome version. This assembly, called Twi-v4-no_bacteria, had 557 scaffolds with a total size of 126.1 Mb and an N50 of 6.7 Mb (Table 2; see Ref. 38 or https://github.com/PalMuc/2Tethya_genomes/tree/main/06-FINAL_Assemblies ENA accession GCA_964030475).

For *T. minuta*, we used "MetaBAT v2.15-25"[37] to identify and separate bacterial contigs from sponge scaffolds, which had produced 23 bins from the assembly. One of the bins, numbered as bin-17, contained the bulk of the assembly, and
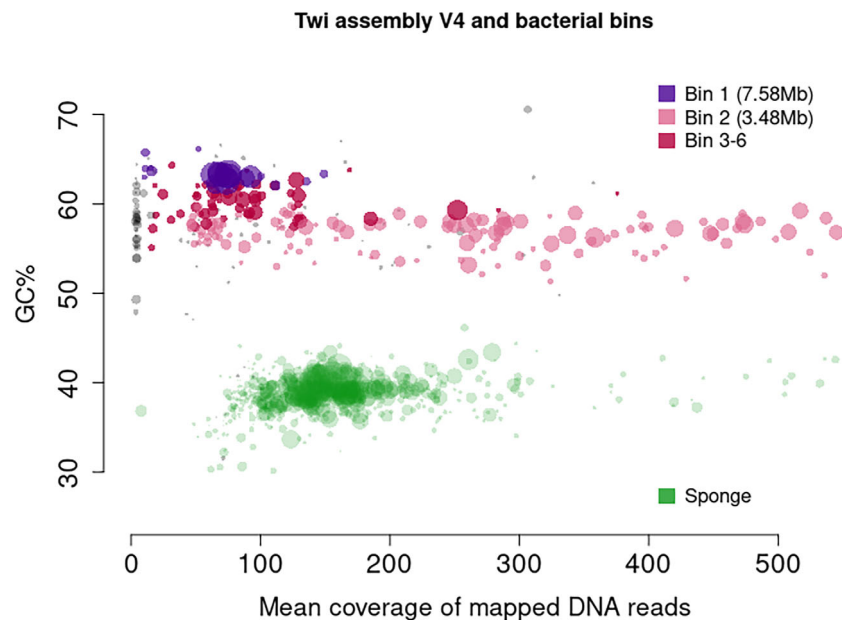

Figure 1. Coverage of mapped DNA reads plotted against GC content of scaffolds of *T. wilhelma* (Twi-v4). MetaBAT v2.15-25 was used for binning. Unidentified scaffolds are shown in grey.
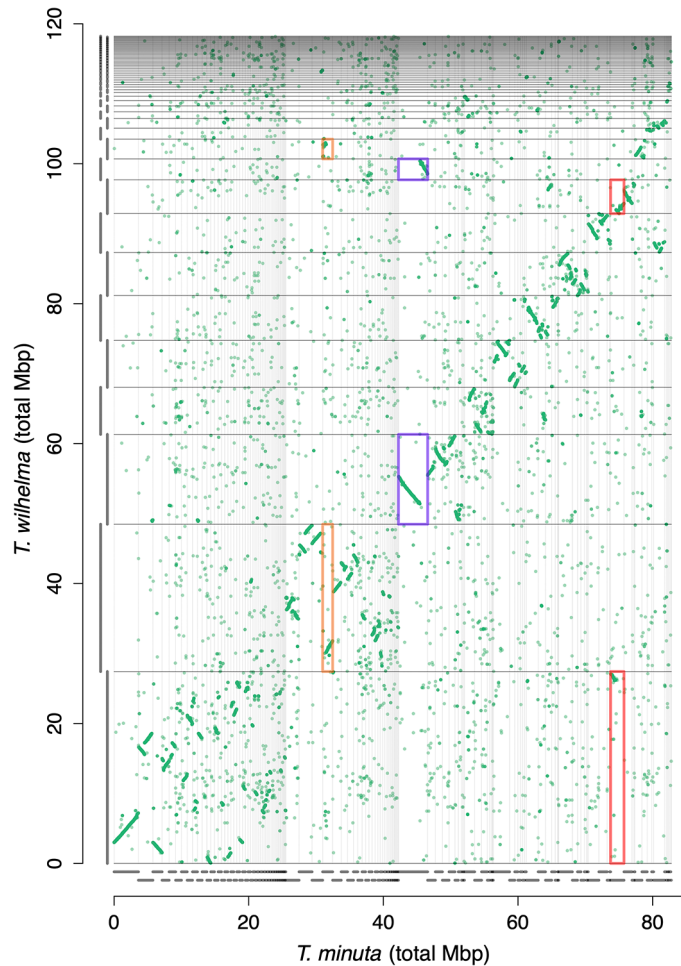
Figure 2. Synteny plot of *Tethya wilhelma* versus *Tethya minuta*. Each point represents a homologous gene between the two species. Bars parallel to each axis show the scaffold size. Scaffolds are arranged from longest to shortest in *T. wilhelma*, with the scaffolds in *T. minuta* sorted to match the *T. wilhelma* scaffold with the most homologs. Several rearrangements are evident, some are shown in the orange, blue, and red boxes, which could result from translocations, inversions on currently incomplete scaffolds, or misassemblies.

was identified as originating from the sponge due to the GC content of 38.3% and substantial RNAseq mapping. This bin (here now called Tmi-v4-no_bacteria, Table 3) had 244 scaffolds with a total size of 86.07 Mb, around 40 Mb smaller than the assembly of *T. wilhelma* (Table 2, see Ref. 38 or https://github.com/PalMuc/2Tethya_genomes/tree/main/06-F-INAL_Assemblies; ENA accession GCA_964030485). Nearly all of the large chromosomal pieces in *T. wilhelma* had matching pieces among the scaffolds of *T. minuta*, as evident on the synteny plot (Figure 2), which suggested that the assembly of *T. minuta* was smaller not because of missing scaffolds or mis-assemblies, but merely from a smaller genome.

RNA extraction, sequencing, and assembly

RNA was extracted from fresh tissue of *Tethya minuta* using TRIzol (Fisher Scientific, Germany, Cat. Nr. 12034977) and chloroform (Carl Roth, Germany, Cat. Nr. 3313.1) precipitation[39] with subsequent quality control on a Bioanalyzer 2100. Libraries were prepared and sequenced twice using one third of a lane of an Illumina HiSeq1500 (100 bp and 50 bp) at the LMU GeneCenter, yielding 137 M read pairs. Reads were assembled *de novo* using Trinity,[40] using default parameters, resulting in an assembly of 151,079 contigs with an average length of 677 bp. This assembly was also used as a training set for *de novo* gene prediction (see below). Transcriptome sequencing and assembly of *Tethya wilhelma* has been described in Francis et al.[20] Statistics of the different sequencing libraries of *Tethya wilhelma* and *Tethya minuta* are given in Table 1.

Table 4. *Tethya wilhelma* (Twi-v4) and *Tethya minuta* (Tmi-v4) (assemblies without bacteria) BUSCO-values for lineage dataset metazoa_odb10.

BUSCO results	Twi-v4-no_bacteria	Tmi-v4-no_bacteria
Total query BUSCOs	954	954
Complete BUSCOs (C)	800 (83.6%)	552 (57.9%)
Complete and single-copy (S)	776 (81.3%)	498 (52.2%)
Complete and duplicated (D)	24 (2.5%)	54 (5.7%)
Fragmented (F)	63 (6.6%)	186 (19.5%)
Missing (M)	91 (9.5%)	216 (22.6%)

Gene annotation

Both *Tethya* species were annotated using AUGUSTUS. For *T. wilhelma*, we used the BRAKER v2.0 pipeline,[41] with the options `--useexisting --species=Tethya_wilh` and including mapped RNA. This predicted a total of 28,113 gene models, which were used for downstream analysis.

For *Tethya minuta*, the assembly Tmi-v4-no_bacteria and the *de novo* Trinity assembly were used as inputs for WebAUGUSTUS.[42] This yielded 22,779 gene models and 33,041 genes (see files 'hints_pred' and 'hints_UTR_pred' at Ref. 38 or https://github.com/PalMuc/2Tethya_genomes/tree/main/05-annotation/tethya_minuta_augustus).

Results

The final version of the *Tethya wilhelma* draft genome assembly (see Table 2, Twi-v4-no_bacteria) without bacterial scaffolds has 557 scaffolds, a length of 126.1 MB, an N50 of 6.7 MB, and contains 1030.7 kb gaps (Ns). The final version of the *Tethya minuta* draft genome assembly (see Table 3, Tmi-v4-no_bacteria) without bacterial scaffolds has 244 scaffolds, a length of 86.07 MB, an N50 of 969.3 kb, and contains 534.5 kb gaps (Ns). BUSCO values for the two assemblies are given in Table 4.

Ethical considerations

For work with sponges (Porifera) no ethical clearing is needed.

Data availability

Raw reads are available from the European Nucleotide Archive under bioproject numbers PRJNA288690, PRJEB53671, for individual accession numbers see Table 1.

The assembled genomes are also available in the European Nucleotide Archive: *Tethya wilhelma* GCA_964030475, *Tethya minuta* GCA_964030485.

Further data on the genome assemblies, including analytical pipelines and scripts, is available in the public repository https://github.com/PalMuc/2Tethya_genomes, archived at Zenodo (https://zenodo.org/doi/10.5281/zenodo.10991740).[38]

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC BY-SA 4.0 DEED) (https://creativecommons.org/licenses/by-sa/4.0/).

Voucher specimens are deposited in the Bavarian State Collection for Paleontology and Geology (SNSB-BSPG, https://bspg.snsb.de) under accession numbers SNSB-BSPG.GW33333 (*T. wilhelma*) and SNSB-BSPG.GW41624 (*T. minuta*).

References

1. de Voogd NJ, Alvarez B, Boury-Esnault N, *et al.*: *World Porifera Database.* World Porifera Database; 2024 [cited 2024 Mar 20].
Reference Source

2. Dohrmann M, Wörheide G: **Dating early animal evolution using phylogenomic data.** *Sci. Rep.* 2017 Jun 15; **7**(1): 3599.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

3. Juravel K, Porras L, Höhna S, *et al.*: **Exploring genome gene content and morphological analysis to test recalcitrant nodes in the animal phylogeny.** *PLoS One.* 2023 Mar 23; **18**(3): e0282444.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

4. Schultz DT, Haddock SHD, Bredeson JV, *et al.*: **Ancient gene linkages support ctenophores as sister to other animals.** *Nature.* 2023 May 17; **618**: 110–117.
Publisher Full Text

5. Telford MJ, Moroz LL, Halanych KM: **Evolution: A sisterly dispute.** *Nature.* 2016 Jan 21; **529**(7586): 286–287.
PubMed Abstract | **Publisher Full Text**

6. Redmond AK, McLysaght A: **Evidence for sponges as sister to all other animals from partitioned phylogenomics with mixture models and recoding.** *Nat. Commun.* 2021 Mar 19; **12**(1): 1–14.
Publisher Full Text

7. De Goeij JM, van Oevelen D, Vermeij MJA, *et al.*: **Surviving in a aarine desert: The Sponge Loop retains resources within coral reefs.** *Science.* 2013 Oct 3; **342**(6154): 108–110.
PubMed Abstract | **Publisher Full Text**

8. Olinger LK, McClenaghan B, Hajibabaei M, *et al.*: **Looking for the sponge loop: analyses of detritus on a Caribbean forereef using stable isotope and eDNA metabarcoding techniques.** *PeerJ.* 2024 Feb 23; **12**: e16970.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

9. Bell JJ, Smith RO, Micaroni V, *et al.*: **Marine heat waves drive bleaching and necrosis of temperate sponges.** *Curr. Biol.* 2023 Jan 9; **33**(1): 158–163.e2.
PubMed Abstract | **Publisher Full Text**

10. Perkins NR, Monk J, Soler G, *et al.*: **Bleaching in sponges on temperate mesophotic reefs observed following marine heatwave events.** *Clim. Change Ecol.* 2022 Dec 1; **3**: 100046.
Publisher Full Text

11. Theissinger K, Fernandes C, Formenti G, *et al.*: **How genomics can help biodiversity conservation.** *Trends Genet.* 2023 Jul; **39**(7): 545–559.
Publisher Full Text

12. Renard E, Leys SP, Wörheide G, *et al.*: **Understanding animal evolution: The added value of sponge transcriptomics and genomics: The disconnect between gene content and body plan evolution.** *Bioessays.* 2018 Sep; **40**(9): e1700237.
PubMed Abstract | **Publisher Full Text**

13. Waldvogel AM, Feldmeyer B, Rolshausen G, *et al.*: **Evolutionary genomics can improve prediction of species' responses to climate change.** *Evol. Lett.* 2020 Feb; **4**(1): 4–18.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

14. Srivastava M, Simakov O, Chapman J, *et al.*: **The *Amphimedon queenslandica* genome and the evolution of animal complexity.** *Nature.* 2010 Jan 1; **466**(7307): 720–726.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

15. Kenny NJ, Francis WR, Rivera-Vicéns RE, *et al.*: **Tracing animal genomic evolution with the chromosomal-level assembly of the freshwater sponge *Ephydatia muelleri*.** *Nat. Commun.* 2020 Jul 27; **11**(1): 1–11.
Publisher Full Text

16. Francis WR, Eitel M, Vargas S, *et al.*: **The genome of the reef-building glass sponge *Aphrocallistes vastus* provides insights into silica biomineralization.** *R. Soc. Open Sci.* 2023 Jun; **10**(6): 230423.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

17. Sará M, Sará A, Nickel M, *et al.*: **Three new species of *Tethya* (Porifera: Demospongiae) from German Aquaria.** *Stuttg Beitr Naturkd.* 2001 Jan 1; **631**: 1–16.

18. Mills DB, Francis WR, Vargas S, *et al.*: **The last common ancestor of animals lacked the HIF pathway and respired in low-oxygen environments.** *elife.* 2018 Feb 6; **7**: e31176.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

19. Flensburg SB, Garm A, Funch P: **The contraction-expansion behaviour in the demosponge *Tethya wilhelma* is light-controlled and follows a diurnal rhythm.** *J. Exp. Biol.* 2022 Dec 7; **225**(24): jeb244751.
PubMed Abstract | **Publisher Full Text**

20. Francis W, Eitel M, Vargas S, *et al.*: **The genome of the contractile demosponge *Tethya wilhelma* and the evolution of metazoan neural signalling pathways.** *bioRxiv.* 2017; p. 120998.
Publisher Full Text

21. Ellwanger K, Nickel M: **Neuroactive substances specifically modulate rhythmic body contractions in the nerveless metazoon *Tethya wilhelma* (Demospongiae, Porifera).** *Front. Zool.* 2006 Apr 27; **3**: 7.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

22. Rivera AS, Hammel JU, Haen KM, *et al.*: **RNA interference in marine and freshwater sponges: actin knockdown in *Tethya wilhelma* and *Ephydatia muelleri* by ingested dsRNA expressing bacteria.** *BMC Biotechnol.* 2011 Jan 1; **11**(1): 67.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

23. Hammel JU, Herzen J, Beckmann F, *et al.*: **Sponge budding is a spatiotemporal morphological patterning process: Insights from synchrotron radiation-based x-ray microtomography into the asexual reproduction of *Tethya wilhelma*.** *Front. Zool.* 2009 Jan 1; **6**(1): 19.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

24. Ellwanger K, Eich A, Nickel M: **GABA and glutamate specifically induce contractions in the sponge *Tethya wilhelma*.** *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 2007 Jan 1; **193**(1): 1–11.
PubMed Abstract | **Publisher Full Text**

25. Fortunato A, Taylor J, Scirone J, *et al.*: **Sponges are highly resistant to radiation exposure and cancer.** bioRxiv. 2021 [cited 2024 Mar 20]; p. 2021.03.17.435910.
Publisher Full Text

26. Vargas S, Caglar C, Büttner G, *et al.*: **Slime away: a simple CTAB-based high molecular weight DNA and RNA extraction protocol for "difficult" invertebrates.** 2021 Jul 26 [cited 2022 Dec 23];
Reference Source

27. Belton JM, McCord RP, Gibcus JH, *et al.*: **Hi-C: a comprehensive technique to capture the conformation of genomes.** *Methods.* 2012 Nov; **58**(3): 268–276.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

28. Putnam NH, O'Connell BL, Stites JC, *et al.*: **Chromosome-scale shotgun assembly using an in vitro method for long-range linkage.** *Genome Res.* 2016 Mar; **26**(3): 342–350.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

29. Boetzer M, Pirovano W: **SSPACE-LongRead: scaffolding bacterial draft genomes using long read sequence information.** *BMC Bioinform.* 2014 Jun 20; **15**: 211.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

30. Luo R, Liu B, Xie Y, *et al.*: **SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler.** *GigaScience.* 2012 Dec 27; **1**(1): 18.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

31. Weisenfeld NI, Kumar V, Shah P, *et al.*: **Direct determination of diploid genome sequences.** *Genome Res.* 2017 May; **27**(5): 757–767.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

32. Zhu BH, Xiao J, Xue W, *et al.*: **P_RNA_scaffolder: a fast and accurate genome scaffolder using paired-end RNA-sequencing reads.** *BMC Genomics.* 2018 Mar 2; **19**(1): 175.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

33. Matthey-Doret C, Baudry L, Cournac A, *et al.*: **koszullab/hicstuff: Use miniconda layer for docker and improved P(s) normalisation.** 2020.
Reference Source

34. Jain M, Olsen HE, Paten B, *et al.*: **The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community.** *Genome Biol.* 2016 Nov 25; **17**(1): 239.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

35. Ruan J, Li H: **Fast and accurate long-read assembly with wtdbg2.** *Nat. Methods.* 2019 Dec 9; **17**: 155–158.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

36. Li H: **Minimap2: pairwise alignment for nucleotide sequences.** *Bioinformatics.* 2018 Sep 15; **34**(18): 3094–3100.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

37. Kang DD, Li F, Kirton E, *et al.*: **MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies.** *PeerJ.* 2019 Jul 26; **7**: e7359.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

38. Wörheide G, Francis W: **PalMuc/2Tethya_genomes. Dataset for: The genomes of the aquarium sponges *Tethya wilhelma* and *Tethya minuta* (Porifera: Demospongiae).** 2024 [cited 2024 May 15].
Reference Source

39. Rio DC, Ares M Jr, Hannon GJ, *et al.*: **Purification of RNA using TRIzol (TRI reagent).** *Cold Spring Harb. Protoc.* 2010 Jun; **2010**(6): pdb.

prot5439.
Publisher Full Text

40. Haas BJ, Papanicolaou A, Yassour M, *et al.*: **De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis.** *Nat. Protoc.* 2013 Aug; **8**(8): 1494–1512.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

41. Brůna T, Hoff KJ, Lomsadze A, *et al*.: **BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database.** *NAR Genom. Bioinform.* 2021 Mar; **3**(1): lqaa108.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

42. Hoff KJ, Stanke M: **WebAUGUSTUS—a web service for training AUGUSTUS and predicting genes in eukaryotes.** *Nucleic Acids Res.* 2013 May 21; **41**(W1): W123–W128.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 26 August 2024

https://doi.org/10.5256/f1000research.169477.r309948

✔ **Bernie Degnan**

School of the Environment, University of Queensland, Queensland, Australia

The responses and the revision looks fine. The revised figure is greatly improved.

Competing Interests: No competing interests were disclosed.

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

Version 1

Reviewer Report 13 July 2024

https://doi.org/10.5256/f1000research.165438.r295815

✔ **Eric Bautista-Guerrero** [iD]

Laboratorio de Ecología Marina, Centro de Investigaciones Costeras, Centro Universitario de la Costa, University of Guadalajara, Guadalajara, Jalisco, Mexico

Comments to the Author

This note represents a significant contribution to the field of sponge biology, advancing our understanding of this organism and providing valuable metagenomic resources. It proposes the use of two species of sponge that can be easily maintained long-term in ex situ culture. The

sponges in question are proposed as model systems for studying the responses of this invertebrate to the effects of climate change.

Furthermore, the sample set is remarkable, and the sequencing and bioinformatics techniques used to improve the draft genome are noteworthy.

Overall, the Genome note presents a high-quality bioinformatic analysis, and it is recommended that it be accepted pending consideration of minor revisions as detailed below.

Keywords

1. First line– Please use italics for the following: Tethya wilhelma, Tethya minuta.

Methods

2. DNA extraction: it would be helpful if a brief explanation the modifications to the CTAB method.

3. In the sentence: "Fragment size was controlled on a 1.5 agarose gel"…., add the percentage should be added after 1.5 (1.5%).

4. Figure 2: Axes of the graph, scientific name of the sponges in italics.

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: I'm an expert in integrative taxonomy and molecular phylogeny of marine sponges. I believe that the genome of these two sponges will contribute significantly to our understanding of how this group of invertebrates responds to climate change.

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 28 Jul 2024

Gert Wörheide

Dear Professor Bautista-Guerrero,
Thank you very much for your constructive comments on our manuscript to which we respond point by point below:

1. First line (Keywords) – Please use italics for the following: Tethya wilhelma, Tethya minuta.
Authors reply: Thanks, correct. However, in the Word File I downloaded for the revision the species names in the keywords are correctly formatted in italics, so this must be a typesetting issue on publisher's side. I will alert them to the fact.

2. DNA extraction: it would be helpful if a brief explanation the modifications to the CTAB method.
Author's reply: Thank for this comment, although the modifications are detailed in the protocol (citation 26) at protocols.io we have now included the following sentence:

The modification concerned the addition of Potassium acetate (KOAc, Sigma-Aldrich, Germany, Cat. Nr. 791733) in step no. 5 of the protocol [26].

3. In the sentence: "Fragment size was controlled on a 1.5 agarose gel"…., add the percentage should be added after 1.5 (1.5%).
Author's reply: Thanks for picking this up, we have added the % after 1.5

4. Figure 2: Axes of the graph, scientific name of the sponges in italics.
Author's reply: Thanks, and sorry for this oversight, this has now been corrected in the revised Figure 2 (see reply to comment 3 by Bernie Degnan (Reviewer 1).

Competing Interests: no competing interests

Reviewer Report 12 July 2024

https://doi.org/10.5256/f1000research.165438.r295816

? **Bernie Degnan**
School of the Environment, University of Queensland, Queensland, Australia

Woerheide et al. provide a strong rationale for the sequencing, assembly and analysis of the genomes of two congeneric demosponges, *Tethya wilhelma* and *T. minuta,* both which are readily found in public marine aquaria. Using a combination long and short read sequencing and Dovetail HiC/Chicago sequencing, the authors substantially improved the original *T. wilhelma* genome

assembly. The *T. minuta* assembly, based on long and short reads only, is of sufficient quality for comparison with *T. wilhelma* genome.

Suggested revisions: 1.The authors should include details about how the sponge DNA was procured by Dovetail for HiC/Chicago as this could be useful for the sequencing of other sponge and marine invertebrate genomes. 2. Several of the accession numbers in Table 1 can not be traced in NCBI - please update. 3. The synteny plot comparing the two *Tethya* genomes (Fig. 2) is difficult to interpret and should be improved/reordered to allow better direct comparison of congener scaffolds.

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?
Partly

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: Marine biology, sponges, genomes, transcriptomes

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 28 Jul 2024
Gert Wörheide

Dear Professor Degnan,
thank you very much for your constructive comments on our manuscript to which we respond point by point below:

1. The authors should include details about how the sponge DNA was procured by Dovetail for HiC/Chicago as this could be useful for the sequencing of other sponge and marine invertebrate genomes.
Authors reply: Unfortunately, we do not have information how DNA was procured by Dovetail. As stated, we sent them four whole sponges and they extracted chromatin for Chicago and Hi-C according to their proprietary protocols. We then only received the data, without any information on wet-bench protocols. We contacted Dovetail in this matter but

have yet to receive a reply.

2. Several of the accession numbers in Table 1 can not be traced in NCBI - please update.
Authors reply: The accession number can all be traced in the European Nucleotide Archive (ENA), to which the data was submitted (we double checked all entries). We have, unfortunately, no influence on the timing of data exchange between partners in the International Nucleotide Sequence Database Collaboration (INSDC), where both ENA and NCBI participate.
We have now included the direct link to the ENA browser to ease access.
https://www.ebi.ac.uk/ena/browser/view/

3. The synteny plot comparing the two Tethya genomes (Fig. 2) is difficult to interpret and should be improved/reordered to allow better direct comparison of congener scaffolds.
Authors reply: Thank you very much for this great suggestion, we have now reordered the plot and highlight some rearrangements in boxes in the plot. We also now provide a much more detailed explanation in the figure caption, i.e.,
Figure 2: Synteny plot of *Tethya wilhelma* versus *Tethya minuta*. Each point represents a homologous gene between the two species. Bars parallel to each axis show the scaffold size. Scaffolds are arranged from longest to shortest in *T. wilhelma*, with the scaffolds in *T. minuta* sorted to match the *T. wilhelma* scaffold with the most homologs. Several rearrangements are evident, some are shown in the orange, blue, and red boxes, which could result from translocations, inversions on currently incomplete scaffolds, or misassemblies.

Competing Interests: no competing interests