

DATA NOTE

The genome sequence of the long-spined sea scorpion,

Taurulus bubalis (Euphrasén, 1786) [version 1; peer review: 2

approved]

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Abstract

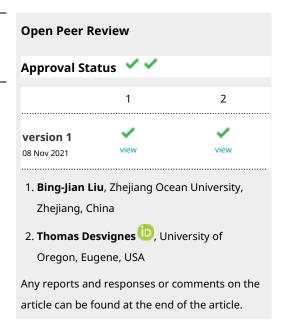
We present a genome assembly from an individual female *Taurulus bubalis* (the long-spined sea scorpion; Chordata; Actinopteri; Perciformes; Cottidae). The genome sequence is 615 megabases in span. The complete assembly is scaffolded into 21 chromosomal pseudomolecules.

Keywords

Taurulus bubalis, long-spined sea scorpion, genome sequence, chromosomal



This article is included in the Tree of Life gateway.



Corresponding author: Darwin Tree of Life Consortium (mark.blaxter@sanger.ac.uk)

Author roles: Potter S: Writing - Original Draft Preparation, Writing - Review & Editing;

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Species taxonomy

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Neoteleostei; Acanthomorphata; Eupercaria; Perciformes; Cottioidei; Cottales; Cottidae; Taurulus; *Taurulus bubalis* (Euphrasén, 1786) (NCBI:txid61643).

Background

The long-spined sea scorpion (Taurulus bubalis, Perciformes: Cottidae), also known as the longspined bullhead or fatherlasher, is named for the distinctive long spine found on its cheek (above the pectoral fin and behind the eye). It is a rocky shore fish found throughout western European waters and around all coasts of Britain and Ireland, from the shore to around 30 m depth. They are also occasionally seen in the Mediterranean. Adult fish can reach around 20 cm long and have a broad head with a large mouth. They are sometimes confused with the bull rout, Myoxocephalus scorpius (also known as the shortspined scorpion fish), but adult bull rout are much larger and lack the distinctive cheek spine (Neal, 2008). Long-spined sea scorpions are ambush predators with cryptic coloration and have a varied diet, primarily focused on crustaceans, but molluscs, fish and polychaetes are also consumed. Most prey are swallowed whole. Individuals grow rapidly during the first two years of life, and may begin to spawn during their second year. All individuals will have spawned by their third year, with spawning occurring between December and March (King & Fives, 1983).

The long-spined sea scorpion has a notable behavioural response to changing environmental conditions: when oxygen tension is lower, individuals will emerge from the water and climb onto the land where they breathe air. They are not, however, very mobile once they have emerged (Davenport & Woolmington, 1981). The long-spined sea scorpion has been classified as "Least Concern" on the IUCN red list (Lorance *et al.*, 2014).

Genome sequence report

The genome was sequenced from one *T. bubalis* of unknown sex collected from Farland Point, Great Cumbrae, North Ayrshire, UK (latitude 55.746815, longitude -4.914907) (Figure 1). A total of 38-fold coverage in Pacific Biosciences single-molecule long reads and 51-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 161 missing/misjoins and removed 6 haplotypic duplications, reducing the assembly length by 0.28% and the scaffold number by 86.10%, and increasing the scaffold N50 by 31.71%.

The final assembly has a total length of 615 Mb in 26 sequence scaffolds with a scaffold N50 of 29.1 Mb (Table 1). The complete assembly sequence was assigned to 21 chromosomal-level scaffolds, representing 21 autosomes (numbered by sequence length) (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.1.2 (Manni *et al.*, 2021) completeness of 98.4% (single 97.6%, duplicated 0.8%) using the actinopterygii_odb10 reference set. While not fully phased, the assembly deposited is of

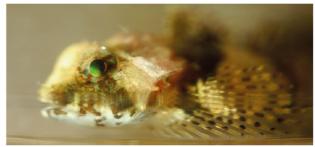






Figure 1. Images of the fTauBub2 specimen taken prior to preservation and processing. The bottom image includes the FluidX storage tubes, length 43.9 mm, for scale.

one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Methods

Sample acquisition and nucleic acid extraction

A single *T. bubalis* of unknown sex (fTaubBub2) was collected by hand from Farland Point, Great Cumbrae, North Ayrshire, UK (latitude 55.746815, longitude -4.914907) by Richard Durbin, University of Cambridge/Wellcome Sanger Institute. The sample was identified by the same individual and preserved on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The fTauBub2 sample was weighed and

Table 1. Genome data for Taurulus bubalis, fTauBub2.1.

Project accession data		
Assembly identifier	fTauBub2.1	
Species	Taurulus bubalis	
Specimen	fTauBub2	
NCBI taxonomy ID	NCBI:txid61643	
BioProject	PRJEB45118	
BioSample ID	SAMEA7522994	
Isolate information	Unknown sex, muscle (genome assembly), fin (RNA-Seq), gill (Hi-C)	
Raw data accessions		
PacificBiosciences SEQUEL II	ERR6412369	
10X Genomics Illumina	ERR6054766-ERR6054769	
Hi-C Illumina	ERR6054770	
Illumina polyA RNA-Seq	ERR6286720	
Genome assembly		
Assembly accession	GCA_910589615.1	
Accession of alternate haplotype	GCA_910589325.1	
Span (Mb)	615	
Number of contigs	268	
Contig N50 length (Mb)	13.0	
Number of scaffolds	26	
Scaffold N50 length (Mb)	22.1	
Longest scaffold (Mb)	50.1	
BUSCO* genome score	C:98.4%[S:97.6%,D:0.8%],F:0.5%, M:1.1%,n:3640	

^{*}BUSCO scores based on the actinopterygii_odb10 BUSCO set using v5.1.2. C= complete [S= single copy, D=duplicated], F=fragmented, M=missing, n=number of orthologues in comparison. A full set of BUSCO scores is available at https://blobtoolkit.genomehubs.org/view/fTauBub2.1/dataset/CAJUUV01/busco.

dissected on dry ice with tissue set aside for Hi-C and RNA sequencing. Muscle tissue was cryogenically disrupted to a fine powder using a Covaris cryoPREP Automated Dry Pulveriser, receiving multiple impacts. Fragment size analysis of 0.01–0.5 ng of DNA was then performed using an Agilent FemtoPulse. High molecular weight (HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. Low molecular weight DNA was removed from a 200-ng aliquot of extracted DNA using 0.8X AMpure XP purification kit prior to 10X Chromium sequencing; a minimum of 50 ng DNA was submitted for 10X sequencing. HMW DNA was sheared into an average fragment size between 12–20 kb in a Megaruptor 3 system with speed setting 30. Sheared DNA was purified by solid-phase reversible

immobilisation using AMPure PB beads with a 1.8X ratio of beads to sample to remove the shorter fragments and concentrate the DNA sample. The concentration of the sheared and purified DNA was assessed using a Nanodrop spectrophotometer and Qubit Fluorometer and Qubit dsDNA High Sensitivity Assay kit. Fragment size distribution was evaluated by running the sample on the FemtoPulse system.

RNA was extracted from fin tissue in the Tree of Life Laboratory at the WSI using TRIzol (Invitrogen), according to the manufacturer's instructions. RNA was then eluted in 50 μ l RNAse-free water and its concentration assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA

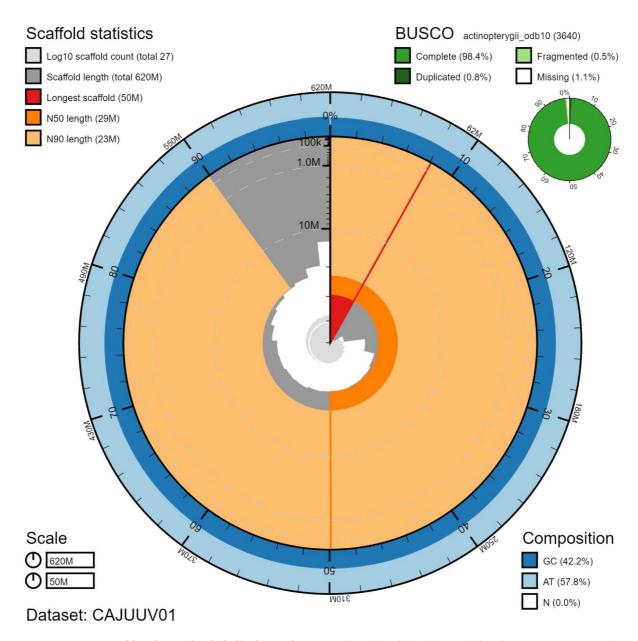


Figure 2. Genome assembly of *Taurulus bubalis*, **fTauBub2.1: metrics.** The BlobToolKit Snailplot shows N50 metrics and BUSCO gene completeness. The main plot is divided into 1,000 size-ordered bins around the circumference with each bin representing 0.1% of the 615,153,901 bp assembly. The distribution of chromosome lengths is shown in dark grey with the plot radius scaled to the longest chromosome present in the assembly (50,132,026 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (29,138,660 and 22,724,154 bp), respectively. The pale grey spiral shows the cumulative chromosome 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 in the actinopterygii_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/fTauBub2.1/dataset/CAJUUV01/snail.

Broad-Range (BR) Assay kit. Analysis of the integrity of the RNA was done using Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Sequencing

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud sequencing libraries were constructed

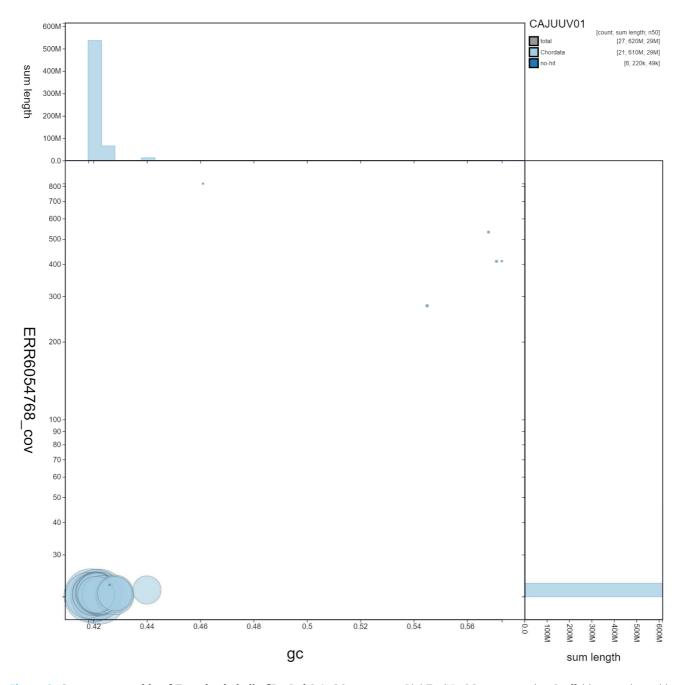


Figure 3. Genome assembly of *Taurulus bubalis,* **fTauBub2.1: GC coverage.** BlobToolKit GC-coverage plot. Scaffolds are coloured by phylum. Circles are sized in proportion to scaffold length Histograms show the distribution of scaffold length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/fTauBub2.1/dataset/CAJUUV01/blob.

according to the manufacturers' instructions. Poly(A) RNA-Seq libraries were constructed using the NEB Ultra II RNA Library Prep kit. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq X (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were

generated from gill tissue using the Arima v2 Hi-C kit and sequenced on HiSeq X.

Genome assembly

Assembly was carried out with Hifiasm (Cheng et al., 2021); haplotypic duplication was identified and removed with

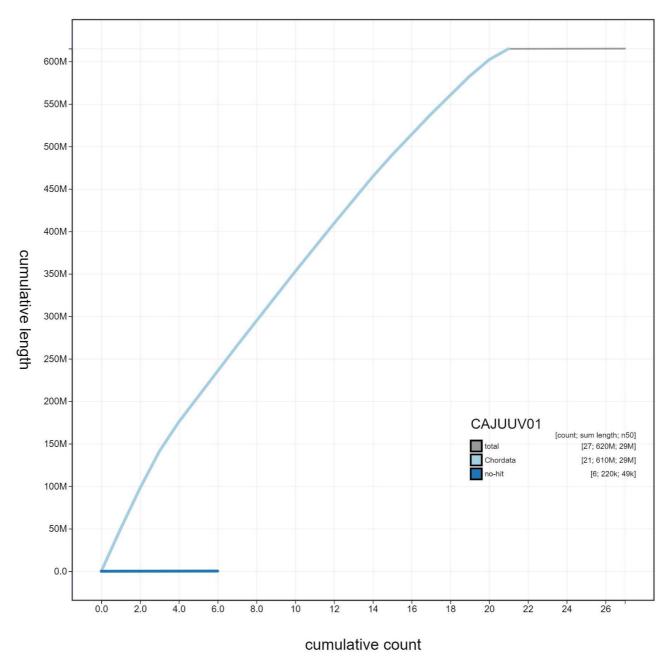


Figure 4. Genome assembly of *Taurulus bubalis***, fTauBub2.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all scaffolds. Coloured lines show cumulative lengths of scaffolds assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/fTauBub2.1/dataset/CAJUUV01/cumulative.

purge_dups (Guan et al., 2020). One round of polishing was performed by aligning 10X Genomics read data to the assembly with longranger align, calling variants with freebayes (Garrison & Marth, 2012). The assembly was then scaffolded with Hi-C data (Rao et al., 2014) using SALSA2 (Ghurye et al., 2019). The assembly was checked for contamination and corrected using the gEVAL system (Chow et al., 2016) as described previously

(Howe *et al.*, 2021). Manual curation (Howe *et al.*, 2021) was performed using gEVAL, HiGlass (Kerpedjiev *et al.*, 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva *et al.*, 2021). The genome was analysed and BUSCO scores generated within the BlobToolKit environment (Challis *et al.*, 2020). Table 3 contains a list of all software tool versions used, where appropriate.

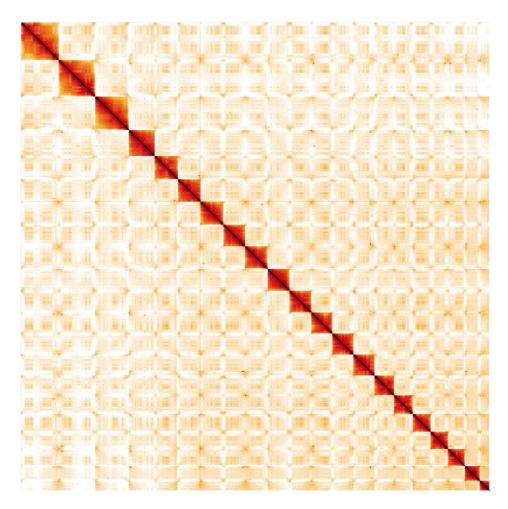


Figure 5. Genome assembly of *Taurulus bubalis***, fTauBub2.1: Hi-C contact map.** Hi-C contact map of the fTauBub2.1 assembly, visualised in HiGlass.

Table 2. Chromosomal pseudomolecules in the genome assembly of *Taurulus bubalis*, fTauBub2.1.

INSDC accession	Chromosome	Size (Mb)	GC%
OU342711.1	1	50.13	42.2
OU342712.1	2	47.70	41.9
OU342713.1	3	43.50	42.1
OU342714.1	4	34.46	41.8
OU342715.1	5	30.39	42.1
OU342716.1	6	29.76	42.1
OU342717.1	7	29.55	42.2
OU342718.1	8	29.27	42
OU342719.1	9	29.14	42.1
OU342720.1	10	28.94	42

INSDC accession	Chromosome	Size (Mb)	GC%
OU342721.1	11	28.35	42.2
OU342722.1	12	28.17	42.3
OU342723.1	13	27.68	42.2
OU342724.1	14	27.45	42.1
OU342725.1	15	25.62	42.1
OU342726.1	16	24.36	42.3
OU342727.1	17	23.58	42.8
OU342728.1	18	22.72	42.8
OU342729.1	19	22.07	42.1
OU342730.1	20	19.29	42.8
OU342731.1	21	12.81	44
OU342732.1	MT	0.02	46.2
-	Unplaced	0.20	54.8

Table 3. Software tools used.

Software tool	Version	Source
Hifiasm	0.12	(Cheng et al., 2021)
purge_dups	1.2.3	Guan et al., 2020
SALSA2	2.2	Ghurye <i>et al.</i> , 2019
longranger align	2.2.2	https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines
freebayes	1.3.1-17-gaa2ace8	Garrison & Marth, 2012
MitoHiFi	1.0	(Uliano-Silva et al., 2021)
gEVAL	N/A	Chow et al., 2016
HiGlass	1.11.6	(Kerpedjiev et al., 2018)
PretextView	0.1.x	https://github.com/wtsi-hpag/PretextView
BlobToolKit	2.6.2	Challis et al., 2020

Ethics/compliance issues

The materials that have contributed to this genome note have been supplied by a Darwin Tree of Life Partner. The submission of materials by a Darwin Tree of Life Partner is subject to the Darwin Tree of Life Project Sampling Code of Practice. By agreeing with and signing up to the Sampling Code of Practice, the Darwin Tree of Life Partner agrees they will meet the legal and ethical requirements and standards set out within this document in respect of all samples acquired for, and supplied to, the Darwin Tree of Life Project. Each transfer of samples is further undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Darwin Tree of Life Partner, Genome Research Limited (operating as the Wellcome Sanger Institute), and in some circumstances other Darwin Tree of Life collaborators.

Data availability

European Nucleotide Archive: Taurulus bubalis (long-spined sea scorpion). Accession number PRJEB45118; https://identifiers.org/ena.embl/PRJEB45118.

The genome sequence is released openly for reuse. The *T. bubalis* genome sequencing initiative is part of the Darwin Tree of Life (DToL) project. All raw sequence data and the assembly

have been deposited in INSDC databases. The genome will be annotated using the RNA-Seq data and presented through the Ensembl pipeline at the European Bioinformatics Institute. Raw data and assembly accession identifiers are reported in Table 1.

Author information

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.4893704.

Members of the Wellcome Sanger Institute Tree of Life programme collective are listed here: https://doi.org/10.5281/zenodo.5377053.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.4790456.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.5013542.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.4783559.

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Version 1

Reviewer Report 21 October 2022

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Thomas Desvignes 🗓



In this article, the authors report on the publicly-available, chromosome-scale genome assembly of the longspined bullhead *Taurulus bubalis*, a marine sculpin from western Europe. The assembly appears to be of great quality using appropriate methods and following the high standard of the Darwin Tree of Life Project. I have only minor comments.

In the abstract the sequenced specimen is referred to as a female, but the first sentence of the genome sequence report and of the methods state that the specimen was of unknown sex. What is the accurate situation? Knowing the sex of the individual would obviously be beneficial.

Is there any information on the karyotype of *T. bubalis* or closely related species to support that the 21 pseudochromosome molecules obtained is the expected number of chromosomes?

I am confused by the statement that the 21 chromosomal-level scaffolds represent "21 autosomes"? What about potential sex chromosomes? Is there any information supporting the hypothesis that the species' sex determination mechanism is environmental? Or is it instead that the analysis of the two haplotypes did not reveal obvious differences in any chromosome pairs? In which case, if the species has a genetically determined sex, the potential sex chromosomes would be homomorphic, although still not autosomes, I believe.

According to Fishbase, "longspined bullhead" is the FAO-accepted common name for *Taurulus bubalis* while "longspined sea-scorpion" is a vernacular name from Ireland and the UK. Please verify and if appropriate replace "sea-scorpion" by "bullhead", also note that there is apparently no hyphen in "longspined".

Similarly, based on Fishbase, the FAO-accepted common name for *Myoxocephalus scorpius* is "shorthorn sculpin" while "bull-rout" is considered a vernacular name.

In the background section, the mentioned spines are preopercular spines. It might be worth simply adding this word because these preopercular spines represent a distinctive character in

sculpins.

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: Fish evolutionary biology.

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 20 September 2022

https://doi.org/10.21956/wellcomeopenres.19189.r52365

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Bing-Jian Liu

National Engineering Laboratory of Marine Germplasm Resources Exploration and Utilization, Zhejiang Ocean University, Zhejiang, China

In this study, the authors have generated a chromosome-scale genome assembly of the long-spined sea scorpion, and the protocols for the genome assembly are well chosen and robust. The Hi-C contact heat map and BUSCO results are quite good, showing a high-quality reference genome.

I notice that the gender of the sample was unknown. Why not determine the gender of the sample? In my opinion, gender is a key piece of information for a genome. Of course, I know very little about the biological characteristics of *Taurulus bubalis*. So if the comment is not appropriate, please ignore it.

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

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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: population genetics/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.