The genome sequence of *Tenthredo notha* Klug, 1814, a sawfly [version 2; peer review: 2 approved]

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**Abstract**

We present a genome assembly from an individual female *Tenthredo notha* (Arthropoda; Insecta; Hymenoptera; Tenthredinidae). The genome sequence is 253 megabases in span. Most of the assembly (99.91%) is scaffolded into 20 chromosomal pseudomolecules. The mitochondrial genome was also assembled and is 19.8 kilobases in length. Gene annotation of this assembly on Ensembl has identified 10,235 protein coding genes.

**Keywords**

*Tenthredo notha*, sawfly, genome sequence, chromosomal, Hymenoptera

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**Open Peer Review**

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| **version 2**   | ✓ | ✓ |
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1. Marko Prous, University of Tartu, Tartu, Estonia
2. Saskia Wutke, University or Eastern Finland, Joensuu, Finland

Any reports and responses or comments on the article can be found at the end of the article.
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Author roles: Falk S: Investigation, Resources; Broad GR: Writing – Original Draft Preparation;

Competing interests: No competing interests were disclosed.

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Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Hymenoptera; Tenthredinoidea; Tenthredinidae; Tenthredininae; Tenthredo; Tenthredo notha (Klug, 1814) (NCBI:txid362091).

Background
*Tenthredo notha* is a yellow- and black-striped sawfly, one of a group of several wasp-mimicking species. It can be separated from close relatives by details of the yellow markings on the abdomen and the black apex to the hind tibia (Benson, 1952; Fekete, 2018), although males can be tricky to identify. The green larvae (with a pale lateral stripe) feed on white clover (*Trifolium repens*) and tufted vetch (*Vicia cracca*); like the food plants, *T. notha* is widely distributed across Britain, although under-recorded. *Tenthredo notha* is found throughout Central, Northern and South-East Europe and Eastwards widely through Russia (Taeger, 1988) and China (Wei et al., 2006).

Flying mainly in July and August, the flight time helps distinguish *T. notha* from the very similar *Tenthredo arcuata* Forster, 1771, which is typically a spring flyer. As with other *Tenthredo* species, adults can often be found on flowers, particularly of Apiaceae.

As the first chromosome-level genome for the subfamily Tenthredininae, this will help with research into the diversification of this large group of often conspicuous sawflies, a particularly North temperate radiation. Previous limited sequence data for *Tenthredo notha* has been used in a study of tenthredinid phylogeny, concentrating on the large subfamily Nematinae (Nyman et al., 2006). There is still much work to do in reconstructing the phylogeny of Tenthredininae.

Genome sequence report
The genome was sequenced from a single *T. notha* (Figure 1) collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.769, longitude -1.339). A total of 77-fold coverage in Pacific Biosciences single-molecule long reads and 262-fold coverage in 10X Genomics read clouds were generated. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly curation corrected 91 missing/misjoins and removed 29 haplotypic duplications, reducing the assembly size by 4.90% and the scaffold number by 70.79%, and increasing the scaffold N50 by 22.36%.

The final assembly has a total length of 253 Mb in 26 sequence scaffolds with a scaffold N50 of 14.0 Mb (Table 1). Of the assembly sequence, 99.91% was assigned to 20 chromosomal-level
scaffolds (numbered by sequence length) (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.2.2 (Manni et al., 2021) completeness of 95.5% (single 94.9%, duplicated 0.6%) using the hymenoptera_odb10 reference set (n=5,991).

The assembly is that of a diploid female. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Metadata for specimens, barcode results, spectra estimates, sequencing runs, contaminants and pre-curation assembly statistics are given at https://links.tol.sanger.ac.uk/species/362091.

**Genome annotation report**

The iyTenNoth1.1 genome has been annotated using the Ensembl rapid annotation pipeline (Table 1; https://rapid.ensembl.org/Tenthredo_notha_GCA_914767705.1/Info/Index). The resulting

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**Figure 2. Genome assembly of Tenthredo notha, iyTenNoth1.2: 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 252,838,151 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 (24,575,054 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 chromosome lengths (13,966,764 and 8,019,486 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 hymenoptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/iyTenNoth1.2/dataset/CAJZBL02/snail.
annotation includes 16,290 transcribed mRNAs from 10,235 protein-coding and 1,484 non-coding genes. There are 1.44 coding transcripts per gene and 6.62 exons per transcript.

Methods
Sample acquisition and nucleic acid extraction
A female *T. notha* (specimen ID Otx000858, ToLID iyTenNoth1) was collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.769, longitude -1.339) from woodland by Steven Falk, Independent Researcher, using a net. The sample was formally identified by the same individual and snap-frozen on dry ice.

DNA was extracted at the Tree of Life laboratory, Wellcome Sanger Institute. The iyTenNoth1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing.
Thorax tissue was disrupted using a Nippi Powermasher fitted with a BioMasher pestle. 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

Figure 4. Genome assembly of *Tenthredo notha*, *iyTenNoth1.2*: 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/iyTenNoth1.2/dataset/CAJZBL02/cumulative](https://blobtoolkit.genomehubs.org/view/iyTenNoth1.2/dataset/CAJZBL02/cumulative).
Table 2. Chromosomal pseudomolecules in the genome assembly of *Tenthredo notha*, *iyTenNoth1.2*.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| OU611906.1      | 1          | 24.58     | 36.8|
| OU611907.1      | 2          | 22.78     | 37.2|
| OU611908.1      | 3          | 21.79     | 37.2|
| OU611909.1      | 4          | 20.43     | 36.8|
| OU611910.1      | 5          | 15.34     | 37.1|
| OU611911.1      | 6          | 15.20     | 36.8|
| OU611912.1      | 7          | 13.97     | 36.8|
| OU611913.1      | 8          | 13.72     | 36.8|
| OU611914.1      | 9          | 10.99     | 37.4|
| OU611915.1      | 10         | 10.77     | 37.4|

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| OU611916.1      | 11         | 10.58     | 36.8|
| OU611917.1      | 12         | 8.88      | 36.7|
| OU611918.1      | 13         | 8.79      | 38.1|
| OU611919.1      | 14         | 8.23      | 38.3|
| OU611920.1      | 15         | 8.14      | 37.6|
| OU611921.1      | 16         | 8.12      | 37.0|
| OU611922.1      | 17         | 8.02      | 37.4|
| OU611923.1      | 18         | 7.91      | 37.5|
| OU611924.1      | 19         | 7.39      | 37.8|
| OU611925.1      | 20         | 6.97      | 37.1|
| OU611926.1      | MT         | 0.02      | 17.2|

Figure 5. *Genome assembly of Tenthredo notha, iyTenNoth1.2: Hi-C contact map*. Hi-C contact map of the *iyTenNoth1.2* assembly, visualised in HiGlass. Chromosomes are shown in size order from left to right and top to bottom. The interactive Hi-C map can be viewed here.
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 abdomen tissue of iyTenNoth1 in the Tree of Life Laboratory at the WSI using the RNA Extraction: Automated MagMax™ mirVana protocol (do Amaral et al., 2023). The RNA concentration was assessed using a Nanodrop spectrophotometer and a Qubit Fluorometer using the Qubit RNA Broad-Range Assay kit. Analysis of the integrity of the RNA was done using the Agilent RNA 6000 Pico Kit and Eukaryotic Total RNA assay.

Protocols developed by the WSI Tree of Life laboratory are publicly available on protocols.io (Denton et al., 2023).

Sequencing
Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud sequencing libraries were constructed according to the manufacturers’ instructions. DNA sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II and Illumina NovaSeq 6000 instruments. RNA sequence data was generated on an Illumina HiSeq 4000 instrument. Hi-C data were generated from head tissue of iyTenNoth1 using the Arima v2.0 kit and sequenced on an Illumina NovaSeq 6000 instrument.

Genome assembly
Assembly was carried out with Hifiasm (Cheng et al., 2021); haplotypic duplication was identified and removed with 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 as described previously (Howe et al., 2021). Manual curation (Howe et al., 2021) was performed using HiGlass (Kerpedjiev et al., 2018) and Pretext. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva et al., 2021), which performed annotation using MitoFinder (Allio et al., 2020). The genome was analysed and BUSCO scores generated within the BlobToolKit environment (Challis et al., 2020). Table 3 contains a list of all software tools versions used, where appropriate.

Genome annotation
The Ensembl gene annotation system (Aken et al., 2016) was used to generate annotation for the Tenthredo notha assembly (GCA_914767705.1). Annotation was created primarily through alignment of transcriptomic data to the genome, with gap filling via protein-to-genome alignments of a select set of proteins from UniProt (UniProt Consortium, 2019).

| Table 3. Software tools used. |
|-----------------------------|
| Software tool | Version | Source |
| Hifiasm | 0.15.1 | Cheng et al., 2021 |
| purge_dups | 1.2.3 | Guan et al., 2020 |
| SALSA2 | 2.2 | Ghurye et al., 2019 |
| longranger align | 2.2.2 | https://support.10xgenomics.com/genome-exome/software/pipelines/latest/advanced/other-pipelines |
| freebayes | v1.3.1-17-gaa2ace8 | Garrison & Marth, 2012 |
| MitoHiFi | 2 | https://github.com/marcelauliano/MitoHiFi |
| HiGlass | 1.11.6 | Kerpedjiev et al., 2018 |
| PretextView | 0.2.x | https://github.com/wtsi-hpag/PretextView |
| BlobToolKit | 3.0.5 | 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: Tenthredo notha. Accession number PRJEB46306; https://identifiers.org/ena.embl/PRJEB46306.

The genome sequence is released openly for reuse. The T. notha 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. Raw data and assembly accession identifiers are reported in Table 1.

Author information
Members of the University of Oxford and Wytham Woods Genome Acquisition Lab are listed here: https://doi.org/10.5281/zenodo.5746938.

Members of the Darwin Tree of Life Barcoding collective are listed here: https://doi.org/10.5281/zenodo.5744972.
Members of the Wellcome Sanger Institute Tree of Life programme are listed here: https://doi.org/10.5281/zenodo.6125027.

Members of Wellcome Sanger Institute Scientific Operations: DNA Pipelines collective are listed here: https://doi.org/10.5281/zenodo.5746904.

Members of the Tree of Life Core Informatics collective are listed here: https://doi.org/10.5281/zenodo.6125046.

Members of the Darwin Tree of Life Consortium are listed here: https://doi.org/10.5281/zenodo.5638618.

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Version 2

Reviewer Report 05 April 2024

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✔️ Saskia Wutke 🌐
Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

Thank you for incorporating the suggested changes into the report.

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

**Reviewer Expertise:** insect genomics and phylogenetics

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 01 April 2024

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✔️ Marko Prous 🌐
Museum of Natural History, University of Tartu, Tartu, Estonia

Thank you for the corrections. I have no further comments.

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

**Reviewer Expertise:** Systematics of sawflies (Hymenoptera).
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 31 May 2022

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Saskia Wutke
Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

This data note describes the sequencing, assembly and annotation of the genome for Tenthredo notha, a sawfly in the family Tenthredinidae, using Pacbio, 10X linked read and Hi-C sequencing. Overall, the dataset is nicely presented and the methods are state-of-the-art and of high quality. Yet, I would wish for a few more details concerning the sample (I was wondering why the sex is unknown) and genome annotation. It is stated that transcriptomic data was used for annotating the genome, but the origin/source of this data is not provided i.e., the transcriptome of which taxa was used?

Additionally, the background section could be more detailed and include also some taxonomic placement to support its relevance.

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: insect genomics and phylogenetics

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.

Marko Prous
Museum of Natural History, University of Tartu, Tartu, Estonia

I don't have anything to say about the genome data. It's a valuable resource and seems to be of good quality (I have already used it to fish out some gene sequences used for other sawflies).

My main concern is poor documentation of the specimen used for sequencing.

Strangely the sex of the specimen is not known. Knowing the sex is important for species identification, so how is it possible that the identifier did not know the sex? It seems that the whole specimen was not destroyed for DNA extraction, then it should be trivial to report the sex (if tip of abdomen is still preserved).

If the specimen is preserved, in which collection is it stored? What is the specimen voucher code/specimen ID (Ox000858 or iyTenNoth1?) and is the specimen labelled accordingly to unambiguously associate the specimen with the sequence data?

In the abstract correct "Halictidae" to Tenthredinidae.

In table 1 clarify specimen number. In GenBank the specimen ID is Ox000858 and iyTenNoth1 is 'tolid' (don't know what is the purpose of this number).

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: Systematics of sawflies (Hymenoptera).

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.