DATA NOTE

The genome sequence of the pebble prominent, Notodonta ziczac (Linnaeus, 1758) [version 1; peer review: 2 approved]

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Abstract
We present a genome assembly from an individual male Notodonta ziczac (the pebble prominent; Arthropoda; Insecta; Lepidoptera; Notodontidae). The genome sequence is 352 megabases in span. The majority of the assembly (99.66%) is scaffolded into 31 chromosomal pseudomolecules, with the Z sex chromosome assembled. The mitochondrial genome was also assembled, and is 18.3 kilobases in length.

Keywords
Notodonta ziczac, pebble prominent, genome sequence, chromosomal, Lepidoptera

This article is included in the Tree of Life gateway.
Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Noctuoidea; Notodontidae; Notodontinae; Notodonta; Notodonta ziczac (Linnaeus, 1758) (NCBI:txid988002).

Background
The pebble prominent (Notodonta ziczac) is a moth of the family Notodontidae. A typical specimen's wingspan is 42 to 52 mm; its forewings are primarily ochreous brown, with the apical area carrying a grey, pebble-shaped marking from which its common name is drawn (Skinner & Wilson, 2009). Its species name ziczac, from the German zickzack, meaning zigzag, comes from the humps on its caterpillars' sixth, seventh and twelfth segments and the posture it assumes at rest, which creates a zigzag-like pattern (Emmet, 1991; Newman, 1869).

It can be found across the Palearctic region, from North Africa to China, within which the species is sometimes divided into three subspecies (Schintlmeister, 2008). It is widely distributed throughout Britain and Ireland, with observations most frequent in southern England and Wales (Randle et al., 2019). Double-brooded in the south and single-brooded in the north, it usually feeds on willow (Salix spp.), and is less commonly observed on poplar (Populus spp.) (Skinner & Wilson, 2009). It is widely distributed throughout Britain and Ireland, with observations most frequent in southern England and Wales (Randle et al., 2019; South, 1977). Its abundance in the UK declined sharply from 1970 to 2016, and shows a consistent downward trend (Randle et al., 2019).

Genome sequence report
The genome was sequenced from one male N. ziczac (ilNotZicz1) collected from Wytham Woods, Oxfordshire (biological vice-county: Berkshire), UK (latitude 51.772, longitude -1.338) by Douglas Boyes, UKCEH, using a light trap in woodland. 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 ilNotZicz1 sample was weighed and dissected on dry ice with tissue set aside for Hi-C sequencing. Thorax 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

Methods
Sample acquisition and DNA extraction
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Figure 1. Image of the Notodonta ziczac (ilNotZicz1) specimen taken prior to preservation and processing. Specimen shown next to FluidX storage tube, 43.9 mm in length.

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### Table 1. Genome data for *Notodonta ziczac*, ilNotZicz1.1.

| Project accession data                  |
|-----------------------------------------|
| Assembly identifier                     | ilNotZicz1.1 |
| Species                                 | *Notodonta ziczac* |
| Specimen                                | ilNotZicz1 |
| NCBI taxonomy ID                        | NCBI:txid988002 |
| BioProject                              | PRJEB46845 |
| BioSample ID                            | SAMEA7746619 |
| Isolate information                     | Male, thorax (genome assembly), head (Hi-C) |

| Raw data accessions                     |
|-----------------------------------------|
| PacificBiosciences SEQUEL II            | ERR6939251 |
| 10X Genomics Illumina                   | ERR6688606-ERR6688609 |
| Hi-C Illumina                           | ERR6688605 |

| Genome assembly                         |
|-----------------------------------------|
| Assembly accession                      | GCA_918843915.1 |
| Accession of alternate haplotype        | GCA_918843885.1 |
| Span (Mb)                               | 352 |
| Number of contigs                       | 55 |
| Contig N50 length (Mb)                  | 11.7 |
| Number of scaffolds                     | 51 |
| Scaffold N50 length (Mb)                | 12.7 |
| Longest scaffold (Mb)                   | 14.9 |
| BUSCO* genome score                     | C:98.9%, S:98.4%, D:0.4%, F:0.3%, M:0.9%, n:5286 |

*BUSCO scores based on the lepidoptera_odb10 BUSCO set using v5.2.2. 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/ilNotZicz1.1/dataset/CAKKNU01.1/busc*o.*

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.

**Sequencing**

Pacific Biosciences HiFi circular consensus and 10X Genomics Chromium read cloud sequencing libraries were constructed according to the manufacturers’ instructions. Sequencing was performed by the Scientific Operations core at the Wellcome Sanger Institute on Pacific Biosciences SEQUEL II (HiFi) and Illumina NovaSeq 6000 (10X) instruments. Hi-C data were generated from head tissue using the Arima Hi-C+ kit and sequenced on NovaSeq 6000.

**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
Figure 2. Genome assembly of *Notodonta ziczac*, ilNotZicz1.1: 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 352,061,436 bp assembly. The distribution of scaffold lengths is shown in dark grey with the plot radius scaled to the longest scaffold present in the assembly (14,880,619 bp, shown in red). Orange and pale-orange arcs show the N50 and N90 scaffold lengths (12,722,079 and 8,864,530 bp), respectively. The pale grey spiral shows the cumulative scaffold 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 lepidoptera_odb10 set is shown in the top right. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilNotZicz1.1/dataset/CAKKNU01.1/snail.
Figure 3. Genome assembly of *Notodonta ziczac*, ilNotZicz1.1: 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/ilNotZicz1.1/dataset/CAKKNU01.1/blob.

described previously (Howe et al., 2021). Manual curation (Howe et al., 2021) was performed using HiGlass (Kerpedjiev et al., 2018) and Pretex. The mitochondrial genome was assembled using MitoHiFi (Uliano-Silva et al., 2021), which performs 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 tool versions used, where appropriate.
Figure 4. Genome assembly of *Notodonta ziczac*, ilNotZicz1.1: 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/ilNotZicz1.1/dataset/CAKKNU01.1/cumulative.

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
**Figure 5.** Genome assembly of *Notodonta ziczac*, ilNotZicz1.1: Hi-C contact map. Hi-C contact map of the ilNotZicz1.1 assembly, visualised in HiGlass. Chromosomes are shown in order of size from left to right and top to bottom. An interactive version of this map is available [here](#).

**Table 2.** Chromosomal pseudomolecules in the genome assembly of *Notodonta ziczac*, ilNotZicz1.1.

| INSDC accession | Chromosome | Size (Mb) | GC%  |
|----------------|-----------|-----------|------|
| OU974037.1     | 1         | 14.88     | 38.5 |
| OU974039.1     | 2         | 14.52     | 38.8 |
| OU974040.1     | 3         | 14.41     | 38.1 |
| OU974041.1     | 4         | 14.05     | 38.3 |
| OU974042.1     | 5         | 13.88     | 37.7 |
| OU974043.1     | 6         | 13.62     | 38.6 |
| OU974044.1     | 7         | 13.07     | 38.4 |
| OU974045.1     | 8         | 13.01     | 38.1 |
| OU974046.1     | 9         | 12.97     | 37.8 |
| OU974047.1     | 10        | 12.92     | 38.2 |
| OU974048.1     | 11        | 12.79     | 38.0 |
| OU974049.1     | 12        | 12.72     | 38.3 |
| OU974050.1     | 13        | 12.42     | 38.5 |
| OU974051.1     | 14        | 11.81     | 38.0 |
| OU974052.1     | 15        | 11.72     | 38.6 |
| OU974053.1     | 16        | 11.70     | 38.1 |
| OU974054.1     | 17        | 11.69     | 38.4 |
| OU974055.1     | 18        | 11.45     | 39.2 |
| OU974056.1     | 19        | 10.88     | 38.7 |
| OU974057.1     | 20        | 10.78     | 39.2 |
| OU974058.1     | 21        | 10.78     | 38.4 |
| OU974059.1     | 22        | 9.26      | 38.9 |
| OU974060.1     | 23        | 9.21      | 39.0 |
| OU974061.1     | 24        | 8.94      | 39.4 |
| OU974062.1     | 25        | 8.86      | 39.3 |
| OU974063.1     | 26        | 8.43      | 39.2 |
| OU974064.1     | 27        | 6.64      | 40.3 |
| OU974065.1     | 28        | 6.64      | 39.7 |
| OU974066.1     | 29        | 6.21      | 40.7 |
| OU974067.1     | 30        | 6.00      | 40.1 |
| OU974038.1     | Z         | 14.56     | 38.2 |
| OU974068.1     | MT        | 0.02      | 19.2 |
| -              | Unplaced  | 1.19      | 45.2 |
Table 3. Software tools used.

| Software tool   | Version     | Source                           |
|-----------------|-------------|----------------------------------|
| Hifiasm         | 0.15.3-r339 | Cheng et al., 2021               |
| purge_dups      | 1.2.3       | Guan et al., 2020                |
| SALSA           | 3.0         | Ghurye et al., 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        | 2.0         | Uliano-Silva et al., 2021        |
| 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             |

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: Notodonta ziczac (pebble prominent). Accession number PRJEB46845; https://identifiers.org/ena.embl/PRJEB46845.

The genome sequence is released openly for reuse. The N. ziczac 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 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 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.

References
Allio R, Schomaker-Bastos A, Roniguier J, et al.: MitoFinder: Efficient Automated Large-Scale Extraction of Mitogenomic Data in Target Enrichment Phylogenomics. Mol Ecol Resour. 2020; 20(4): 892-905. PubMed Abstract | Publisher Full Text | Free Full Text
Challis R, Richards E, Rajan J, et al.: BlobToolKit - Interactive Quality Assessment of Genome Assemblies. G3 (Bethesda). 2020; 10(4): 1361-74. PubMed Abstract | Publisher Full Text | Free Full Text
Cheng H, Concepcion GT, Feng X, et al.: Haplotype-Resolved de Novo Assembly Using Phased Assembly Graphs with Hifiasm. Nat Methods. 2021; 18(2): 170-75. PubMed Abstract | Publisher Full Text | Free Full Text
Emmet AM: The Scientific Names of the British Lepidoptera: Their History and Meaning. Harley Books. 1991. Reference Source
Garrison E, Marth G: Haplotype-Based Variant Detection from Short-Read Sequencing. 2012. Publisher Full Text
Ghurye J, Rhee A, Walenz BP, et al.: Integrating Hi-C Links with Assembly Graphs for Chromosome-Scale Assembly. PloS Comput Biol. 2019; 15(8): e1007273. PubMed Abstract | Publisher Full Text | Free Full Text
Guan D, McCarthy SA, Wood J, et al.: Identifying and Removing Haplotypic Duplication in Primary Genome Assemblies. Bioinformatics. 2020; 36(9): 2896-98. PubMed Abstract | Publisher Full Text | Free Full Text
Howe K, Chow W, Collins J, et al.: Significantly Improving the Quality of Genome Assemblies through Curation. Gigascience. 2021; 10(1). PubMed Abstract | Publisher Full Text | Free Full Text
Kerpedjiev P, Abdennur N, Lekschas F, et al.: HiGlass: Web-Based Visual Exploration and Analysis of Genome Interaction Maps. Genome Biol. 2018; 19(1): 125. PubMed Abstract | Publisher Full Text | Free Full Text

Manni M, Berkeley MR, Seppey M, et al.: BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Mol Biol Evol. 2021; 38(10): 4647–54. PubMed Abstract | Publisher Full Text | Free Full Text

Newman E: An Illustrated Natural History of British Butterflies and Moths. London: Glaisher. 1869. Reference Source

Randle Z, Evans-Hill LJ, Parsons MS, et al.: Atlas of Britain and Ireland's Larger Moths. Pisces Publications, Newbury. 2019. Reference Source

Rao SSP, Huntley MH, Durand NC, et al.: A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. Cell. 2014; 159(7): 1665–80. PubMed Abstract | Publisher Full Text | Free Full Text

Schintlmeister A: Notodontidae. Palaearctic Macrolepidoptera. Vol. 1. Stenstrup: Apollo Books. 2008. Reference Source

Skinner B, Wilson D: Colour Identification Guide to the Moths of the British Isles. 2009. Reference Source

South R: The Moths of the British Isles. F Warne. 1977. Reference Source

Uliano-Silva M, Nunes JGF, Krasheninnikova K, et al.: marcelauliano/MitoHiFi: mitohifi_v2.0. 2021. Publisher Full Text
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Christopher M. Ward
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The data note by the late Boyes et al., describes the full genome sequence of Notodonta ziczac (Linnaeus, 1758). The data note is concise and in line with others of its kind, providing a lucid description of the organism and of the assembly. Therefore, I have no hesitation to support its indexing.

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: Adaptation, insect-hostplant arms race, grapevine genetics

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.
This is an important data note by the late Douglas Boyes and others. The species is in steep decline and there is thus an immediate impetus to make this genome assembly public. I see no problems with the way the paper is written or presented, and is in-line with many of the other genome notes. I fully support and approve the indexing of this Data Note.

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:** Lepidoptera evolution, 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.