DATA NOTE

The genome sequence of the large tortoiseshell, *Nymphalis polychloros* (Linnaeus, 1758) [version 1; peer review: 3 approved]

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**Abstract**
We present a genome assembly from an individual female *Nymphalis polychloros* (the large tortoiseshell; Arthropoda; Insecta; Lepidoptera; Nymphalidae). The genome sequence is 398 megabases in span. The majority of the assembly is scaffolded into 32 chromosomal pseudomolecules, with the W and Z sex chromosome assembled.

**Keywords**
*Nymphalis polychloros*, large tortoiseshell, genome sequence, chromosomal

This article is included in the Tree of Life gateway.

**Open Peer Review**

| Approval Status | 1 | 2 | 3 |
|-----------------|---|---|---|
| version 1       | ✔ | ✔ | ✔ |
| 16 Sep 2021     | view | view | view |

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Any reports and responses or comments on the article can be found at the end of the article.
Species taxonomy
Eukaryota; Metazoa; Ecdysozoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Papilionoidea; Nymphalidae; Nymphalinae; Nymphalis; Nymphalis; *Nymphalis polychloros* (Linnaeus, 1758) (NCBI: txid171594).

Introduction
The large tortoiseshell, also known as the black-legged tortoise-shell or elm nymphalid, is a widespread but rare butterfly in woodlands across continental Europe, North Africa and Central Asia. Once common in England and Wales, *N. polychloros* went extinct in Southern Britain in the 1960s for unknown reasons and is currently classified as ‘vulnerable’ in several European countries (Maes et al., 2020). It is listed as Least Concern in the IUCN Red List Category (Europe) (van Swaay et al., 2010). However, recent sightings of a breeding colony in Dorset in 2021 suggest that this species is once again resident in the UK. It is morphologically very close to both the small tortoise-shell, *Aglais urticae*, and the scarce tortoise-shell, *N. xanthomelas*, in adult appearance. The species uses a wide variety of host plants such as *Pyrus*, *Prunus*, *Salix*, *Ulmus*, *Crataegus*, and others. It is univoltine and overwinters as an adult. (Lorković, 1941) reported a karyotype of 31 chromosomes and the genome size estimated for its relative, *Aglais io*, is 363.5 Mb (Mackintosh et al., 2019).

Genome sequence report
The genome was sequenced from a single female *N. polychloros* (Figure 1) to 36-fold coverage in Pacific Biosciences single-molecule long reads and 84-fold coverage in 10X Genomics read clouds. Primary assembly contigs were scaffolded with chromosome conformation Hi-C data. Manual assembly correction corrected two missing/misjoins, reducing the scaffold number by 5.31%. The final assembly has a total length of 398 Mb in 38 sequence scaffolds with a scaffold N50 of 14 Mb (Table 1). Of the assembly sequence, 100% was assigned to 32 chromosomal-level scaffolds, representing 30 autosomes (numbered by sequence length), and the W and Z sex chromosome (Figure 2–Figure 5; Table 2). The assembly has a BUSCO v5.1.2 (Simão et al., 2015) completeness of 98.8% using the lepidoptera_odb10 reference set. While not fully phased, the assembly deposited is of one haplotype. Contigs corresponding to the second haplotype have also been deposited.

Methods
The female *N. polychloros* specimen SC_NP_345 was collected using a net from Somiedo, Brana de Mumian, Asturias, Spain.

**Figure 1.** Fore and hind wings of *Nymphalis polychloros* specimen from which the genome was sequenced. (A) Dorsal surface view of wings from specimen SO_NP_354 (IlNympPoly1) from Somiedo, Spain used to generate Pacific Biosciences and 10X genomics data. (B) Ventral surface view of wings from specimen SO_NP_354 from Somiedo, Spain, used to generate Pacific Biosciences and 10X genomics data.
Table 1. Genome data for Nymphalis polychloros, ilNymPoly1.1.

| Project accession data                              |
|-----------------------------------------------------|
| Project accession data                              |
| Assembly identifier                                  | ilNymPoly1.1                                         |
| Species                                             | Nymphalis polychloros                                |
| Specimen                                            | ilNymPoly1                                           |
| NCBI taxonomy ID                                    | NCBI:txid171594                                      |
| BioProject                                          | PRJEB43012                                           |
| BioSample ID                                        | SAMEA7523140                                         |
| Isolate information                                 | Female, whole organism                               |

| Raw data accessions                                  |
|-----------------------------------------------------|
| Raw data accessions                                  |
| PacificBiosciences SEQUEL II                        | ERR6590585                                           |
| 10X Genomics Illumina                               | ERR6054433-ERR6054436                                |
| Hi-C Illumina                                       | ERR6054437                                           |
| RNAseq PolyA Illumina                               | ERR6286714                                           |

| Genome assembly                                     |
|-----------------------------------------------------|
| Genome assembly                                     |
| Assembly accession                                  | GCA_905220585.1                                      |
| Accession of alternate haplotype                    | GCA_905220575.1                                      |
| Span (Mb)                                           | 398                                                 |
| Number of contigs                                   | 45                                                  |
| Contig N50 length (Mb)                              | 14                                                  |
| Number of scaffolds                                 | 38                                                  |
| Scaffold N50 length (Mb)                            | 14                                                  |
| Longest scaffold (Mb)                               | 17                                                  |
| BUSCO* genome score                                 | C:98.8%[S:98.6%,D:0.2%],F:0.3%,M:0.8%,n:5286       |

*BUSCO scores based on the lepidoptera_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/ilNymPoly1.1/dataset/CAJNAJ01/busc](https://blobtoolkit.genomehubs.org/view/ilNymPoly1.1/dataset/CAJNAJ01/busc).

DNA was extracted from thorax tissue at the Wellcome Sanger Institute (WSI) Scientific Operations core from the whole organism using the Qiagen MagAttract HMW DNA kit, according to the manufacturer’s instructions. RNA was extracted (also from thorax 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 μl RNase-free water and its concentration RNA assessed using a Nanodrop spectrophotometer and Qubit Fluorometer using the Qubit RNA 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.
Pacific Biosciences HiFi circular consensus and 10X Genomics read cloud DNA sequencing libraries, in addition to PolyA RNA-Seq libraries, were constructed according to the manufacturers’ instructions. DNA and RNA sequencing was performed by the Scientific Operations core at the WSI on Pacific Biosciences SEQUEL II (HiFi), Illumina HiSeq X (10X) and Illumina HiSeq 4000 (RNA-Seq) instruments. Hi-C data were generated from abdomen tissue using the Arima v2.0 kit and sequenced on Illumina NovaSeq.

Assembly was carried out with Hifiasm (Cheng et al., 2021); haplotypic duplication was identified and removed with
Figure 3. Genome assembly of *Nymphalis polychloros*, ilNymPoly1.1: GC coverage. BlobToolKit GC-coverage plot. Chromosomes are coloured by phylum. Circles are sized in proportion to chromosome length. Histograms show the distribution of chromosome length sum along each axis. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilNymPoly1.1/dataset/CAJNAJ01/blob.

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 was performed using purge_dups (Guan et al., 2020).
**Figure 4. Genome assembly of Nymphalis polychloros, ilNymPoly1.1: cumulative sequence.** BlobToolKit cumulative sequence plot. The grey line shows cumulative length for all chromosomes. Coloured lines show cumulative lengths of chromosomes assigned to each phylum using the buscogenes taxrule. An interactive version of this figure is available at https://blobtoolkit.genomehubs.org/view/ilNymPoly1.1/dataset/CAJNAJ01/cumulative.

The materials that have contributed to this genome note were supplied by a Tree of Life collaborator. The Wellcome Sanger Institute employs a process whereby due diligence is carried out proportionate to the nature of the materials themselves, and the circumstances under which they have been/are to be collected and provided for use. The purpose of this is to address and...
mitigate any potential legal and/or ethical implications of receipt and use of the materials as part of the research project, and to ensure that in doing so we align with best practice wherever possible.

The overarching areas of consideration are:

- Ethical review of provenance and sourcing of the material;
- Legality of collection, transfer and use (national and international).

Each transfer of samples is undertaken according to a Research Collaboration Agreement or Material Transfer Agreement entered into by the Tree of Life collaborator, Genome Research Limited (operating as the Wellcome Sanger Institute) and in some circumstances other Tree of Life collaborators.
Table 2. Chromosomal pseudomolecules in the genome assembly of *Nymphalis polychloros*, iINymPoly1.1.

| INSDC accession | Chromosome | Size (Mb) | GC% |
|-----------------|------------|-----------|-----|
| HG992242.1      | 1          | 16.56     | 34.4|
| HG992243.1      | 2          | 16.45     | 33.7|
| HG992244.1      | 3          | 16.03     | 34.1|
| HG992245.1      | 4          | 15.91     | 33.8|
| HG992246.1      | 5          | 15.83     | 34.1|
| HG992247.1      | 6          | 15.48     | 34  |
| HG992248.1      | 7          | 15.41     | 33.3|
| HG992249.1      | 8          | 15.04     | 34.3|
| HG992250.1      | 9          | 14.99     | 34.1|
| HG992251.1      | 10         | 14.77     | 35.2|
| HG992252.1      | 11         | 14.06     | 33.4|
| HG992253.1      | 12         | 13.93     | 33.6|
| HG992254.1      | 13         | 13.74     | 33.9|
| HG992255.1      | 14         | 13.53     | 33.9|
| HG992256.1      | 15         | 13.45     | 33.9|
| HG992257.1      | 16         | 12.92     | 33.6|
| HG992258.1      | 17         | 12.55     | 34  |
| HG992259.1      | 18         | 12.34     | 34.2|
| HG992260.1      | 19         | 11.88     | 34.6|
| HG992261.1      | 20         | 11.42     | 34.1|
| HG992262.1      | 21         | 10.92     | 34.8|
| HG992263.1      | 22         | 10.46     | 34.2|
| HG992264.1      | 23         | 10.26     | 34.4|
| HG992265.1      | 24         | 10.09     | 36  |
| HG992266.1      | 25         | 9.27      | 34.3|
| HG992267.1      | 26         | 8.82      | 34.8|
| HG992268.1      | 27         | 7.95      | 38.3|
| HG992269.1      | 28         | 7.29      | 36  |
| HG992270.1      | 29         | 6.82      | 36.7|
| HG992271.1      | 30         | 6.08      | 38.3|
| HG992272.1      | W          | 4.33      | 37.3|
| HG992241.1      | Z          | 18.34     | 33.4|
| HG992273.1      | MT         | 0.02      | 20.3|
| -               | Unplaced   | 1.22      | 38.6|

Data availability
European Nucleotide Archive: Nymphalis polychloros (large tortoiseshell). Accession number PRJEB42956; https://identifiers.org/ena.embl:PRJEB42956.

The genome sequence is released openly for reuse. The *N. polychloros* 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.

Acknowledgements
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.4783586.

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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Current Peer Review Status: ✔️ ✔️ ✔️

Version 1

Reviewer Report 03 May 2023

https://doi.org/10.21956/wellcomeopenres.19000.r49891

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This report describes a near-complete genome assembly for the butterfly *Nymphalis polychloros*, which will be an excellent resource for the study of butterfly genetics and diversification. It is worth mentioning that a high-quality genome annotation is available for the closely related species *Nymphalis (Aglais) io*. I recommend mentioning Zhang et al. 2021, for its clarification of the genus status in the corresponding clade.

References
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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:** Butterfly evolutionary genomics and development

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.
In the last sentence of the manuscript's Introduction, "(Lorković, 1941) reported a karyotype of 31 chromosomes and the genome size estimated for its relative, Aglais io, is 363.5Mb (Mackintosh et al., 2019)." We see that the genome size of Nymphalis polychloros has not been reported in previous studies. It is suggested that the authors should increase the analysis of genome size prediction based on GCE or other tools in the Methods, and this is a good guide for subsequent genome assembly or haplotypic selection.

1. In the part of “Genome sequence report”, the W and Z sex chromosomes were mentioned to be assembled in this work. However, how to identify sex chromosomes in the Methods was not stated clearly, and the authors are suggested to clearly introduce the method for distinguishing sex chromosomes in this study.

2. The results of genome assembly probably depend on the setting of parameters used in the software, thus the authors are suggested to add the main parameters of tools in Table 3.

3. For some quality control analysis in the Methods, can the authors describe the changes in data volume before and after filtering in the text? For example, “haplotypic duplication was identified and removed with purge_dups (Guan et al., 2020).” “Manual curation was performed using Manual curation was performed using gEVAL, HiGlass (Kerpedjiev et al., 2018) and Pretext.”

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:** the phylogeny and evolutionary history of some butterfly groups.

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.

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**Quentin Rougemont**
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This data note by Lohse and colleagues reports the genome sequence of a butterfly in the large family Nymphalidae, well known for harbouring other iconic butterfly models for metapopulation dynamics or for the study of wing coloration. This adds a reference genome for a group of butterflies with interesting biology such as adult overwintering and long distance migration.

The genome presented is of very high quality. I see nothing problematic with the assembly which offers excellent contiguity. My comments are minor but may reflect the perspective of someone involved in assembling similar genomes.

It may make no difference, but wondered why the length was initially estimated to the 363Mb of the peacock butterfly *Aglais io*, while the comma butterfly *Polygonia c-album* appears more closely related, and has a published, slightly higher genome size estimated to 373Mb (Celorio-Mancera et al. 2021 Genome Biol. Evol. 13:evab054)¹

Table 2 reports 1.22Mb of unplaced scaffolds, and the text reports 38 sequence scaffolds. Yet the authors report that 100% of the assembly was assigned to 32 chromosomal-level scaffolds. I was wondering what constitutes the difference between those statistics (large heterozygous tracts/haplotypes?)

Hi-C data was generated from abdomen tissue. Since this is a wild and therefore mated female, the abdomen is likely to contain recombinant gametes from one or several unknown males. I assume that this is unlikely to introduce large errors for scaffolding, but wondered whether the authors could perhaps give a few words on this possible issue, since it is a question that frequently arises when using abdominal female tissue from wild-caught individuals.

This assembly is made from the DNA of a wild-caught specimen. Therefore it would be interesting...
to provide details on its observed heterozygosity. Dealing with heterozygosity is a recurrent issue in genome assembly.

Similarly, chosen parameter values would be good to provide for all packages and softwares (such as hifiasm, freebase, etc), perhaps as an additional column for table 3. Those values are essential for reproducibility, but also very useful for people assembling similar genomes.

It would be interesting to provide details of the improvements allowed by the different steps (for instance the polishing step, by giving stats before and after). Again this would be useful for other users and generally for assembly of similar genomes. This could take the form of a table.

The note presents the generation of RNAseq data, which is great, but the data is not (yet) used for annotation. I wondered why include this in the methods if it is actually not analysed.

Status/justification: This species the large tortoiseshell is a fairly common though elusive butterfly in its predominantly continental European range. I understand that the Darwin tree of life effort is motivated by sequencing “British taxa” and the status of this species in Britain may have influenced its position on the priority list. However, the aim of a reference genome probably goes beyond that. Abundance and conservation status of taxa are very variable depending on how far from the range margin one stands! From a broader perspective, the large tortoiseshell is a forest species with a broad European distribution. It has a relatively poorly known ecology compared to closely related species. And interesting question marks remain regarding the origins and status of its genetic structure (vicariance, speciation?). *Nymphalis* as a genus also has unclear relationships with other genera such as *Polygonia* and *Kaniska*. Perhaps a reference genome could stimulate interesting research on those aspects which could make a better "justification" for sequencing it than the recent sighting of a colony in Dorset where the species is teetering on its range margin.

References
1. Celorio-Mancera M, Rastas P, Steward R, Nylin S, et al.: Chromosome Level Assembly of the Comma Butterfly (Polygonia c-album ). *Genome Biology and Evolution*. 2021; 13 (5). Publisher Full Text

**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:** Population genomics
We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.