# A high-coverage draft genome of the mycalesine butterfly *Bicyclus anynana*

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## Abstract

**Background:** The mycalesine butterfly *Bicyclus anynana*, the 'Squinting bush brown', is a model organism in the study of lepidopteran ecology, development and evolution. Here, we present a draft genome sequence for *B. anynana* to serve as a genomics resource for current and future studies of this important model species.

**Findings:** Seven libraries with insert sizes ranging from 350 bp to 20 kb were constructed using DNA from an inbred female and sequenced using both Illumina and PacBio technology. 128 Gb raw Illumina data were filtered to 124 Gb and assembled to a final size of 475 Mb (~260X assembly coverage). Contigs were scaffolded using mate-pair, transcriptome and PacBio data into 10,800 sequences with an N50 of 638 kb (longest scaffold 5 Mb). The genome is comprised of 26% repetitive elements, and encodes a total of 22,642 predicted protein-coding genes. Recovery of a BUSCO set of core metazoan genes was almost complete (98%). Overall, these metrics compare well with other recently published lepidopteran genomes.

**Conclusions:** We report a high-quality draft genome sequence for *Bicyclus anynana*. The genome assembly and annotated gene models are available at LepBase (http://ensembl.lepbase.org/index.html).

**Keywords:** *Bicyclus anynana*, Squinting bush brown, Nymphalidae, nymphalid, satyrid, lepidopteran genome.

## Data description

The squinting bush brown butterfly, *Bicyclus anynana*, is a member of the remarkably speciose nymphalid subtribe Mycalesina, which is distributed across the Old World tropics (Figure 1). *B. anynana* is an important model organism for the study of lepidopteran ecology, development, speciation, behaviour, and evolution [1-6]. *B. anynana* are found primarily in woodland habitats across East Africa (from southern Sudan in the north to Swaziland in the south), and adults are typically observed flying close to the ground where they feed on fallen fruit [1]. Strikingly, *B. anynana* exhibits seasonal polyphenism, a form of phenotypic plasticity whereby individuals that develop during the wet season differ in both behaviour, appearance and life history to those that develop during the dry season [7-9]. Wet season butterflies are smaller, have shorter lifespans, are more active, and show larger and more conspicuous eyespots on their wings in comparison to dry season individuals. The genetic basis of this plasticity, and its impacts on various other life-history and developmental characteristics, are ongoing research questions to which the availability of a *B. anynana* reference genome will contribute [10-12].

### Sampling and sequencing

Genomic DNA was extracted from a *B. anynana* female that had been inbred via seven generations of brother-sister matings. The captive laboratory stock population from which these individuals originated was established in 1988 from 80 wild-caught

individuals, and has been maintained at large effective population sizes to minimise the loss of genetic diversity [1]. Two short-insert libraries with insert sizes of 350 and 550 bp were constructed using Illumina TruSeq Nano reagents and sequenced (125 base, paired end) on an Illumina HiSeq2500 at Edinburgh Genomics (Edinburgh, UK). DNA from a sister to this focal animal was used to construct four long-insert (mate-pair) libraries with insert sizes of 3 and 5 kb (two of each) at the Centre for Genomic Research, University of Liverpool (Liverpool, UK); libraries of both insert-sizes were then sequenced on an Illumina HiSeq2500 and an Illumina MiSeq at Edinburgh Genomics (Table 1). DNA from a female descendent of the same inbred line was used to construct two long read libraries with insert sizes of 10 and 20 kb, sequenced on the PacBio platform at the Genome Institute of Singapore at 20x coverage using 16 P6 SMRT cells. All raw data have been deposited in the Short Read Archive under the accessions given in Table 1.

A total of 128.2 Gb raw Illumina data were filtered for low-quality bases and adapter contamination using Skewer v0.2.2 [13], and both raw and trimmed reads were inspected using FastQC v0.11.4 [14]. Only 4 Gb data (3.1%) were discarded, indicating the high quality of the raw data. Kmer frequency distributions were estimated using the "kmercountexact" program from the BBMap v36.02 package [15], and showed two major coverage peaks at ~105X and ~210X (Figure 2). The first peak (105X) represents the proportion of the genome that is heterozygous, and has an approximate span of 87.7 Mb (18.4% of the genome; calculated as one half of the area under the 105X curve, from 50X to 150X). The expected proportion of heterozygous sites given seven brother-sister (full-sib) matings is 0.75^7 = 13.3%, or 63.5 Mb. Thus, the greater than expected heterozygosity is likely to be due primarily to selection against highly inbred individuals during the course of the inbreeding regime [16].

## Contaminant filtering and assembly

Short-insert libraries were screened for the presence of contaminant reads using Taxon-Annotated GC-Coverage (TAGC) plots, or “blobplots” [17]. An initial draft assembly was constructed using CLC assembler (CLCBio, Copenhagen) and compared to the NCBI nucleotide database (nt) using Megablast v2.3.0+ [18], and against the UniRef90 protein database using Diamond v0.7.10 [19]. Read coverage for each contig was calculated by mapping both libraries to the CLC assembly using CLC mapper (CLCBio, Copenhagen), and blobplots were generated using Blobtools v0.9.19.4 [20] using the “bestsumorder” rule for taxonomic annotation of contigs (Figure 3). Contigs that showed a substantially different coverage relative to that of the main cluster of contigs and/or good hits to sequences annotated as non-Arthropoda were classed as putative contaminants. A total of 237,394 pairs of reads (~59 Mb) that were classed as either “mapped/mapped” or “mapped/unmapped” to a putative contaminant were subsequently discarded from further analysis.

Filtered libraries were reassembled using the heterozygous-aware assembler Platanus v1.2.4 [21], with default parameters. Contigs were further scaffolded with the mate pair libraries using SSPACE v3.0 [22] and with 35,747 assembled *B. anynana* transcripts using a combination of L_RNA_scaffolder [23] and SCUBAT v2 [24]. The transcripts were assembled using Trinity v. 20140717 [25] from ca. 2 x $10^9$ paired end RNA-Seq reads sequenced from thorax and abdomen tissue of 72 outbred *B. anynana* females of the standard captive laboratory stock population (Oostra et al., in preparation). A final round of scaffolding was performed with PacBio long reads (fastq files error-corrected using the RS_Preaassembler.2 protocol) using SSPACE-LongRead v1.1 [26]. Finally, gaps between scaffolds were filled using GapFiller v1.10 [27] and PBJelly v15.8.24 [28].

Our final assembly (v1.2) comprised 10,800 scaffolds spanning a total of 475.4 Mb, with a scaffold N50 of 638 kb (Table 2). The genome-wide proportion of G+C was 36.5%, while the number of undetermined bases (N's) was 5.8 Mb (~1.2% of the total span). We determined assembly completeness by mapping both genomic and transcriptomic reads from *B. anynana* (SRA whole genome sequencing accessions ERR1102671-8, and transcriptome accessions ERR1022636-7, ERR1022640-1, and ERR1022644-5, downloaded October 2016) to the genome using BWA mem v0.7.12 [29] and STAR v020201 [30] respectively. Over 99% of reads from the two short-insert libraries mapped to the assembly, suggesting that the vast majority of the genome represented by these data has been assembled. In addition, 94.9% of RNA-Seq reads mapped to the assembly, suggesting that the majority of transcribed genes are present. Gene-level completeness was assessed using CEGMA v2.5 [31] and BUSCO v2.0 [32]. The proportion of CEGMA genes "completely" recovered (*n* = 248) was 81%, increasing to 97% when partially recovered genes are included. The recovery of BUSCO genes specific to the metazoa (*n* = 978) was higher, at 98% for complete genes, increasing to 99% when partial genes are included. An almost complete set (99.2%) of BUSCO genes specific to the Arthropoda (*n* = 1,066) was also recovered. In addition, CEGMA indicated a duplication rate of 1.1 while BUSCO estimated only ~2% genes were present in multiple copies. The high complete CEGMA/BUSCO scores suggest a good assembly that has captured the majority of core metazoan/Arthropod genes in full-length, and that the fragmentation of genes across multiple scaffolds is low. In addition, the low duplication rates suggest that most genes are present in single copy, and thus that the genome does not include significant duplicated segments representing alternative haplotypes.

## Annotation

Prior to gene prediction, we masked the *B. anynana* assembly for repetitive elements to minimise the number of spurious open-reading frames due to low-complexity repeat

regions or transposable elements. Repetitive motifs in the *B. anynana* assembly were modelled *ab initio* using RepeatModeler v1.0.5 (http://www.repeatmasker.org/RepeatModeler.html). Repeats occurring within genuine coding regions were excluded by querying the proteins from a previous *B. anynana* assembly (v0.1) versus the RepeatModeler database using BLAST, removing any sequences showing a match at *E*-value $\leq$ 1e-10 threshold. The filtered RepeatModeler database was combined with known repeats from the Lepidoptera using RepBase v20.05 [33] and input to RepeatMasker v4.0.5 [34] to mask the assembly. Overall, approximately one quarter of the assembly (122.6 Mb) was masked from gene prediction (Table 3).

**Table 3:** Major types of repeat content for *B. anynana*.

| Repeat type | Span (Mb) | Proportion of genome |
|---|---|---|
| SINE | 10.8 | 2.3% |
| LINE | 15.3 | 3.2% |
| LTR elements | 1.1 | 0.2% |
| DNA elements | 0.8 | 0.2% |
| Small RNA | 10.8 | 2.3% |
| Unclassified | 86.2 | 18.1% |
| **Total** | 122.6 | 25.8% |

Gene finding was performed following a two-pass approach [35]. Initial gene-models were constructed with MAKER v2.31 [36], using HMMs derived from SNAP [37] and GeneMark-ES v4.3 [38] in conjunction with a recently published *B. anynana* transcriptome as evidence [39]. MAKER gene-models were then passed to AUGUSTUS v3.0.3 [40] for refinement, resulting in an initial set of 26,722 predicted protein-coding genes. A set of basic filters was applied to remove likely spurious gene models (Table 4), resulting in the deletion of 4,080 gene models. Protein sequences

from the filtered 22,642 genes were annotated using BLAST searches versus UniRef90 and the NCBI non-redundant protein database (nr), and domains/motifs were described using InterProScan5 [41]. Summary statistics for the 22,642 predicted gene models are given in Table 5.

**Table 4:** Number of genes in potential error categories.

| Category | Description | Number of genes |
|---|---|---|
| **(a)** | Single-exon | 7112 |
| **(b)** | Small exon (< 9 bp) | 1866 |
| **(c)** | Small intron (≤ 40 bp) | 45 |
| **(d)** | Short (CDS < 120 bp) | 127 |
| **(e)** | No hit to *nr* | 6532 |
| **(f)** | Duplicate (≥ 98% identity over ≥ 98% query length) | 822 |
| **Total**[1] | | 4080 |

[1]Defined as the non-redundant total of the intersection of each category (a) to (d) with category (e), plus the shorter of any duplicates identified in category (f).

## Comparison to other lepidopteran genomes

To ascertain the relative quality of the *B. anynana* v1.2 assembly, we compared our results to nine other published lepidopteran genomes available on LepBase (http://lepbase.org/) [42]: *Bombyx mori* ASM15162v1 [43], *Danaus plexippus* v3 [44], *Heliconius melpomene* Hmel2 [45,46], *Lerema accius* v1.1 [47], *Melitaea cinxia* MelCinx1.0 [48], *Papilio glaucus* v1.1 [49], *Papilio polytes* Ppol 1.0 [50], *Papilio xuthus* Pap_xu_1.0 [50] and *Plutella xylostella* DBM_FJ_v1.1 [51]. The *B. anynana* v1.2 assembly was of high quality compared to other published genomes, with the majority of the genome represented in a relatively small number of scaffolds despite being only marginally smaller than the largest lepidopteran genome, *B. mori* (Figure 4a). Interestingly, *B. anynana* v1.2 encodes the highest number of proteins of the 10

species compared (Figure 4b). Despite measures to eliminate potentially spurious ORFs caused by annotation error or by duplication, *B. anynana* encodes ~3,250 more genes than the diamondback moth *P. xylostella*, and ~10,400 more than the swallowtail *P. polytes*. It is tempting to attribute the apparently high number of genes to the developmental plasticity and alternative seasonal forms with divergent morphologies and life histories in *B. anynana*. However, it remains to be determined whether the number of genes predicted in *B. anynana* is a function of its larger genome size or unusual life-history characteristics, or if further curation of the v1.2 gene models will reduce the number of inferred genes.

**Concluding remarks**

We present a high-coverage, high quality draft assembly and annotation of the mycalesine butterfly *B. anynana*. The assembly will be a core resource for ongoing analyses of population genomics, discovery of *cis*-regulatory elements of wing patterning and other genes, functional genetics and functional ecology of complex gene families, and the evolution of novel and plastic lifecycle strategies in lepidopterans and other arthropods.

## Availability of supporting data

All raw sequence data have been deposited in the Short Read Archive (SRA) and are available for download using the accession numbers provided in Table 1. The *B. anynana* v1.2 assembly, as well as final predicted gene-models and protein annotations, are publicly available for viewing and download via LepBase [42], an Ensembl [52] genome database for the Lepidoptera (http://ensembl.lepbase.org/index.html). Data supporting the manuscript, including annotations as well as BUSCO and CEGMA results, are also available via the

*GigaScience* database, GigaDB [53]. A previous *B. anynana* assembly (nBa.0.1) is also available on LepBase.

## Abbreviations

BUSCO: Benchmarking Universal Single-Copy Orthologs; CEGMA: Core Eukaryotic Genes Mapping Approach; CDS: Coding sequence; ORF: Open reading frame.

## Competing interests

The authors declare that they have no competing interests.

## Author contributions

PMB and MB designed the study; AM and BRW collected samples and produced the inbred line; AEVH, IJS and HC extracted DNA samples; RWN, BE and MB worked on the genome assembly and annotation; VO, BJZ, CW and MS contributed transcriptome data; AM, HC and MLA contributed PacBio data; SK and RJC uploaded the assembly to LepBase. RWN, VO, AM, PMB and MB wrote the manuscript. All authors have read and approved the final version of the manuscript.

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# Tables

Tables 1, 2 and 5 are in landscape orientation and can be found as additional files at the end of this manuscript.

# Figure legends

**Figure 1:** Wet-season morph of *Bicyclus anynana* (picture credit: William H. Piel and Antónia Monteiro).

**Figure 2:** Kmer frequency distribution for *B. anynana* short-insert libraries ($k$ = 31). The bimodality of the distribution, with peaks at approximately 105X and again at 210X, is the result of heterozygosity in the sequence data.

**Figure 3:** Taxon-annotated GC-coverage plots for **(a)** draft and **(b)** final *B. anynana* genome assemblies. Each contig/scaffold in the assembly is represented as a circle,

coloured according to the best match to taxonomically annotated sequence databases (see legends) and distributed according to the proportion GC (*x*-axis) and read coverage (*y*-axis). The upper- and right-hand panels show the distribution of the total span (kb) of contigs/scaffolds for a given coverage (upper panel) or GC (right panel) bin. The heterozygosity in the sample is evident in the bimodal coverage distribution seen in (a). The cluster of orange-coloured contigs at a lower coverage and higher GC than the main cloud were likely derived from contaminant *Enterococcus* present in the sample. The final assembly, (b), shows the effective collapse of heterozygous regions, the removal of contaminant sequences and the scaffolding of contigs into long contiguous sequences. Note that only taxon annotations with a span > 1 Mb are shown in the legend for clarity.

**Figure 4:** Assembly and gene prediction comparison among 10 lepidopteran genomes. **(a)** Cumulative assembly curves showing the relationship between the number of scaffolds (*x*-axis) and the cumulative span of each assembly (*y*-axis), coloured by species. Higher quality assemblies are represented by an almost-vertical line (e.g., *H. melpomene* Hmel2 assembly in black), indicating a relatively small number of scaffolds is required to reach the final genome span; conversely, a long tail indicates the assembly includes a large number of smaller scaffolds. The curve for *B. anynana* (brown and bold) suggests a good assembly for this species, with the majority of the assembly comprised of relatively few scaffolds. **(b)** *B. anynana* v1.2 encodes the greatest number of genes of the 10 genomes, and is particularly different from *B. mori*, which is of equivalent length. Species names/colours are as follows: “bicyclus” (brown), *B. anynana*; “bombyx” (blue), *B. mori*; “danaus” (light green), *D. plexippus*; “heliconius” (black), *H. melpomene*; “lerema” (dark green), *L. accius*; “melitaea” (orange), *M. cinxia*; “glaucus” (red), *P. glaucus*; “polytes” (pink), *P. polytes*; “xuthus” (violet), *P. xuthus*; “plutella” (grey), *P. xylostella*.

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# Tables

**Table 1:** Data counts and library information.

| Library type | Platform | Read length | Insert size (expected) | Number of reads (raw) | Number of reads (trimmed) | Number of bases (trimmed) | SRA run accessions |
|---|---|---|---|---|---|---|---|
| Short insert | Illumina HiSeq2500 | 125 bp paired-end | 350 bp | 271808057 pairs | 267241712 (98.3%) | 66334099834 (97.6%) | ERR1102671-2, ERR1102675-6 |
| Short insert | Illumina HiSeq2500 | 125 bp paired-end | 550 bp | 241050065 pairs | 234269871 (97.2%) | 57913474128 (96.1%) | ERR1102673-4, ERR1102677-8 |
| Mate pair | Illumina HiSeq2500 | 100 bp paired-end | 3 kb | 77105680 pairs | 31848200 (41.3%) | 5758856502 (37.3%) | ERR1750945 |
| Mate pair | Illumina MiSeq | 100 bp paired-end | 3 kb | 5641764 pairs | 2170610 (38.5%) | 397993018 (35.3%) | ERR754051 |
| Mate pair | Illumina HiSeq2500 | 100 bp paired-end | 5 kb | 77614870 pairs | 45676725 (58.9%) | 8203769131 (52.8%) | ERR1750946 |
| Mate pair | Illumina | 100 bp | 5 kb | 7939601 pairs | 4734000 (59.6%) | 861352793 (54.2%) | ERR754052 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | MiSeq | paired-end | | | | | |
| Long read | PacBio P6 | 0.80-50 kb | 10 kb | 1388796 | 1199064 (86.3%) | 4086394966 | ERR1797559-74 |

**Table 2:** Summary of *B. anynana* genome assembly and comparison to selected lepidopteran genomes.

| | ***B. anynana*** | ***B. mori*** | ***D. plexippus*** | ***H. melpomene*** | ***M. cinxia*** |
|---|---|---|---|---|---|
| **Assembly version** | v1.2 | ASM15162v1 | v3 | Hmel2 | MelCinx1.0 |
| **Span** | 475.4 Mb | 481.8 Mb | 248.6 Mb | 275.2 Mb | 389.9 Mb |
| **Contigs** | | | | | |
| Number | 23699 | 88673 | 10682 | 3100 | 48180 |
| N50[1] | 78.7 kb | 15.5 kb | 111.0 kb | 328.9 kb | 14.1 kb |
| NumN50[2] | 1543 | 8075 | 548 | 214 | 7366 |
| **Scaffolds** | | | | | |
| Number | 10800 | 43379 | 5397 | 795 | 8261 |
| N50 | 638.3 kb | 4008.4 kb | 715.6 kb | 2102.7 kb | 119.3 kb |
| NumN50 | 194 | 38 | 101 | 34 | 970 |
| N90 | 99.3 kb | 61.1 kb | 160.5 kb | 273.1 kb | 29.6 kb |
| NumN90 | 909 | 258 | 366 | 176 | 3396 |
| Shortest / longest | 201 b / 5 Mb | 53 b / 16.2 Mb | 300 b / 6.2 Mb | 394 b / 9.4 Mb | 1.5 kb / 668 kb |
| G+C content | 36.5% | 37.7% | 31.6% | 32.8% | 32.6% |

| **NNNs** | | | | | |
|---|---|---|---|---|---|
| Span | 5.8 Mb (1.2%) | 50.1 Mb (10.4%) | 6.7 Mb (2.7%) | 986 kb (0.4%) | 28.9 Mb (7.4%) |
| N50 | 1.4 kb | 5.0 kb | 2.5 kb | 2.4 kb | 1.4 kb |
| **CEGMA**[3] (*n* = 248) | **C:**81.1%; **D:**1.1; **F:**97.2% | **C:**76.6%; **F:**96.8% | **C:**90.3%; **F:**96% | **C:**88.7%; **F:**96.8% | NA |
| **BUSCO**[3] (*n* = 1066) | **C:**98.3%; **D:**1%; **F:**99.2% | **C:**97.5%; **D:**0.5%; **F:**98.4% | **C:**97.4%; **D:**8.6%; **F:**98.5% | **C:**98.8%; **D:**0.7%; **F:**99.3% | **C:**85.7%; **D:**0.2%; **F:**91.8% |

[1]N50: the length of the contig/scaffold at which 50% of the genome span is accounted, given a list of sequences sorted by length. [2]numN50: the number of sequences required to reach the N50 sequence. [3]CEGMA / BUSCO notation: **C**, proportion (%) genes completely recovered; **D**, duplication rate; **F**, proportion (%) genes at least partially recovered (including complete genes); ***n***, number of queries. Note that duplication rate (D) for CEGMA is given as the average number of (complete) genes recovered, whereas for BUSCO it is the proportion of complete genes recovered multiple times. BUSCO values are based on comparisons to the Arthropoda gene set.

**Table 5:** Summary of *B. anynana* gene prediction.

| | ***B. anynana*** | ***B. mori*** | ***D. plexippus*** | ***H. melpomene*** | ***M. cinxia*** |
|---|---|---|---|---|---|
| **Assembly version** | v1.2 | ASM15162v1 | v3 | Hmel2 | MelCinx1.0 |
| **Number of CDS** | 22642 | 19618 | 15130 | 13178 | 16668 |
| Mean length | 1.4 kb | 1.6 kb | 1.4 kb | 1.3 kb | 958 bp |
| Median length | 1.2 kb | 1.2 kb | 981 bp | 927 bp | 693 bp |
| Min/max | 84 bp / 28.3 kb | 23 bp / 60.3 kb | 9 bp / 58.9 kb | 45 bp / 46.4 kb | 6 bp / 45.4 kb |
| **Introns** | | | | | |
| Mean number per gene | 4.4 | 9.9 | 5.7 | 5 | NA[1] |
| Length (mean/median) | 1.3/0.6 kb | 2.4/0.8 kb | 795/280 bp | 960/416 bp | NA |
| **Exons** | | | | | |
| Length (mean/median) | 208/126 bp | 283/161 bp | 206/149 bp | 284/157 bp | NA |
| **Number of single-exon genes** | 3571 | 1744 | 1461 | 3113 | NA |
| **Transcript GC** | 49.2% | 48.3% | 46.5% | 43% | 41.7% |
| **Gene frequency[2] (genes per Mb)** | 47.7 | 32.1 | 60.9 | 55.5 | NA |

[1]GFF for *M. cinxia* not available; [2]Defined as the number of genes divided by the total genome span (Mb).

Figure 1

Click here to download Figure Fig1_B_anynana.pdf

Figure 2

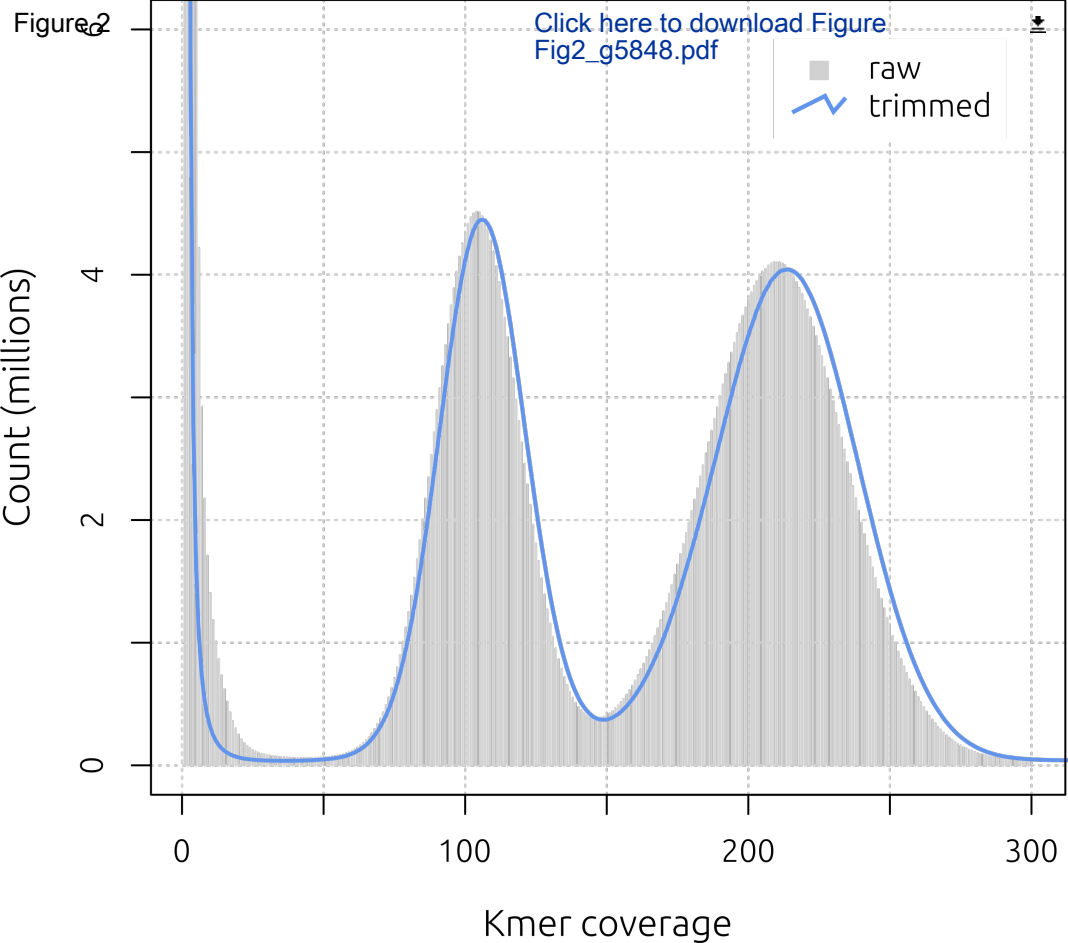
Click here to download Figure
Fig2_g5848.pdf
raw
trimmed
Count (millions)
0
2
4
6
0
100
200
300
Kmer coverage

Figure 3

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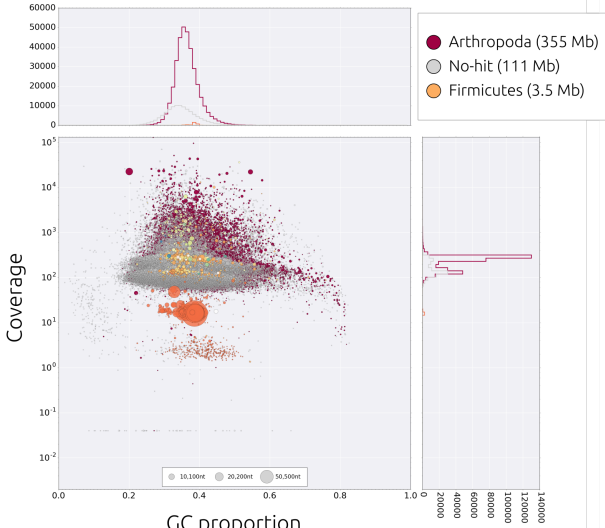


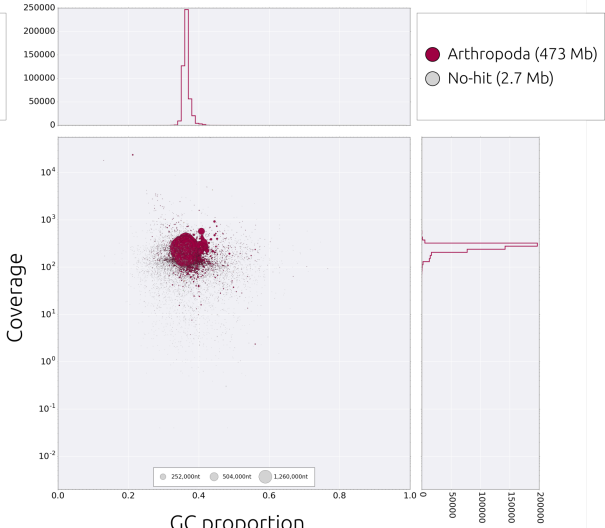

Figure 4

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Fig4_g5525.pdf