

Supplementary material:

SM1: Details of genome assembly pipelines and parameters

The paired-end reads were filtered using Trimmomatic v0.36 (Bolger *et al.* 2014). Adapters were removed using adapters provided by Trimmomatic. Leading and trailing bases below quality 3 were removed. Reads were scanned using a 4-base sliding window and trimmed when the average quality dropped below 20. Reads were discarded if the size dropped below 60 bp. (parameters: PE ILLUMINACLIP:TruSeq-PE.fa:2:30:10:4 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 AVGQUAL:20 MINLEN:60).

The mate-pair reads were filtered using NxTrim v0.4.1 (O'Connell *et al.* 2015) (parameters: --preserve-mp --separate). As a result, mate-pair reads flagged as MP and UNKNOWN were concatenated following the authors' suggestion. Finally, the concatenated mate-pair reads were trimmed using Trimmomatic with the same parameters described previously.

We employed non-standard methods for genome assemblies due to uneven coverage produced by PCR-based whole-genome amplification (Chen *et al.* 2013; Oyola *et al.* 2014). Only the overlapped libraries were used for contig assemblies. Filtered paired-end reads were merged into single reads by overlap detection using BBDiff v36.59 (Bushnell 2014) and the module BBMerge (parameters: minoverlap=15 mismatches=0 ecct strict). The merged reads were then concatenated with the rest of the single reads and normalization was performed using the module BBNorm with a target average depth of 65x.

Normalized data were *de novo* assembled using SPAdes v3.10.1 (Bankevich *et al.* 2012) (parameters: --careful -k 21,33,55,77,99,111,127). Contigs were then scaffolded with SSPACE v3.0 (Boetzer *et al.* 2012) using the remaining libraries and gap-filled with GapCloser v1.12-r6, a module of the SOAP package (Luo *et al.* 2012) with default parameters. Then, the genome assemblies were decontaminated using BlobTools v1.0 (Laetsch & Blaxter, 2017) under the taxrule "bestsumorder". Hit files were generated after a blastn v2.7.1+ against the NCBI nt database (v 2016-06), searching for hits with an e-value below 1e-25 (parameters: -max_target_seqs 10 -max_hsps 1 -evalue 1e-25). Scaffolds without hits to metazoans were filtered out from the assemblies.

The completeness of genomes assemblies was assessed with BUSCO v3.0.2 (Seppey *et al.* 2019) against the *arthropoda_odb9* lineage and the --long option.

SM2: Annotation of protein coding genes

Raw paired-end RNA-sequence reads were trimmed according to quality with Trimmomatic v0.36 (Bolger *et al.* 2014) using the maximum information approach with tolerant parameters.

Any reads with a sequence length of < 80 bp after trimming were discarded (parameters: adapters.fa:2:30:12:1:true LEADING:3 TRAILING:3 MAXINFO:40:0.4 MINLEN:80). All trimmed RNA-sequence reads were then mapped against the genomes using STAR v2.5.3a (Dobin *et al.* 2013) under the "2-pass mapping" mode and default parameters. Then, the STAR outputs were used to produce transcriptome assemblies using Trinity v2.5.1 (Haas *et al.* 2013) "genome guided" mode (parameters: --genome_guided_max_intron 100000 --SS_lib_type RF --jaccard_clip). Finally, the transcriptome assemblies were filtered following Trinity developers recommendations

(<https://github.com/trinityrnaseq/trinityrnaseq/wiki/Trinity-FAQ>):

Briefly, filtered RNA-seq reads were mapped back against the transcriptomes using Kallisto v0.43.1 (Bray *et al.* 2016) with options --bias and --rf-stranded then transcripts with at least 1 TPM in any samples were retained.

Protein coding genes were predicted using MAKER v2.31.8 (Holt & Yandell 2011) in a 2-iterative way described in Campbell *et al.* (2014) with minor modifications following author recommendations. Only scaffolds above 500bp were annotated. Prior to gene prediction, MAKER used RepeatMasker v4.0.7 (Tarailo-Graovac & Chen 2009) for masking repetitive regions. For the first iteration, genes were predicted using Augustus v3.2.3 (Stanke *et al.* 2006) trained with the BUSCO v3.0.2 (Seppey *et al.* 2019) results (SM1). A combination of UniProtKB/Swiss-Prot (release 2018_01) and the BUSCO *arthropoda_odb9* proteome were used as protein evidence. The Trinity assembled mRNA-seq reads (described above) were used as transcript evidence. The resulting gene models were then used to retrain Augustus as well as SNAP v2013.11.29 (Korf 2004) and a second iteration was performed. Subsequently, predicted protein coding genes were functionally annotated using Blast2GO v5.5.1 (Conesa *et al.* 2005; Götz *et al.* 2008) with default parameters against the NCBI *non-redundant arthropods* protein database (v 2018-10).

SM3: Estimation of genome size and heterozygosity by genome profiling analysis

Raw paired-end resequencing reads were trimmed using the same strategy as described in SM2. K-mer frequencies were computed using KMC v3.1.1 (Kokot *et al.* 2017). Genome sizes and heterozygosities were estimated with GenomeScope v2.0 (Ranallo-Benavidez *et al.* 2020) using parameters recommended by the authors.

Table S1: Overview of RNA sequencing reads from ostracods in GenBank.

No full assemblies or annotations are available from these studies. # of genes = total number of orthologous genes used for phylogenetic or gene expression studies, respectively.

Order	Species	GenBank accession number	Sequencing technique	# of genes	Study purpose	Reference
Mydocopida	<i>Vargula tsujii</i>	SRX532406	454	261	Phylogeny of pancrustaceans	Oakley <i>et al.</i> 2012
Mydocopida	<i>Euphilomedes morini</i>	SRX1097309	454	261	Phylogeny of pancrustaceans	Oakley <i>et al.</i> 2012
Mydocopida	<i>Alternochelata lizardensis</i>	PRJNA436031	Illumina		1KITE project	unpublished
Mydocopida	<i>Vargula hilgendorfii</i>	SRX884494	Illumina		1KITE project	unpublished
Mydocopida	<i>Vargula hilgendorfii</i>	DRX059606	Illumina			unpublished
Mydocopida	<i>Eusarsiella sp.</i>	SRX2085852	Illumina	244	Phylogeny of pancrustaceans	Lozano-Fernandez <i>et al.</i> 2019
Mydocopida	<i>Cylindroleberidinae</i>	SRX2085850	Illumina	244	Phylogeny of pancrustaceans	Lozano-Fernandez <i>et al.</i> 2019
Mydocopida	<i>Rutiderma sp.</i>	SRX2458829	Illumina	277	Phylogeny of pancrustaceans	Schwenter <i>et al.</i> 2018
Halocyprida	<i>Conchoecia obtusata</i>	SRX2458823	Illumina	277	Phylogeny of pancrustaceans	Schwenter <i>et al.</i> 2018
Podocopida	Undetermined; family Cypridinae	JL207200	454	822	Phylogeny of pancrustaceans	Von Reumont <i>et al.</i> 2012
Podocopida	<i>Heterocypris incongruens</i>	ICLE01000001	Illumina	4	Expression of antioxidant genes	Hiki <i>et al.</i> 2019
Podocopida	<i>Pontocypris mytiloides</i>	SRX4048873	Illumina	277		unpublished
Podocopida	<i>Paranesidea sp.</i>	SRX2085851	Illumina	277	Phylogeny of pancrustaceans	Schwenter <i>et al.</i> 2018
Podocopida	<i>Pterygocythereis sp.</i>	SRX2458816	Illumina	277	Phylogeny of pancrustaceans	Schwenter <i>et al.</i> 2018

Table S2: Origin of biological material.

All species were collected in Belgium in 2018. The species printed in blue is a putative ancient asexual, species printed in red are sexually reproducing.

Species	Population name	Habitat
<i>Cyprideis torosa</i>	Dievengat	Natural brackish lake
<i>Darwinula stevensoni</i>	Zaventem	Artificial lake
<i>Notodromas monacha</i>	Overijse	Artificial lake

Table S3: Statistics of ostracod genome sequence data.

bp = basepairs. G = giga. The coverage is estimated from final assembly sizes. The species printed in blue is a putative ancient asexual, species printed in red are sexually reproducing. Coverage is provided in %.

Species	Data type	Insert size (bp)	Yield (Gbp)	Coverage
<i>Cyprideis torosa</i>	Paired-end 2x150 bp	250-300	45.8	136.9
		350	41.3	123.3
		550	16.2	48.5
	Mate-pair 2x150 bp	3000	13.6	40.4
		5000	12.2	36.5
<i>Darwinula stevensoni</i>	Paired-end 2x150 bp	250-300	51.8	135.5
		350	39.1	102.2
		550	13.0	34.0
	Mate-pair 2x150 bp	3000	18.7	48.9
		5000	12.2	32.0
<i>Notodromas monacha</i>	Paired-end 2x150 bp	250-300	36.9	98.0
		350	51.3	136.2
		550	15.0	39.9
	Mate-pair 2x150 bp	3000	15.5	41.1
		5000	13.4	35.6

Table S4: Statistics of ostracod transcriptome sequence data.

Data type: paired-end 2x 101bp. bp = basepairs. G = giga. The species printed in blue is a putative ancient asexual, species printed in red reproduce sexually.

Species	Description	Yield (Gbp)
<i>Cyprideis torosa</i>	Pool 1 (40 males and females)	7.2
	Pool 2 (40 males and females)	8.2
	Pool 3 (40 males and females)	10.1
	Pool 4 (40 males and females)	9.8
<i>Darwinula stevensoni</i>	Pool 1 (40 females)	6.7
	Pool 2 (40 females)	7.1
	Pool 3 (40 females)	7.4
<i>Notodromas monacha</i>	Pool 1 (40 females)	3.8
	Pool 2 (40 males)	20.2

Table S5: Statistics of ostracod resequencing data.

Data type: paired-end 2x 101bp. bp = basepairs. G = giga. The coverage is estimated from final assembly sizes. The species in blue is a putative ancient asexual, species printed in red reproduce sexually.

Species	Description	Yield (Gbp)	Coverage
<i>Darwinula stevensoni</i>	Female DS_02	21.7	56.7
	Female DS_05	23.9	62.5
<i>Notodromas monacha</i>	Female Nm_F03	20.9	55.6
	Female Nm_F07	24.0	63.8

Table S6: Statistics of genome assemblies of ostracod species.

Σ represents the sum of all scaffolds in million basepairs (Mbp). The average N50 was calculated per scaffold in kilo basepairs (Kbp). The BUSCO score is the proportion of conserved single copy ortholog genes among arthropods. N is the proportion of unknown nucleotides (gaps) in the assembly. The species in blue is a putative ancient asexual, species printed in red reproduce sexually.

Species	Σ [Mbp]	N50 [kbp]	BUSCO [%]	Ns [%]
<i>Cyprideis torosa</i>	334.9	19.0	91.8	1.3
<i>Darwinula stevensoni</i>	382.1	56.4	95.8	0.9
<i>Notodromas monacha</i>	376.7	42.3	94.4	1.6

Table S7: Statistics of protein coding gene annotations and other gene features in ostracod genomes.

bp = basepairs. The genome coverage is the proportion of each genome covered by genes. Red species reproduce sexually, while the species indicated in blue is a putative ancient asexual.

Species	Gene count	Transcript count	Average gene length [bp]	Genome coverage [%]
<i>Cyprideis torosa</i>	17776	18069	4734	25.1
<i>Darwinula stevensoni</i>	15453	15922	7558	30.6
<i>Notodromas monacha</i>	13771	14294	7040	25.7

Table S8: Annotations and gene features of crustacean genomes of the last four years and of the current study.

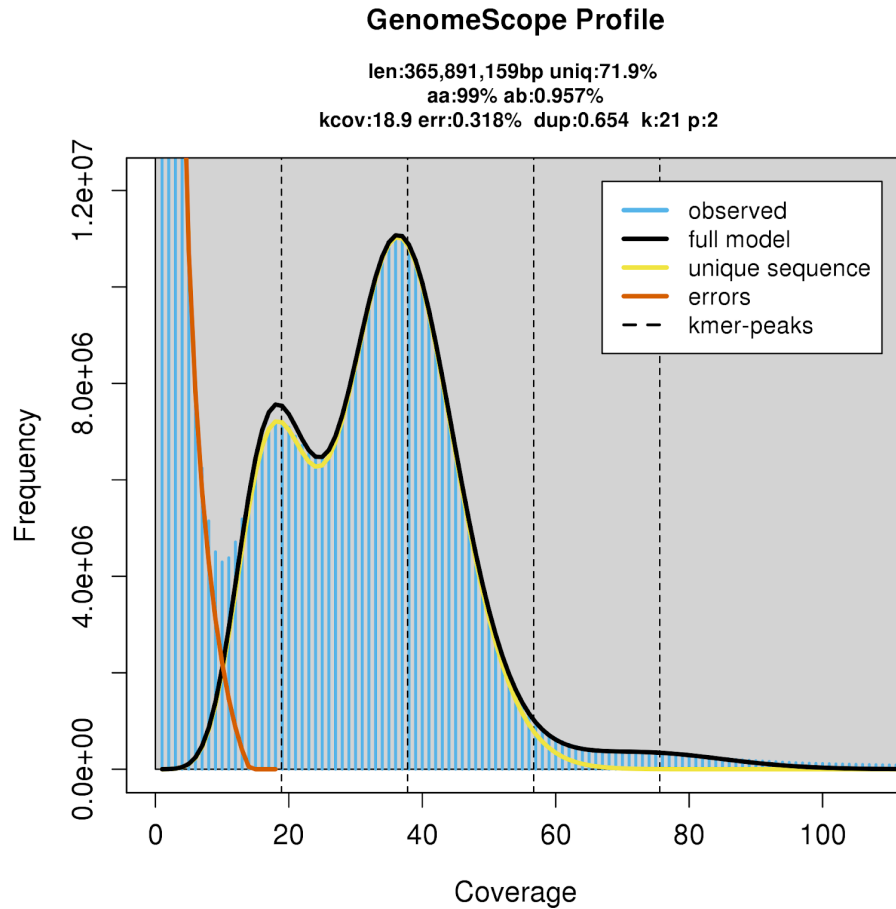
Average gene, intron and exon sizes are provided in base pairs.* including all transcripts from different experiments and life stages. n. i. = no information.

Class	Order	Species	Predicted genes	Gene size	Intron size	Exon size	Predicted transcripts	Reference
Branchiopoda	Diplostraca	<i>Daphnia pulex</i>	18,440	2,998	223	237	177,598*	Ye <i>et al.</i> 2017
Branchiopoda	Diplostraca	<i>D. magna</i>	15,721	2,407	392	n. i.	25,997	Lee <i>et al.</i> 2019
Branchiopoda	Notostraca	<i>Lepidurus arcticus</i>	10,718	n. i.	269	233	n. i.	Savojardo <i>et al.</i> 2018
Branchiopoda	Notostraca	<i>L. apus lubbocki</i>	16,383	n. i.	258	253	n. i.	Savojardo <i>et al.</i> 2018
Branchiopoda	Spinicaudata	<i>Eulimnadia texana</i>	17,667	n. i.	n. i.	n. i.	23,965	Baldwin-Brown <i>et al.</i> 2017
Copepoda	Cyclopoida	<i>Apocylops royi</i>	29,730	n. i.	n. i.	n. i.	45,756	Jørgensen <i>et al.</i> 2019
Copepoda	Cyclopoida	<i>Oithona nana</i>	15,359	2,477	779	391	n. i.	Madoui <i>et al.</i> 2017
Copepoda	Harpaticoida	<i>Tigriopus californicus</i>	14,233	4,760	n. i.	n. i.	14,233	Barreto <i>et al.</i> 2018
Copepoda	Harpaticoida	<i>T. japonicus</i>	25,143	4,174	n. i.	n. i.	n. i.	Jeong <i>et al.</i> 2020
Copepoda	Harpaticoida	<i>T. kingsejongensis</i>	12,772	6,443	n. i.	n. i.	20,392	Kang <i>et al.</i> 2017
Ostracoda	Podocopida	<i>Cyprideis torosa</i>	17,776	4,734	749	272	18,069	Current study
Ostracoda	Podocopida	<i>Darwinula stevensoni</i>	15,453	7,558	961	235	15,922	Current study
Ostracoda	Podocopida	<i>Notodromas monacha</i>	13,771	7,040	805	264	14,294	Current study
Malacostraca	Amphipoda	<i>Parhyale hawaiiensis</i>	28,165	20,000	5,400	n. i.	26,715	Kao <i>et al.</i> 2016
Malacostraca	Isopoda	<i>Armadillidium vulgare</i>	19,051	8,639	1,506	213	n. i.	Chebbi <i>et al.</i> 2019
Malacostraca	Decapoda	<i>Cherax quadricarinatus</i>	19,494	9,768	n. i.	n. i.	n. i.	Tan <i>et al.</i> 2020
Malacostraca	Decapoda	<i>Eriocheir japonica sinensis</i>	12,772	n. i.	1,693	226	n. i.	Tang <i>et al.</i> 2020
Malacostraca	Decapoda	<i>Palaeomon carinicauda</i>	65,772	1,386	n. i.	n. i.	n. i.	Li <i>et al.</i> 2019
Malacostraca	Decapoda	<i>Penaeus monodon</i>	36,685	n. i.	n. i.	n. i.	n. i.	Van Quyen <i>et al.</i> 2020
Malacostraca	Decapoda	<i>Litopenaeus vannamei</i>	25,596	n. i.	n. i.	259	n. i.	Zhang <i>et al.</i> 2019
Malacostraca	Decapoda	<i>Marsupenaeus japonicus</i>	16,716	n. i.	n. i.	n. i.	n. i.	Yuang <i>et al.</i> 2018
Malacostraca	Decapoda	<i>Procambarus virginalis</i>	> 21,000	6,700	2,000	300	22,338	Gutekunst <i>et al.</i> 2018

Figure S1 A-D: Results of GenomeScope analyses.

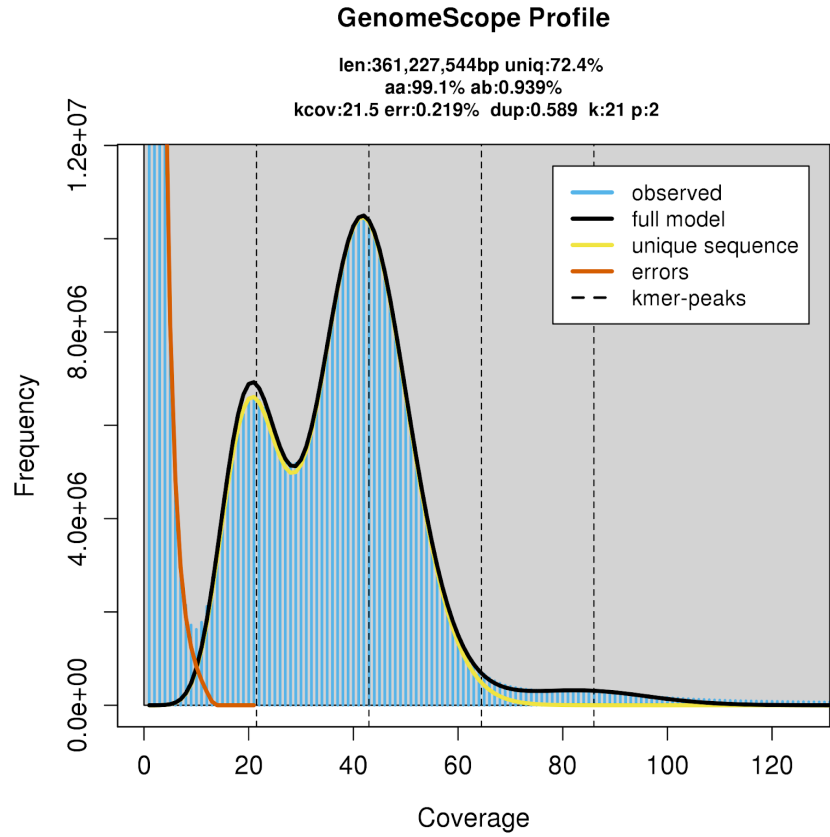
Two individuals each of *Darwinula stevensoni* (Figure S1 A-B) and *Notodromas monacha* (Figure S1 C-D) were resequenced and their reads analyzed to estimate genome sizes and heterozygosities.

Figure S1 A – DS_02



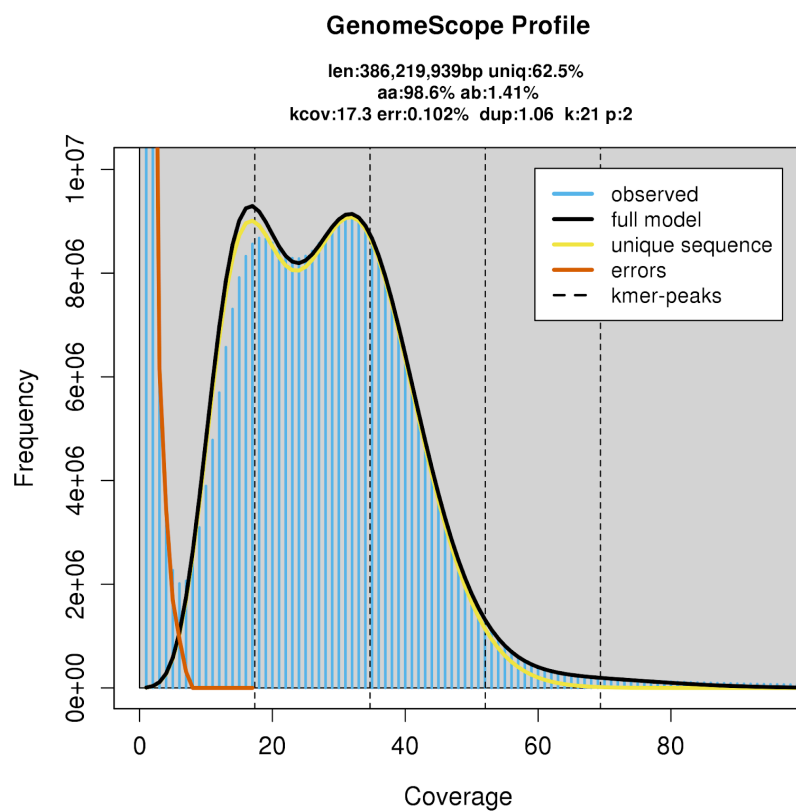
property	min	max
Homozygous (aa)	99.0133%	99.0724%
Heterozygous (ab)	0.9276%	0.9867%
Genome Haploid Length	365,167,484 bp	365,891,159 bp
Genome Repeat Length	102,728,873 bp	102,932,457 bp
Genome Unique Length	262,438,611 bp	262,958,702 bp
Model Fit	77.2855%	98.7670%
Read Error Rate	0.3181%	0.3181%

Figure S1 B – DS_05



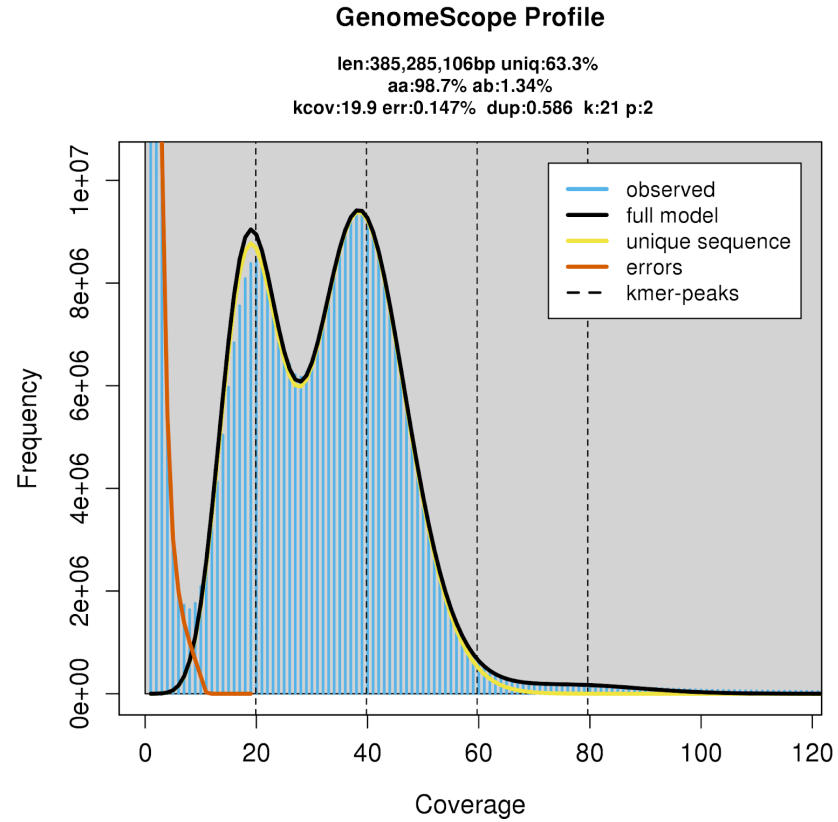
property	min	max
Homozygous (aa)	99.0465%	99.0755%
Heterozygous (ab)	0.9245%	0.9535%
Genome Haploid Length	360,688,136 bp	361,227,544 bp
Genome Repeat Length	99,559,402 bp	99,708,293 bp
Genome Unique Length	261,128,735 bp	261,519,252 bp
Model Fit	77.5377%	98.1080%
Read Error Rate	0.2192%	0.2192%

Figure S1 C – Nm_F03



property	min	max
Homozygous (aa)	98.5730%	98.6159%
Heterozygous (ab)	1.3841%	1.4270%
Genome Haploid Length	385,081,521 bp	386,219,939 bp
Genome Repeat Length	144,297,289 bp	144,723,876 bp
Genome Unique Length	240,784,232 bp	241,496,063 bp
Model Fit	64.0406%	97.5643%
Read Error Rate	0.1021%	0.1021%

Figure S1 D – Nm_F07



property	min	max
Homozygous (aa)	98.6515%	98.6778%
Heterozygous (ab)	1.3222%	1.3485%
Genome Haploid Length	384,661,719 bp	385,285,106 bp
Genome Repeat Length	141,131,428 bp	141,360,147 bp
Genome Unique Length	243,530,291 bp	243,924,959 bp
Model Fit	65.1164%	98.0966%
Read Error Rate	0.1470%	0.1470%

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