A High-quality Oxford Nanopore Assembly of the Hourglass Dolphin (*Lagenorhynchus cruciger*) Genome

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Abstract

The hourglass dolphin (*Lagenorhynchus cruciger*) is a small cetacean species of the Southern Ocean, with significance to iwi Māori (*Māori tribes*) of Aotearoa New Zealand as taonga (*treasured/valued*). Due to the remoteness and difficulty of surveying Antarctic waters, it remains one of the least-studied dolphin species. A recent stranding of an hourglass dolphin represented a rare opportunity to generate a genome assembly as a resource for future study into the conservation and evolutionary biology of this species. In this study, we present a high-quality genome assembly of an hourglass dolphin individual using a single sequencing platform, Oxford Nanopore Technologies, coupled with computationally efficient assembly methods. Our assembly strategy yielded a genome of high contiguity (N50 of 8.07 Mbp) and quality (98.3% BUSCO completeness). Compared to other Delphinoidea reference genomes, this assembly has fewer missing BUSCOs than any except *Orcinus orca*, more single-copy complete BUSCOs than any except *Phocoena sinus*, and 20% fewer duplicated BUSCOs than the average Delphinoidea reference genome. This suggests that it is one of the most complete and accurate marine mammal genomes to date. This study showcases the feasibility of a cost-effective mammalian genome assembly method, allowing for genomic data generation outside the traditional confines of academia and/or resource-rich genome assembly hubs, and facilitating the ability to uphold Indigenous data sovereignty. In the future the genome assembly presented here will allow valuable insights into the past population size changes, adaptation, vulnerability to future climate change of the hourglass dolphin and related species.

Introduction

The hourglass dolphin (*Lagenorhynchus cruciger)* is a small cetacean species that inhabits pelagic Antarctic and sub-Antarctic waters. Although its northernmost range includes Aotearoa New Zealand, where it is considered a taonga (*treasured)* species by iwi Māori (*Māori tribes*), the hourglass dolphin is strongly associated with the Antarctic Convergence, and rarely found in close proximity to land masses (Acevedo, Garthe, and González 2017; Dellabianca et al. 2012; Santora 2012). Although not an uncommon species (Goodall et al. 1997), due to the remoteness and difficulty of surveying Antarctic waters, it is one of the least studied species of dolphin (Dellabianca et al. 2012; Goodall et al. 1997), with information largely limited to observations from living animals: group size, locality, acoustics, and presence of calves (Goodall 1997; Acevedo, Garthe, and González 2017; Dellabianca et al. 2012; Tougaard and Kyhn 2009; Kyhn et al. 2009; Thiele, Chester, and Gill 2000; Todd and Williamson 2022).

For an enigmatic oceanic species such as the hourglass, the few beach stranding events that have occurred have provided important opportunities to learn more about the physiology and other phenotypes such as diet preference (e.g. (Goodall et al. 1997; Fernandez et al. 2003; Marchesi et al. 2016; Peters et al. 2022). Physical specimens from a stranding also provide an opportunity to obtain genetic material for genome sequencing. These genomes can be a pathway towards understanding past population size changes, adaptation, and vulnerability to changing climate - an aspect particularly important for the hourglass dolphin in the rapidly changing Antarctic environment (MacLeod 2009). In addition, considerable uncertainty remains around the evolutionary relationships between Lissodelphininae - the subfamily of true dolphins to which the hourglass dolphin belongs (Goodall et al. 1997; Vollmer et al. 2019; Harlin-Cognato and Honeycutt 2006; Cope 1866; McGowen 2011; Banguera-Hinestroza et al. 2014; Leduc, Perrin, and Dizon 1999). Genomic data could be particularly useful in resolving such taxonomic confusion, as demonstrated recently in resolving the taxonomic placement of the pygmy right whale (*Caperea marginata*) (Dutoit et al. 2022).

However, the generation and analysis of genomic data can exacerbate pre-existing inequities around who has access to the technology to generate such genetic resources, who gets to decide how these resources are looked after, and who benefits from the generation of such resources (Mc Cartney et al. 2022; Te Aika et al. 2023). This situation is particularly heightened when it comes to sequencing species that are taonga (*treasured*) by Indigenous peoples. In these cases, the tikanga (*protocols*) around protecting and ensuring the safety of tissues and

genetic resources is paramount for upholding Indigenous data sovereignty (Robbins et al. 2023; Jennings et al. 2023).

One pathway toward upholding Indigenous data sovereignty, and ensuring that capacity-building can occur within the communities where rare species are found, is to develop methods for sequencing and genome assembly that are achievable locally. Here, we provide evidence that this is currently possible for mammalian-sized genomes. We present a genomic assembly for an individual hourglass dolphin that relies solely on sequence from the Oxford Nanopore Technologies sequencing platform. We compare multiple basecallers (Guppy and Dorado) and assemblers (Raven, Nextdenovo, and Goldrush), obtaining the most contiguous genome using the Dorado basecaller and the computationally efficient Raven assembler. For most mammalian species, obtaining data such as that utilised in our study is achievable on a single Oxford Nanopore PromethION flow cell for approximately $1500 USD. With the use of a low-overhead RAM assembler such as Raven it is possible to implement the complete assembly pipeline on a high-end gaming laptop (although close to 128 Gb of RAM is required and this is available only on high-end laptop computers). Furthermore, we show that this assembly is, on average, more complete than any other published Delphinoidea genome (fewer missing BUSCOs and more complete single copy BUSCOs), including the highly curated bottlenose dolphin (*Tursiops truncatus*).

While reduced capital outlay and sequencing costs have already allowed for a more equitable distribution of the capability to generate genomic resources, this has rarely extended to include organisms with large or complex genomes. The results here suggest that current genomic technologies can be applied to enhance the ability of Indigenous communities to maintain sovereignty over tissues and data collected from their taonga (*treasured*) species, including rarely sampled species such as the hourglass dolphin.

Methods and Materials

Specimen Collection

On the 5th of August 2020, an individual hourglass dolphin was reported beached at Orepuki Beach in Te Waewae Bay, Murihiku, Aotearoa New Zealand. This individual was given the customary name Hārua-tai, reflecting its connection to the rough seas of the Southern Ocean.

With the permission and full blessing of the tribal authority, Ōraka-Aparima Runaka, we retrieved and necropsied the dolphin on the 29th of September to recover a full suite of samples from major organs including the heart. The customary name, Hārua-tai, was used in addition to a scientific coding system in order to link the whakapapa (*connections*) of the materials recovered from this animal. Cardiac tissue was stored at -80°C until permission from Ōraka-Aparima was granted to undertake this co-designed study to assemble the genome of the hourglass dolphin.

DNA Isolation and Sequencing

We divided the cardiac tissue into two subsamples (21 mg and 25 mg) and isolated DNA from each using the Monarch Genomic DNA Purification Kit. We lysed the tissue for a total of 6.5 hours, agitating at 600 rpm for the first 30 minutes and 300 rpm thereafter. During both these lysis-agitation steps, we incubated the sample at 56ºC. We prepped each DNA sample for Oxford Nanopore sequencing using the ligation sequencing kit (SQK-LSK114) according to the manufacturer's instructions. We sequenced the samples on two sequential days on two PromethION R10.4.1 flow cells on a P2 Solo instrument for 68.5 hours and 72 hours. We stored the DNA at -20C for three months and performed a third sequencing run using the latter sample, which had exhibited a higher read N50 (7.2 kilobase pairs (kbp) vs. 9.5 kbp).

Bioinformatics Pipeline Overview

We implemented a straightforward strategy for basecalling and filtering, assembly, assessment of assembly contiguity and completeness, haplotig purging polishing, annotation, and variant calling and phasing (**Fig. 1**).

Basecalling and Filtering

We basecalled the fast5 files using *Guppy* v.6.3.9. with the dna_r10.4.1_e8.2_400bps_sup model. We also converted fast5 files to pod5 files using *pod5 convert* from the pod5 package of tools. We basecalled the pod5 files using *dorado* v0.3.0 (*Dorado* 2023) and the dna_r10.4.1_e8.2_400bps_sup@v4.1.0 model and converted the resultant .bam file to .fastq format using *samtools fastq* v1.17 (Danecek et al. 2021). To remove control DNA, we first mapped all reads to the control lambda phage genome using *minimap2* (Li 2018) and removed all mapped reads using *samtools view -f 4*. We filtered the fastq files using *chopper* (De Coster and Rademakers 2023) to retain only reads with average quality scores above 10 and lengths

greater than 2 kbp. We also cropped 50 bp from the head and tail of each read. Using 16 threads, this process took 20 minutes and minimal RAM. For downsampling the read data to 90Gb, we used filtlong (Wick and Menzel 2017), prioritising read length (--length_weight 10).

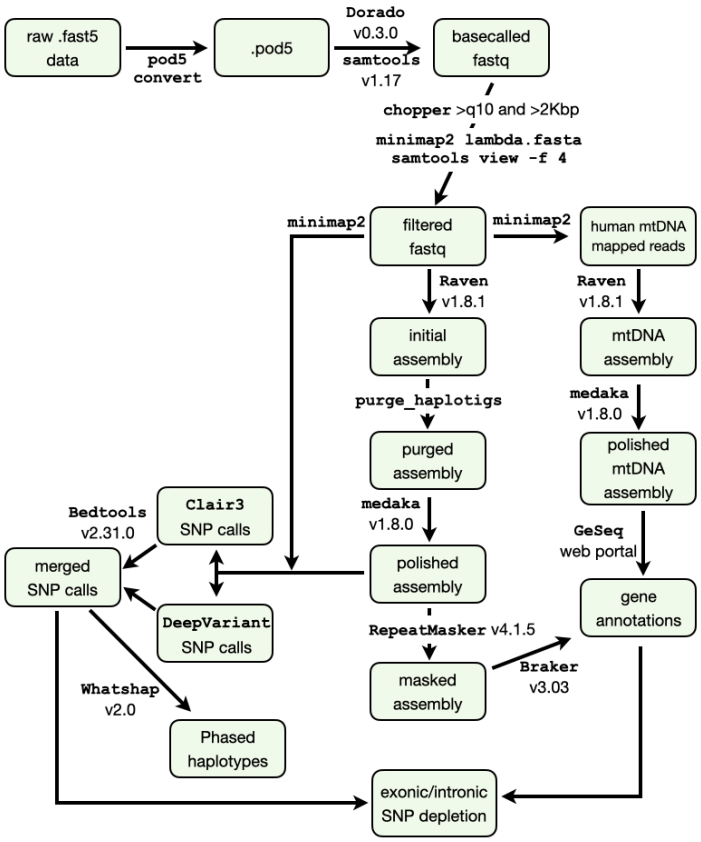

Figure 1. Flowchart of Raven-based assembly. Each software program is noted in Courier serif font; boxes show the results of each step; see methods for details on versions and specific arguments.

Assembly

We then assembled both the Dorado-called and Guppy-called data using three different assemblers with RAM requirements less than 400 GB, and which are optimised for Oxford Nanopore data: Goldrush v1.0.1 (Wong et al. 2023), Nextdenovo v2.5.2 (Hu et al. 2023), and

Raven v1.8.1 (Vaser and Šikić 2021). Using Raven with the Dorado data took a total time of 16 hours and a maximum of 153 Gb RAM, averaging approximately 130 Gb of RAM (**Supp. Fig. 1**)

For mitochondrial genome assembly, we mapped all reads to the human mtDNA sequence, filtered those reads using chopper (De Coster and Rademakers 2023) to include only reads between 14Kbp and 17Kbp (the approximate size of the full length mitochondrial genome), and subsampled this filtered set using *SeqKit* (Shen et al. 2016) to include only 5% of the reads (due to the extremely high coverage). We assembled these reads using Raven.

We calculated NG50 using Quast v5.2.0 (Gurevich et al. 2013), assuming a genome size of 2.384 Gbp.

Haplotig Purging and Assembly Polishing

We used purge_haplotigs (Roach, Schmidt, and Borneman 2018) to remove contigs from the assembly that had lower than expected coverage or which were highly similar to other contigs; these were judged as being haplotigs.

We used medaka v1.8.0 (*Medaka* 2023) to polish the assembly using the *r1041_e82_400bps_sup_v4.1.0* model.

In the most contiguous assembly, Dorado-basecalled data assembled with Raven, we found a short contig of 1,396 bp, with the next smallest contig at almost 25 Kbp). Upon blasting this sequence we found it was 99% identical to a sequence from *Bos taurus*. We removed this contig from the assembly, a likely contaminant from other lab samples. We also used Kraken2 (Wood et al. 2019) to determine the taxon assignment for all other contigs in the assembly and found that all were vertebrate in origin, suggesting no microbial contamination. In addition, given the above contaminant *B. taurus* contig, we used mash (Ondov et al. 2016) to calculate approximate distances for each contig to the reference *Bos taurus* genome and the *Tursiops truncatus* genome using 500,000 min-hashes. All contigs were closer to *T. truncatus* than *B. taurus*, although 84 contigs were too small to accurately measure distance using mash. In these cases we mapped the contigs to each reference genome using minimap2 -x lr:hq (Li 2018). For each contig, we found the longest aligned region to either genome and tested whether the *B. taurus* or the *T. truncatus* alignment was longer. The *T. truncatus* alignments were longer except for three contigs with short aligned regions. To test the identity of these contigs, we blasted them against the nr database. The three contigs ranged from 93% to 97% identity to other Delphinid

genomes. Thus, we concluded that no other contigs besides the short 1,396 bp above were contaminants.

To map the correspondence of the *L. cruciger* hourglass assembly contigs to the autosome and sex chromosomes of the *T. truncatus* assembly, we aligned the *L. cruciger* assembly to the *T. truncatus* assembly using quarTeT Assembly Mapper (Lin et al. 2023).

Repeat Regions

We masked repeats using RepeatMasker v4.1.5 (Bogdahn 2015) with the Dfam v3.7 repeat element database, nhmmscan version 3.3.2, and taxa search limited to mammals. We identified telomeric repeats (TTAGGG) using SeqKit (Shen et al. 2016). To calculate kmer repetition in contigs we used jellyfish 2.2.10 (Marçais and Kingsford 2011). For each contig, we counted the frequency of all 21-mers, and then calculated the fraction of 21-mers that were present once vs. more than once. We used SeqKit (Shen et al. 2016) to count N content across regions masked by RepeatMasker.

Assembly Completeness

We assessed assembly completeness using compleasm.py (Huang and Li 2023) and the odb10 versions of five databases: Laurasiatheria, Cetartiodactyla, Eutheria, Mammalia, and Eukarya. We compared the assembly here to nine additional Delphinoidea assemblies (**Supp. Table 1**) to which we applied compleasm in the same way.

Annotation

We annotated the masked genome using Braker v.3.0.3 (Hoff et al. 2016; Brůna et al. 2021; Hoff et al. 2019; Brůna, Lomsadze, and Borodovsky 2020; Buchfink, Xie, and Huson 2015; Gotoh 2008; Lomsadze et al. 2005; Stanke et al. 2008; Iwata and Gotoh 2012; Stanke et al. 2006) and the OrthoDB11 Vertebrate database of protein families (March 3 2023) (Kuznetsov et al. 2023) downloaded from https://bioinf.uni-greifswald.de/bioinf/partitioned_odb11/.
We annotated the mitochondrial genome using GeSeq (Tillich et al. 2017) with the BLAT reference sequences set to all Delphinid mtDNA genomes in RefSeq.

Variant Calling and Phasing

We used Clair3 v1.0.4 (Zheng et al. 2022) to call variants using the Clair3 *r1041_e82_400bps_sup_v410* model. We observed a clear dip in the quality of SNV calls at a quality score of 14 (**Supp. Fig. 2**), and using *rtg vcffilter* v3.12.1 (Cleary et al. 2014), we filtered the calls to include only those with qualities above 14. We also used DeepVariant (Poplin et al. 2018) with *--model_type=ONT_R104*. In this case there was a clear dip in the quality of calls at a quality score of 20 (**Supp. Fig. 2**), and retained only calls with scores above that. Finally, we obtained a set of high quality calls by intersecting these two call sets using *bcftools* v1.17 (Danecek et al. 2021) *norm* and *isec*. We then used bedtools v2.31.0 (Quinlan and Hall 2010) to find intersections between individual bed files of genic, exonic, and intronic regions and these variant calls.

We phased variant calls using *Whatshap* v2.0 (Martin et al. 2016). To visualise the phasing we used *whatshap haplotag* and IGV v2.1.62 (Thorvaldsdóttir, Robinson, and Mesirov 2013).

Results

Using Dorado, overall we obtained 8.51 million reads and 39.6 Gbp; 4.96 million reads and 31.3 Gbp; and 20.4 million reads and 110.2 Gbp of data from the three Oxford Nanopore runs, respectively. The N50 values for these runs were 7.2 kilobase pairs (Kbp), 9.5 kbp, and 8.4 kbp. After pooling these three runs and filtering, using Dorado, 20.3 million reads and 142.6 Gbp remained, with an N50 of 9.04 kbp; using Guppy and filtering the pooled data resulted in 21.1 million reads and 145.9 Gbp, with an N50 of 8.88 kbp. Using both datasets and all three assemblers (Goldrush, Raven, and Nextdenovo, we found that contiguity differed considerably (**Table 1**), with the Dorado-basecalled Raven assembly having the longest contig NG50 and maximum contig length (8.08 Mbp and 39.1 Mbp, respectively. Both of these are almost 50% longer than the least contiguous method, Guppy with Goldrush.

We obtained 31 Mbp of reads (subsampled to include only 5% of total number) between 14 Kbp and 17 Kbp that mapped to the human mitogenome, with an average length of 15,923 bp. Using Dorado-basecalled data and Raven (the most successful whole-genome combination), we assembled a circular 16,389 bp mitochondrial contig. This was 96.9% identical to the 16,392 bp Pacific white-sided dolphin *L. obliquidens* mitogenome (Lee et al. 2018) and 96.6% identical to

the 16,371 bp Heaviside's dolphin *C. heavisidii* mitogenome (Hassanin et al. 2012), the two closest matches in the NCBI database.

Table 1. Assembly contiguity across basecallers and assemblers. The statistics below are for assemblies before polishing, haplotig purging, or contaminant removal.

Assembler	Basecaller	Number of contigs	Total length (Gbp)	Min. contig length	Max. contig length (Mbp)	NG50 (Mbp)
Goldrush	Guppy	40,014	2.57	1	28.7	5.68
Goldrush	Dorado	29,863	2.44	1	24.5	6.29
Nextdenovo	Guppy	1,090	2.32	15,220	28.4	6..55
Nextdenovo	Dorado	1,098	2.32	16,390	27.5	6.42
Raven	Guppy	1,289	2.45	1,380	24.9	7.32
Raven	Dorado	1,243	2.44	1,379	39.1	8.08
Raven	Dorado Q16	1,187	2.40	10,313	30.6	6.38

Assembly Completeness Across Assemblers

We quantified assembly completeness using compleasm (Huang and Li 2023), a new implementation of BUSCO (Simão et al. 2015) using the Laurasiatheria odb10 database. Again we found that the Dorado-basecalled Raven assembly had the highest number of complete single copy BUSCOs and the fewest fragmented or missing BUSCOs compared to the other five baseball-assembly combinations. (**Supp. Table 2**). Due to the Dorado-based Raven assembly having the highest contiguity and BUSCO completeness, we used this assembly as the basis for the remainder of the analyses here.

Haplotig Purging and Assembly Polishing

We performed a single round of polishing and purging of haplotigs, resulting in a final assembly 2.384 Gbp in length, with 894 contigs, an NG50 of 8.074 Mbp, an L50 of 89, a maximum contig length of 39.03 Mbp, and a minimum contig length of 24,985 bp. This assembly size is similar to the total chromosomally-scaffolded portions of other delphinid species such as the bottlenose dolphin *Tursiops truncatus* (2.343 Gbp), the common dolphin *Delphis delphinus* (2.364 Gbp), the white-beaked dolphin *Lagenorhynchus albirostris* (2.404 Gbp) and the long-finned pilot whale *Globicephala melas* (2.364 Gbp). However, it is considerably smaller when including the unplaced scaffolds of these assemblies (2.637, 2.774, 2.767, and 2.651 Gbp, respectively). This

may be due to the small repetitive contigs in this *L. cruciger* assembly being artifactually collapsed.

To estimate the copy number of each contig, we mapped the raw reads back onto the assembly and filtered the mapped reads to include only those with a mapping quality greater than 20. We found that non-repetitive contigs (see below) had largely bimodal read depths, with the majority having a depth of approximately 52 and a smaller number having a depth of approximately 26 (**Fig. 2**), which we inferred were contigs belonging to the X and Y chromosomes in this male individual. We confirmed this by aligning the contigs to the *T. truncatus* genome assembly (a female individual). We found that out of the 129 non-repetitive contigs with median depth less than 30, 112 aligned to the X chromosome, four did not align, ten aligned to unplaced scaffolds, and three aligned to autosomes.

However, the *T. truncatus* is a female individual and has no Y chromosome. Of the four unaligned contigs (176 kbp, 103 kbp, 74 kbp, and 70 kbp in length), the top blast hits by bit score were, respectively: *Bos taurus* chr Y (80% identity across the alignment, but 93% identity to *D. delphis* chr 12); *G. melas* chr 19 (95% identity); *Balaenoptera acutorostrata* chr 19 (81% identity), and *D. delphis* chr Y (92% identity). Thus, it appears that some unmapped contigs in this hourglass assembly are homologous to other species' Y chromosomes. The match of a single contig with both the *B. taurus* Y chromosome and *D. delphis* chr 12 suggests that other assemblies may have sex chromosome contigs assigned to autosomal contigs.

A number of contigs had lower coverage of high-quality mapped reads, as well as GC percentages in a very narrow range (**Fig. 2**). These contigs also tended to be smaller (less than 500 kbp). When we quantified nucleotide diversity in these using kmer content, we found that it was extremely low (**Fig. 2A**), with the majority having less than 50% of all 21-mers being unique (**Methods**). For this reason, we suspected that they had been artificially collapsed during assembly, resulting in very few reads mapping with high quality. As expected, when we calculated depth based on all mapped reads rather than just high quality mappers, these contigs exhibited very high coverage (**Fig. 2B**). Upon alignment to the *Tursiops truncatus* genome, which is one of the most well-curated delphinoid assemblies, we found that 181 out of the 254 highly repetitive contigs (unique kmer content less than 90%) mapped to unplaced *T. tursiops* scaffolds, and were enriched for the telomeric motif TTAGGG (Zhong et al. 1992): 235 out of 254 were more than four-fold enriched for this motif compared to only 16 of the 640

non-repetitive contigs. This suggests that some of these are artifactually collapsed subtelomeric or telomeric regions.

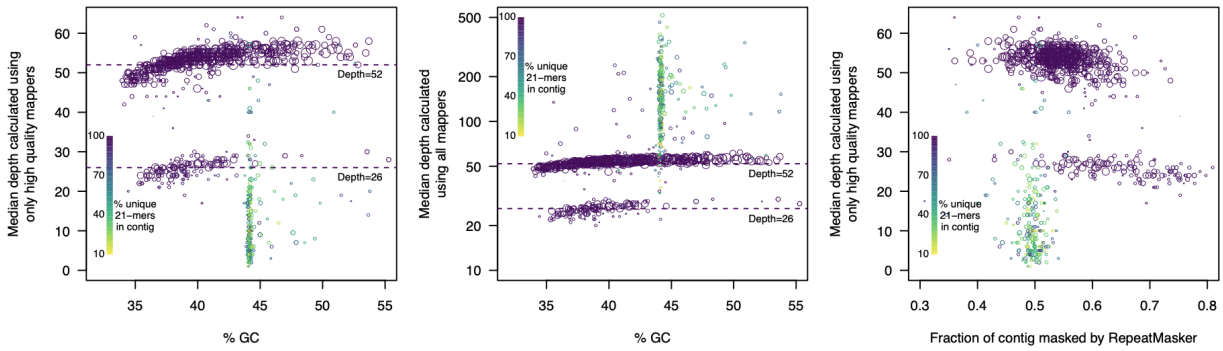

Figure 2. Sequencing depth, GC content, and repetitiveness varies across contigs. A. GC-content vs. depth calculated using high quality mappers. Each contig is represented by a circle that is scaled to the log of contig size. Depth was largely bimodal, with the lower mode (approximately 26) corresponding to the X and Y chromosomes in this male individual. A slight bias toward lower depth in very GC-poor contigs was apparent (visible as an upward slope as % GC increases). A small number of contigs exhibited very similar %GC values (approximately 44%); these contigs had low nucleotide diversity as measured by kmer diversity (green and yellow circles). The low nucleotide diversity of these contigs also resulted in very few high quality mappers and thus low depth. **B**. **GC-content vs. depth calculated using all mapped reads.** The same plot as in (**A**) but when all mapped reads are included. In this case, contigs with low nucleotide diversity exhibited extremely high depth, suggesting that most arose from hard-to-resolve genomic regions that were collapsed during assembly. Note that the scale of the y-axis is linear for (**A**) and logarithmic for (**B**) due to the high coverage values for some repetitive contigs (up to 500-fold). **C. Masked contig fraction vs. depth**. Colours are identical to (**A**) and (**B**). The x-axis indicates the fraction of each contig that was designated by RepeatMasker as containing repetitive elements (e.g. LINEs, SINEs, LTRs) and thus masked with Ns. Contigs that have low nucleotide diversity are depleted for repetitive genomic elements (green points), with most having approximately 50% repetitive element content as opposed to almost 60% for most autosomal contigs.

To further characterise these problematic contigs, we examined the repeat element content. We first used RepeatMasker to determine repetitive genomic elements. 51% of the genome was designated as consisting of repetitive elements, including 111 Mbp of SINE elements (4.68% of the total genome), 704 Mbp of LINE elements (29.6%), and 236 Mbp of LTR elements (9.90%). Notably, the low nucleotide diversity contigs (few unique kmers) were depleted for repetitive

elements (**Fig. 2C**). This supports the hypothesis that these are not collapsed due to repetitive elements; rather they are collapsed due to extremely low nucleotide diversity and are possibly telomeric, centromeric, or parts of the Y chromosome.

Annotation

We annotated the repeat-masked genome with Braker3, resulting in 129,464 exons and 104,187 introns with mean lengths of 157 bp and 1,509 bp, respectively. 25,329 protein coding genes in total were annotated, with an average length of 268 amino acids and a maximum length of 4,065 amino acids. This number of protein coding genes is considerably larger than the number annotated in *T. truncatus* (19,240) despite this assembly having lower BUSCO scores. This is most likely due to differences in annotation methods, as *T truncatus* was annotated using the NCBI eukaryotic genome annotation pipeline, which relies on Prosplign and Gnomon.

The annotated mitochondrial genome had the expected 22 tRNAs, 12S and 16S ribosomal RNAs, and 13 coding sequences.

Table 2. Assembly completeness across Delphinoidea. Each column indicates the total number of single, duplicated, missing, or fragmented BUSCOs as found by Compleasm.

Species	Single copy	Duplicated	Missing	Fragmented
Phocoena sinus	12072	80	59	23
Lagenorhynchus cruciger	12056	96	48	34
Stenella coeruleoalba	12055	87	73	19
Delphinus delphis	12049	94	69	22
Orcinus orca	12048	129	41	16
Lagenorhynchus obliquidens	12043	112	53	26
Tursiops truncatus	12028	119	61	26
Lagenorhynchus albirostris	12013	135	55	31
Globicephala melas	12002	148	62	22
Tursiops aduncus	11969	157	67	41

Completeness of Final Assembly

To check the accuracy and completeness of the final polished and purged assembly, we repeated the compleasm analysis. The final assembly exhibited slightly improved BUSCO

scores in Laurasiatheria (98.37%) and averaged 98.3% single copy complete BUSCOs across five different lineages (**Supp. Table 3**).

We compared the completeness of the hourglass assembly to nine other Delphinidae assemblies, which we selected on the basis of their being the most contiguous and complete assemblies in this family (all are designated as reference genomes by NCBI). However, we also excluded several genomes designated as reference quality as they had far fewer single copy complete BUSCOs, including *Steno bredanensis*, *Grampus griseus*, and *Sousa chinensis*. We found that the hourglass assembly had more complete and single copy BUSCOs (12,056; **Table 2**) than any other assembly except the vaquita, *Phocoena sinus* (12,072); correspondingly it had fewer missing BUSCOs (48) than any other assembly except the orca, *Orcinus orca* (41). 33 of these BUSCOs were missing from all Delphinoidea assemblies (**Fig. 3**), and were likely lost in the ancestral lineage. If these were indeed lost, this suggests the quality of the hourglass assembly is even higher than first appears: excluding the 33 BUSCOs missing in all, while the hourglass is missing only 15 out of 12,201, the average reference-level Delphinoidea assembly is missing 26.

The number of duplicated BUSCOs in the hourglass was 20% lower than average (96 vs. 116); however, the number of fragmented BUSCOs was 30% higher than average (34 vs. 26). This may be an indication that the Oxford Nanopore data results in an increased number of uncorrected small insertions and deletions resulting in truncated reading frames, compared to the combination of PacBio and Illumina data used for most of the other assemblies (**Supp. Table 1**).

We found that the BUSCO results correlated with patterns expected from the evolutionary relationships of the taxa. 50 BUSCOs were present as single copy and complete in all taxa except the most diverged taxon, the vaquita; similarly, the vaquita contained 13 single copy and complete BUSCOs that were not single copy and complete in all other taxa (**Fig. 3**). Nine single copy complete BUSCOs were not single copy-complete in both the hourglass and *Lagenorhynchus obliquidens*, the Pacific white-sided dolphin, but were present as single copy-complete in all others. The hourglass and Pacific white-sided dolphin are likely sister species to the exclusion of *Lagenorhynchus albirostris*, although additional phylogenetic analyses are needed to confirm this. Similar patterns were apparent for both *Tursiops* sister species.

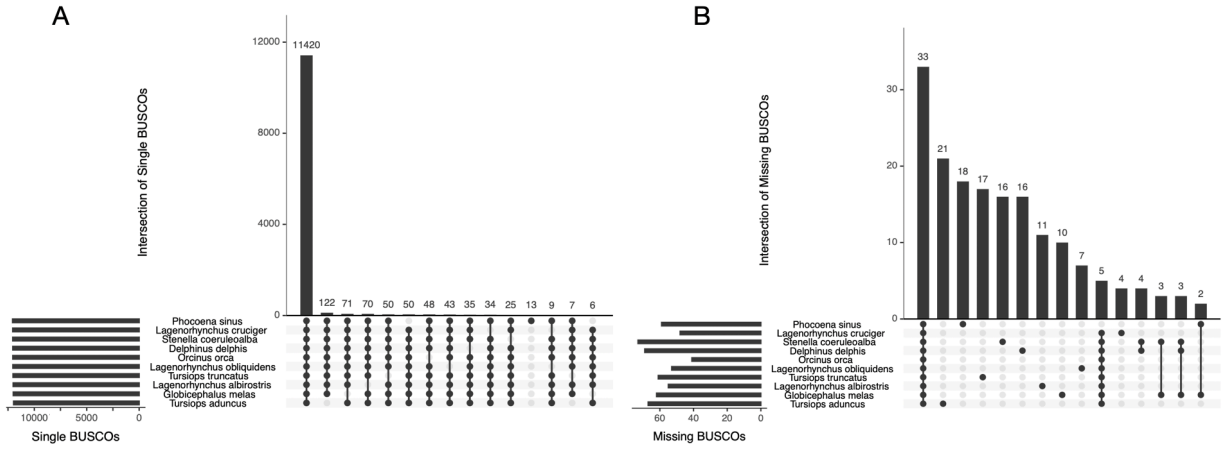

Figure 3. Upset plots of BUSCO overlaps across Delphinoidea assemblies. A. Intersection of single copy and complete BUSCOs using the Laurasiatheria ODB10 database. Each black point on a vertical line indicates taxa that share a set of BUSCOs, while each grey point indicates that the set of BUSCOs is missing from that taxon. The top bars show the total number of BUSCOs in each set (for example, 11,420 BUSCOs are present as single copy-complete in all ten taxa; 122 are single copy-complete in all taxa except *T. aduncus*). The left bars indicate the total number of single copy complete BUSCOs in each taxon. **B. Intersection of missing BUSCOs.** As in (A), overlaps in BUSCO sets are indicated by black points and taxa that are not part of that set are in grey. 33 BUSCOs are missing from all Delphinoidea, most of which were likely lost in the ancestor of these taxa. For both plots, only the 15 intersections with the most BUSCOs are shown, as there are a large number of intersections that contain only one or two BUSCOs.

Variant Calling and Phasing

We called heterozygous sites using both Clair3 and DeepVariant. These have been shown to be the most accurate Oxford Nanopore variant callers, with median indel F1 scores above 99.5% and SNP F1 scores at or above 99.99% at 50X depth in bacterial genomes (Hall et al. 2024).

Clair3 called 4,848,349 million SNVs, 620,899 insertions, and 516,291 deletions. When we filtered these to qualities above 14, this resulted in 4,223,575 SNVs, 208,561 insertions and 278,677 deletions. DeepVariant called 6,869,645 SNVs, 1,069,178 insertions and 3,381,036 deletions, which when filtered to those with quality 20 or more, resulted in 4,188,921 SNVs, 327,370 insertions, and 287,574 deletions. Overlapping the two call sets resulted in a total of 4,021,582 high quality SNPs, 181,409 insertions, and 246,147 deletions (note that the

designation of calls as insertions or deletions arbitrarily depends on the state of these positions in the haploid genome assembly).

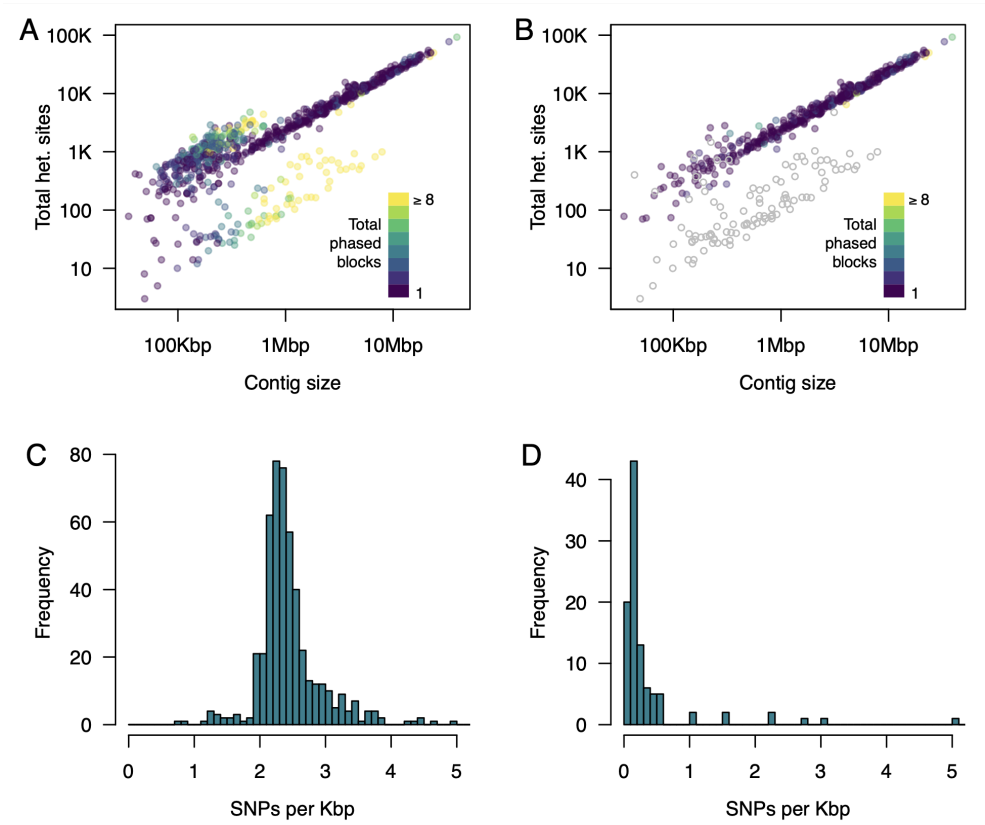

Figure 4. The majority of contigs are phased into a single block. A. Number of phase blocks per contig relative to contig size and the number of heterozygous sites in each contig. The majority of contigs that are not fully phased into a single block have low levels of polymorphism (green and yellow points) except for a small number of very large contigs that could not be fully phased (upper left points above the diagonal). **B**. The same plot as in panel (A) but with repetitive contigs removed (defined here as contigs with less than 90% unique 21-mers and putative sex chromosome contigs (coverage less than 30) coloured in grey. The sex chromosome contigs are inferred as having approximately 20-fold lower levels of polymorphism, likely resulting from a combination of assembly errors, false positive calls, and the homologous pseudoautosomal regions in the X and Y chromosomes. **C**. The number of SNPs per Kbp in non-repetitive contigs (unique 21-mers greater than 90%) and with greater than 45-fold coverage of high quality mappers (presumed autosomal contigs). Most autosomal contigs have approximately 2.4 heterozygous SNPs per Kbp with very little deviation from this average. D. The number of SNPs in non-repetitive contigs with less than 30-fold depth (presumed sex chromosome contigs). These contigs averaged 0.11 SNPs per Kbp.

We also examined the distribution of polymorphic sites across the genome. We hypothesised that due to selection, exons should harbour fewer SNPs relative to introns and intergenic regions, and that exons should harbour few indels. Of the high confidence SNP calls, 0.551% were within exons. This contrasts with the 0.853% of the genome that is annotated as exonic. 6.83% SNPs calls were within introns, closely matching the 6.60% of the genome that is intronic. For indels, only 0.245% lay within exons, a 3.5-fold depletion compared to genome-wide. In addition, exons were depleted for indels that were not multiples of three bp in length; this was not true for introns (**Supp. Fig. 3**). Surprisingly, we observed little depletion for 1 bp indels in exons. These may be due to miscalls present in Oxford Nanopore data at homopolymeric runs, or to segregating slightly deleterious mutations. Additional analyses of the locations, types, and functional effects of these indels would be required to yield insight into this.

Finally, we used Whatshap (Shafin et al. 2021) to phase the genome. 55% of all contigs were phased into a single block, and 80% were phased into three or fewer blocks **(Fig. 4**). Thus, despite relatively low levels of polymorphisms (0.1%) in many contigs (**Fig. 4C** and **4D**; **Supp. Fig. 4**), successful phasing was possible. However, without additional information, it is difficult to say whether there was a substantial amount of haplotype switching in these phased blocks.

Conclusions

The genome assembly we present here is one of the most complete of any cetacean, with BUSCO completeness above 98%. Despite relying solely only on Oxford Nanopore data, this quality exceeds many recent cetacean assemblies, even those relying on multiple sequence technologies, most frequently PacBio and Illumina (**Table 2**; **Supp. Table 1**).The use of the Raven genome assembler decreased the required computational resources to just above the size of the read dataset (close to 150 Gb RAM for the 142 Gbp dataset). The required RAM is easily decreased by decreasing the size of the data set (e.g. 98 Gb RAM for a 90 Gbp dataset (**Supp. Fig. 1**). This decreases both sequencing and compute costs while not severely compromising assembly quality, although a full assessment of the effects of changes in coverage and filtering is beyond the scope of what we present here. Low compute cost with few memory limitations is also available via the cloud. However, for the particular assembly presented here, the feasibility and desirability for this as a solution is dependent on how indigenous data sovereignty is implemented: for example, here and in other cases there may be a strong desire to ensure all data are collected and analysed within country or even more locally.

In 2020, the Vertebrate Genome Project estimated $20,000 USD for a mammalian genome, a two-fold reduction from an estimated $40,000 USD in 2018 (Morin et al. 2020). Here we present a high quality genome, from DNA to curated assembly, for less than a tenth of that price, and consuming considerably less computational resources. Overall, the results here illustrate the potential for inexpensive, and more importantly, accessible, assembly of large genomes, enabling community participation and data sovereignty (Garrison et al. 2019).

Finally, this high-quality genome should assist considerably in resolving taxonomic uncertainties in the subfamily Delphininae. In addition, by providing a comprehensive genomic resource, this study will contribute to a deeper understanding of cetacean evolution and facilitate informed conservation efforts for this enigmatic species.

Data availability Statement

The base-called sequence data, assembly, and annotations have been deposited at the Aotearoa Genomic Database Repository (https://data.agdr.org.nz/). The data will be made available for scientific and conservation-related purposes on behalf of Ōraka-Aparima Rūnaka, with requests for commercial applications deferred to them. A github repository outlining the steps of the assembly and the code and data necessary for plotting the figures presented here is available (https://github.com/osilander/hourglass-assembly).

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Conflict of Interest

OKS has previously received travel and accommodation expenses to speak at an Oxford Nanopore Technologies' conference.

Funder Information

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Author Contributions

NM assisted in DNA isolation and performed DNA sequencing; JLR performed DNA isolation and assisted in DNA sequencing; AW assisted in genome assembly, analyses, and in drafting the manuscript; AA assisted in drafting manuscript and communication with Ōraka-Aparima; ROS supported the dissection and facilitated ongoing discussions with Ōraka-Aparima; MJ provided materials, input on data availability, and guidance from Ōraka-Aparima; KS performed dissection, tissue isolation, and manuscript drafting; OKS performed genome assembly, analyses, and drafted the manuscript; all authors edited and approved the manuscript.

Supplementary Tables and Figures

Supplementary Table 1. List of other Delphinoidea genomes.

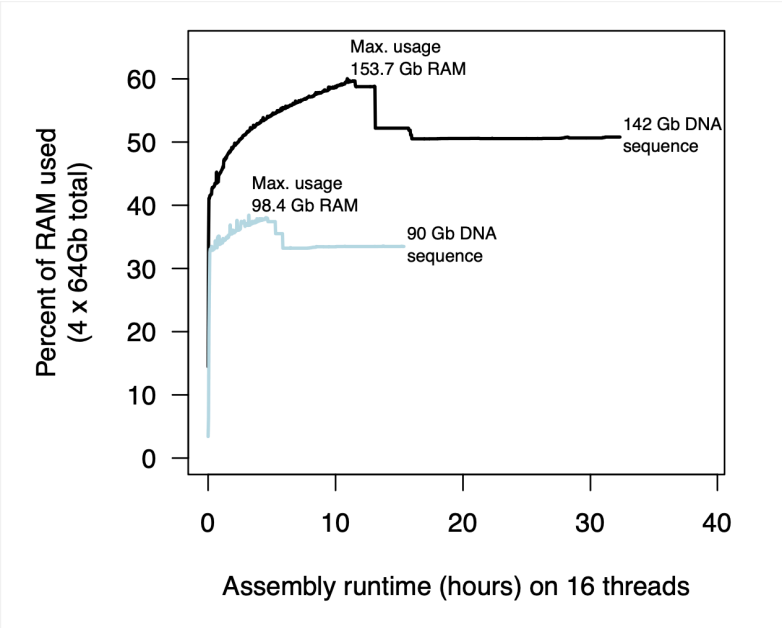
Organism	Assembly ID	Date	Contigs	Technology
Tursiops aduncus	GCA_003227395.1	Jun 14, 2018	44,281	Illumina HiSeq; Illumina NovaSeq
Lagenorhynchus obliquidens	GCA_003676395.1	Oct 23, 2018	21,792	Illumina HiSeq; Illumina NovaSeq
Phocoena sinus	GCA_008692025.1	Sep 26, 2019	272	PacBio Sequel I; Illumina NovaSeq; Arima Genomics Hi-C; Bionano Genomics DLE-1
Tursiops truncatus	GCA_011762595.1	Mar 27, 2020	1,035	PacBio Sequel I CLR; Illumina NovaSeq; Arima Genomics Hi-C; Bionano Genomics DLS
Orcinus orca	GCA_937001465.1	May 3, 2022	570	PacBio and Hi-C
Lagenorhynchus albirostris	GCA_949774975.1	Apr 8, 2023	1,479	PacBio and Arima2 Hi-C
Delphinus delphis	GCA_949987515.1	May 1, 2023	1,598	PacBio and Arima2 Hi-C
Stenella coeruleoalba	GCA_951394435.1	Jun 16, 2023	1,630	PacBio and Arima2 Hi-C
Globicephala melas	GCA_963455315.1	Sep 21, 2023	2,076	PacBio and Arima2 Hi-C

Supplementary Table 2. BUSCO completeness in Laurasiatheria across basecallers and assemblers.

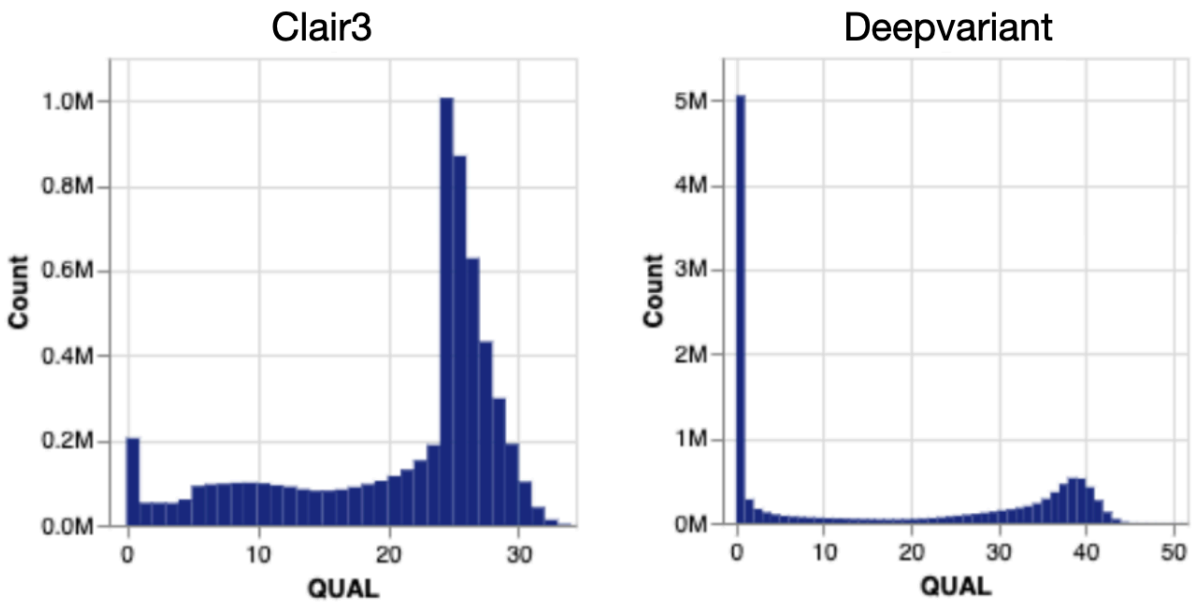
Assembler	Basecaller	Single copy	Duplicate	Fragmented	Missing
Goldrush	Guppy	11,795 (96.4%)	210 (1.72%)	98 (0.80%)	131 (1.07%)
Goldrush	Dorado	11,826 (96.7%)	191 (1.56%)	92 (0.75%)	124 (1.01%)
Nextdenovo	Guppy	11,937 (97.6%)	118 (0.96%)	82 (0.67%)	96 (0.78%)
Nextdenovo	Dorado	11,936 (97.6%)	116 (0.95%)	78 (0.64%)	104 (0.85%)
Raven	Guppy	12,017 (98.2%)	109 (0.89%)	58 (0.47%)	50 (0.41%)
Raven	Dorado	12,024 (98.3%)	107 (0.87%)	55 (0.45%)	48 (0.39%)
Raven	Dorado Q16	12,016 (98.2%)	104 (0.85%)	58 (0.47%)	56 (0.46%)

Supplementary Table 3. BUSCO completeness of the polished Dorado-basecalled Raven hourglass assembly relative to four mammalian lineages and to all eukaryotes.

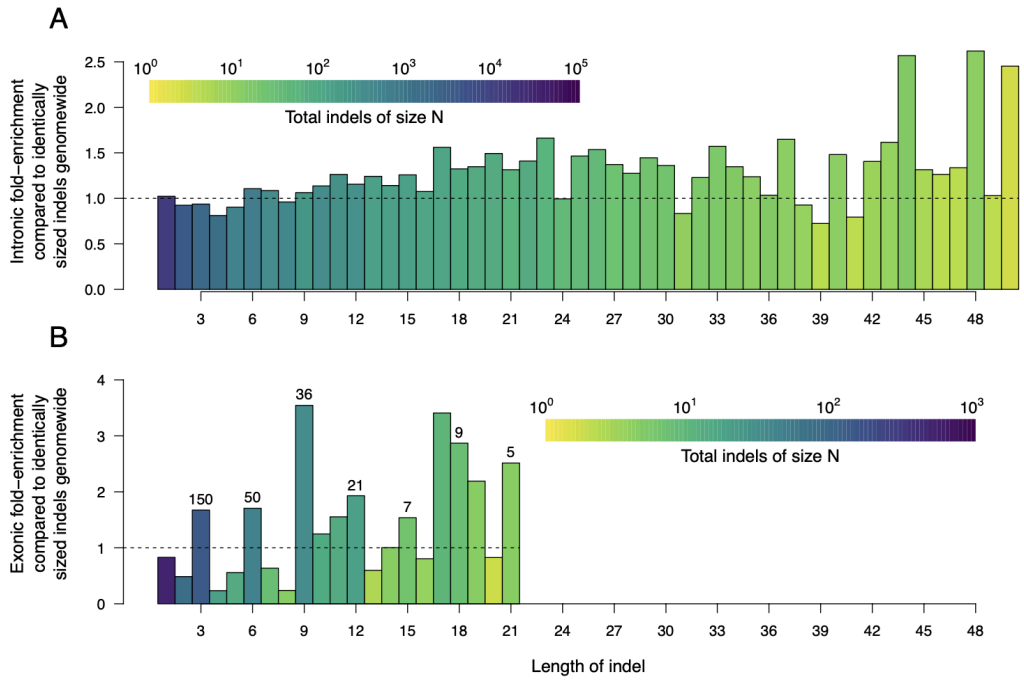
Lineage	Single copy and complete	Duplicated	Fragmented	Missing
Laurasiatheria	12,056 (98.55%)	96 (0.78%)	34 (0.28%)	48 (0.39%)
Cetartiodactyla	13,118 (98.37%)	118 (0.88%)	67 (0.50%)	32 (0.24%)
Eutheria	11,171 (98.29%)	75 (0.66%)	49 (0.43%)	70 (0.62%)
Mammalia	9,088 (98.50%)	63 (0.68%)	34 (0.37%)	41 (0.45%)
Eukarya	250 (98.04%)	5 (1.96%)	0 (0%)	0 (0%)



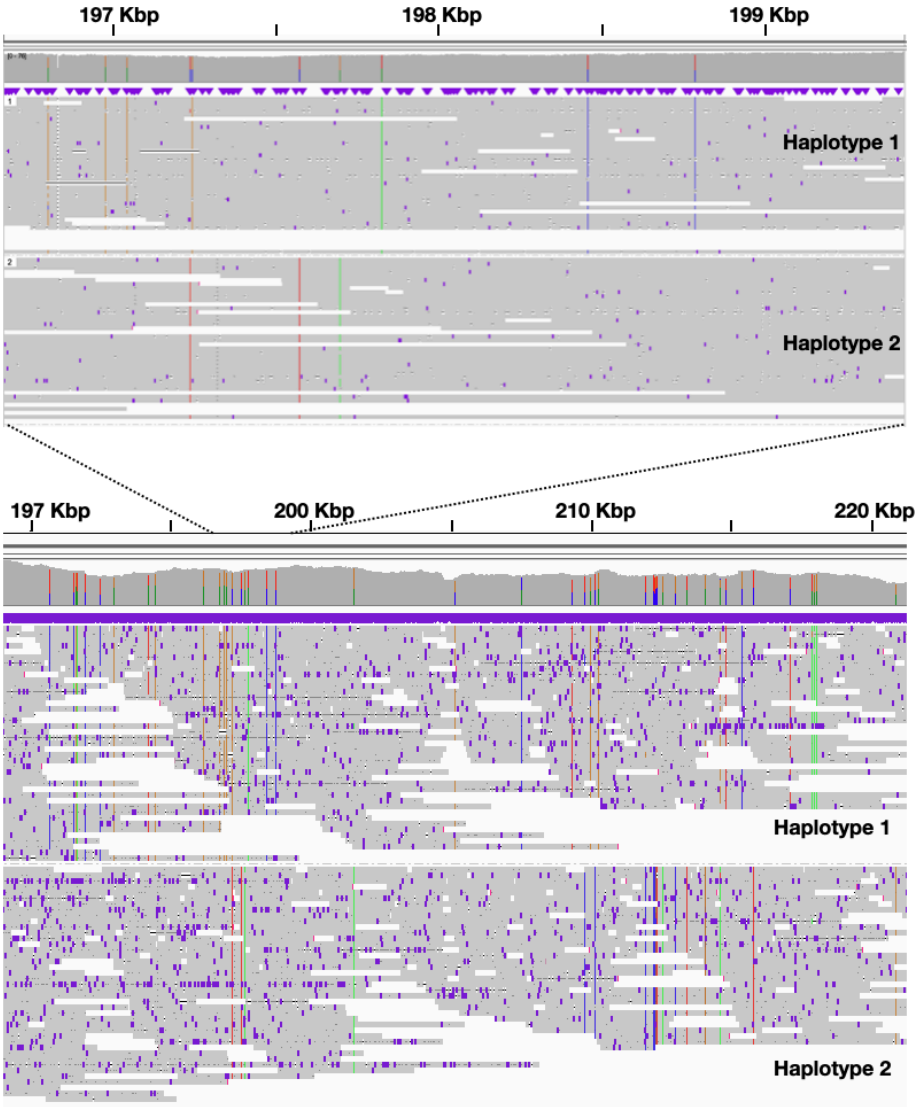
Supplementary Figure 1. RAM usage during Raven genome assembly. The plot shows the amount of RAM used by Raven when performing assembly with 16 threads and using sequencing datasets with either 142 Gbp (the full dataset presented here) or 90 Gbp (a subset filtered by prioritising longer reads). The assembly from the 142 Gbp dataset required a maximum of 153.7 Gb of RAM, beyond the scale of most consumer laptops. The initial assembly from 142 Gbp data had 1,243 contigs, 2.44 Gbp total size, and an N50 of 8.08 Mbp. The initial assembly from the 90 Gbp dataset was slightly lower quality (1,621 contigs, 2.47 Gbp total length, N50 5.85 Mbp), but required less than 100 Gb total RAM. Current high end laptops have up to 128 Gb of RAM (for example, the HP ZBook Fury 16 G10, configurable with 128 Gb RAM and an A1000 GPU for less than $3,000 USD).



Supplementary Figure 2. Genotype quality scores for Clair3 and DeepVariant. Clair3 calls have a minimum around q14; Deepvariant at around q20. We filtered both callsets to include only variants with higher qualities than this, respectively, and then merged the two filtered callsets to form a set of high quality genotype calls.



Supplementary Figure 3. Enrichment and depletion of specific indel sizes in introns and exons relative to all genomic locations. A. The ratio of the number of indels of each size in introns relative to the number of indels of that size genome-wide. If there is no depletion or enrichment, the expected ratio is one (dotted line). Instead, there is a slight depletion of small intronic indels (two to five bp) and an enrichment of larger intronic indels (between five and 25 bp). Overall, we found a slight enrichment of indels in introns compared to the remainder of the genome (7.21% observed vs. 6.60% expected). **B**. The analogous plot as in (A) but for exonic regions. Here there is a specific depletion of indels that are not multiples of three bp. This is expected, as indels that are not multiples of three change the reading frame. For both panels, the colour of the bar indicates the total number of indels at that size (on a log scale). For panel (B), above each bar that is a multiple of three, we show the total number of indels that are of that size. For example, we observed a total of 150 three bp indels in exons. Relative to the total genome-wide number of three bp deletions and normalising for the fraction of indels in exonic regions, we would have expected approximately 1/2 that number (dotted line). The total fraction of indels across all sizes in exonic and intronic regions must sum to one; thus, if some indel sizes are depleted, others must necessarily be enriched. We do not show the relative depletion or enrichment for exonic indels beyond 21 bp as these are small in number and fold-enrichment or depletion varies widely. Note that both the y-axes scales and colour scales are different on each plot.



Supplementary Figure 4. Phased haplotigs at 1 Kbp (top) and 10 Kbp (bottom) scales. Long Oxford Nanopore 10.4.1 chemistry reads allow phasing across the genome, despite relatively low levels of polymorphism in some regions (e.g. fewer than ten SNPs in the 10 kbp region between 200 Kbp and 210 Kbp above).

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