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# An integrated chromosome-scale genome assembly of the Masai Giraffe (Giraffa camelopardalis tippelskirchi)

--Manuscript Draft--

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| **Abstract:** | Background<br><br>The Masai giraffe (Giraffa camelopardalis tippelskirchi) is the largest-bodied giraffe and the world's tallest terrestrial animal. With its extreme size and height, the giraffe's unique anatomical and physiological adaptations have long been of interest to diverse research fields. Giraffes are also critical to ecosystems of sub-Saharan Africa, with their long neck serving as a conduit to food sources not shared by other herbivores. Although the genome of a Masai giraffe has been sequenced, the assembly was highly fragmented and unsuitable for the analysis of chromosome evolution. Herein we report an improved giraffe genome assembly to facilitate evolutionary analysis of the giraffe and other ruminant genomes.<br><br>Findings<br><br>Using SOAPdenovo2 and 170 Gbp of Illumina paired-end and mate-pair reads we generated a 2.6 Gbp female Masai giraffe genome assembly, with a scaffold N50 of 3 Mbp. The incorporation of 114.6 Gbp of Chicago library sequencing data resulted in a HiRise SOAPdenovo + Chicago assembly with an N50 of 48 Mbp and containing 95% of expected genes according to BUSCO analysis. Using the Reference-Assisted Chromosome Assembly tool, we were able to order and orient scaffolds into 42 predicted chromosome fragments (PCFs). Using fluorescence in situ hybridization we placed 153 cattle BACs onto giraffe metaphase spreads to assess and assign the PCFs on 14 giraffe autosomes and the X chromosome. In this assembly, 21,621 protein-coding genes were identified using both de novo and homology-based predictions.<br><br>Conclusions<br><br>We have produced the first chromosome-scale genome assembly for a Giraffidae species. This assembly provides a valuable resource for the study of artiodactyl evolution and for understanding the molecular basis of the unique adaptive traits of giraffes. In addition, the assembly will provide a powerful resource to assist conservation efforts of Masai giraffe, whose population size has declined by 52% in recent years. |
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| Response to Reviewers: | Dear Mr Zauner,<br><br>We are very grateful for your time and the time of four referees who reviewed our manuscript. We were pleased to find that the paper could be accepted for publication in GigaScience just after some minor modifications.<br><br>As you pointed in your email, one of the main issues raised by most of the reviewers was to include a wider comparison with the publicly available giraffe genome assembly. We agree with all the reviewers that a more detailed comparison would be beneficial to illustrate the improvements gained by our new effort, however, we encountered several issues while trying to do so. The main one being the lack of publicly available gene annotations for the Agaba et al., 2016 assembly. This has impeded us to benchmark our gene annotation and assess the improvement. We addressed all the reviewer's comments and our detailed responses are included below.<br><br>We also submit the modified manuscript with all changes highlighted as well as the modified tables and supplementary files.<br><br>Sincerely yours,<br><br>Marta Farré<br>Denis Larkin<br>Harris Lewin<br><br><br>Reviewer reports: |

Reviewer #1:
The manuscript is about resequencing the genome of the Masai Giraffe and constructing a high quality and contiguity chromosome-level reference assembly for the species. The goal was to improve over the previous assembly (Agaba et al. 2016), so that the resulting genome sequence would be applicable for evolutionary studies and species conservation. The authors used a well-selected set of cutting-edge next generation genomics technologies (short- and long-read sequencing; proximity ligation sequencing) and bioinformatics tools to achieve the goal. The manuscript is very well written and clear.
However, while reading the manuscript, I was very positive and enthusiastic in the beginning but disappointed in the end. To me, the manuscript looked like a well-written and detailed methodological manual about how to generate a high-quality chromosome-level annotated genome assembly for any mammalian species. I could recommend it for this purpose to any graduate student and postdoc. Disappointment was because I learned very little about the Masai Giraffe as such. Maybe this was the intention of the authors and in accordance with the profile of the journal. If so, this is a beautiful methodological paper on how to sequence, assemble and annotate mammalian genomes. If not, the authors should provide more information specific to the species.

Response: We thank the reviewer for his/her thoughtful comments. We fully agree with the reviewer that Giraffe is an iconic animal and that our chromosome-scale assembly will lead to new insights into its biology. However, we decided to publicly release the genome as soon as possible to allow the rest of the community access to this genome. Following GigaSicence Data Note style format, only a short description of the assembly should be included. As stated in GigaScience webpage: “One of the aims of a Data Note is to incentivize and more rapidly release data before subsequent detailed analysis has been carried out.”

Specific comments:
Comment 1. Like any other re-sequencing project, the manuscript should provide a comparison with the previous assembly together with examples illustrating the improvement (filling gaps or improving gene models, etc.).

Response: In reality, our project was not a resequencing project. The primary sequencing and de novo assembly of short reads was completed well before the paper by Agaba was published. However, our goal was always to produce a chromosome-scale assembly for the giraffe, and this process took longer than expected. While we agree with the reviewer that a more comprehensive comparison with the previous assembly would be beneficial, gene annotations for the Agaba et al., 2016 version were not publicly released. As such, we were not able to compare gene models between both assemblies. To compare the contiguity of the assemblies, we performed a pair-wise alignment between the publicly available assembly and our assembly and identified the discordant scaffolds. We included a new supplementary table (Supplementary Table 5) reporting these scaffolds. As the reviewer will see, only 18 scaffolds from the Agaba assembly were split when aligned to our assembly, and 11,005 joins were introduced, showing that our assembly is more contiguous.

Comment 2. It would be worth mentioning that together with the study by Agaba et al., there are now sequences of 3 female Masai Giraffe genomes.

Response: We thank the reviewer for this suggestion. Although in Agaba et al., 2016 the authors mentioned a second giraffe assembly, only one was fully described in their Supplementary Table 2 and deposited in GenBank. Therefore, we do not feel confident saying that there are now three giraffe genome assemblies publicly available.

Comment 3. The authors mention in Introduction about the specific biological features and adaptations of the Masai Giraffe, but do not use the improved sequence assembly to show this. If genome-wide analysis of signatures of selection is a too big task (and it likely is), the authors should revisit the genes under selection as pointed out by Agaba et al. (2016), and demonstrate how the new assembly improves this information.

Response: As mentioned above, the primary purpose of this data note is to describe

the details of the first chromosome-scale assembly for any giraffe. The genome-wide analysis for signatures of selection is beyond the scope of this article and will be considered elsewhere. We were not able to revisit the genes under selection indicated in the Agaba et al., 2016 publication because the authors did not provide the location of these genes nor the gene models from them, making it impossible to compare gene annotations.

Comment 4. The authors mention in Introduction about the use of the assembly for conservation efforts of the giraffe but do not show in the manuscript how it will be done.

Response: We thank the reviewer for their suggestions. We included a new section in the manuscript, entitled “Conclusions”. In this section, we give examples of cases where the availability of a genome assembly has fostered conservation genomics approaches.

Comment 5. Fig. 1: please specify whether this is the photo of the sequenced individual or just a representative of a species

Response: This photo is of a representative of the species and not the individual that was sequenced. We have clarified it in the figure legend. It now reads:
“Figure 1. A representative adult female Masai giraffe (Giraffa camelopardalis tippelskirchi) in the Masai Mara national park, Kenya. Picture taken by Bjørn Christian Tørrissen, licence CC BY-SA 3.0.”

Reviewer #2:
The paper sets out to give a chromosome-scale assembly for the Masai giraffe, which is achieved. The results presented give a de novo assembly and chromosomal analyses.
I have a few general comments and specific queries.

Comment 1. It would have been great to draw out a few unique observations about the genes specifically referring to giraffe.
The abstract mentions "many missing fragments and fragmented genes" when introducing the previously published giraffe genome. Which were these fragmented genes, and have they been improved?
No comparison with previous genome apart from BUSCO numbers. Which areas were improved the most? Is everything else the same?
Which genes were missing before? Are they in repeat regions? Are they any of the adaptation genes mentioned in the previous assembly paper?

Response. We thank the reviewer for the suggestion. However, Agaba and colleagues did not make publicly available their gene annotations. Moreover, as reported in their publication, genes were annotated in the giraffe assembly using only lastZ: “We used gene annotations for assemblies of the cow and dog genomes to identify putative coding regions for giraffe and okapi, as follows. We downloaded gene models for the cow assembly called bosTau4 from Ensembl (www.ensembl.org). The union of all intervals annotated as coding, as well as 150 bp flanks, were used to extract sequence from the bosTau4 assembly, forming the mapping target. Giraffe reads were mapped to the target using lastz. Mapping required an alignment with at least 60% of the read length as matched bases, and at least 3 matches better than the second best alignment.”

As such, we believe that our de novo and homology-based gene annotation is more reliable. More importantly, we included the GFT file with the annotation as part of this manuscript, making it accessible to all the researcher community.

Comment 2. I would have liked a summary figure of all of the chromosomes, as the data provided does not give this.

Response. A supplementary figure (Suppl. Fig 1) includes all the giraffe chromosomes compared to the cattle genome and with the placed SOAPdenovo and SOAPdenovo+Chicago scaffolds. One of these chromosomes can also be found in Figure 2. Moreover, in figure 2 the reviewer can find the giraffe karyotype showing the

placement of BACs used to assemble the genome as well as a Circos plot comparing giraffe and cattle chromosomes.

Comment 3. Data analyses: Why hg19 and not GRCh38?
Genome annotation: Ensembl 64 used. Very old, almost 10 years old. Ens 64 is no longer supported in the browser. For GRCh37 why not use Ens 75?
Which version of SwissProt was used of the analysis?

Response. We agree with the reviewer that Ensembl 64 is an old version; however, the protein coding gene annotations in cattle, horse and mouse did not change significantly between Ensembl 64 and Ensembl 75, as shown in the table below. Only the number of protein coding genes annotated in the human genome increased. However, because we used a combination of de novo and homology-based annotations with four different species gene sets, we believe that our final merged gene set using GLEAN represents a comprehensive and reliable gene annotation set, containing similar number of genes than other ruminant genomes.

Table 1. Number of protein-coding genes in each species used for the homology-based step in Ensembl 64 and 75 versions.

| Species | Ensembl 64 | Ensembl 75 | Difference |
|---|---|---|---|
| Cattle (UMD3.1) | 24,616 | 24,621 | 5 |
| Human (hg19) | 21,181 | 22,827 | 1,646 |
| Mouse (NCBIm38) | 22,711 | 22,753 | 42 |
| Horse (EquCab2) | 20,436 | 20,449 | 13 |

Comment 4. Figure 3: I'd like a better legend explaining this. It's not very clear.

Response. We have expanded Figure 3 legend. It now reads:

“Figure 3. Benchmarking of genome completeness for the four giraffe assemblies using BUSCO. The BUSCO dataset of the mammalia_odb9 including 4,104 genes was used to assess the completeness of the four giraffe genome assemblies, as well as the previously published giraffe genome (ASM165123v1 [9]). The newly released cattle (ARS−UCD1.2, GCA_002263795.2) and goat (ARS1, GCA_001704415.1) assemblies are included for comparison.

Comment 5. Supp table 5: There are a few errors in this table.
Mean exon per gene. I am assuming this is an error as the numbers are in thousands of exons per gene?
CDS is in bp. Shouldn't that be in amino acids as it refers to protein?
"Final" row at end of table. Not clear why this number is or how it was derived.

Response. We thank the reviewer for pointing us to this error. As the reviewer suggested, it is indeed an error and we have corrected the table. We renamed the “Final” row to “GLEAN”, as we used this tool to combine the de novo and the homology-based annotations.

Reviewer #3:
Summary: In this manuscript, Farre et al. detail the generation of a new reference for the Masai Giraffe using a combination of short read sequence data, Dovetail Hi-C and reference-guided scaffold correction. The assembly statistics, as presented, show higher degrees of scaffold continuity and BUSCO completeness than the previous Giraffe reference. It's highly likely that this assembly will be of use to the community and that Giraffe represents an interesting leaf in the Artiodactyla clade. Still, I found several areas where the manuscript did not provide enough context or details on the analysis.

Comment 1. Pg 5 Line 23: The details of the PCR chimera check need further fleshing

out. Did the authors use genomic DNA as the template or sequencing libraries? Since not all SF joint boundaries were tested via PCR amplification, it would be helpful to supply a supplementary table showing which boundaries were tested. Finally, how was the 158X physical coverage threshold determined?

Response. We thank the reviewer for the suggestions. To clarify, PCRs were performed using genomic DNA as a template and the mapping of the sequencing libraries was used to establish the 158X physical coverage. A new supplementary table has been included indicating the scaffold ID, the PCR results and the read physical coverage. We have amended the text and it now reads:
“Chimerism was evaluated using PCR amplification of Masai giraffe DNA with primers that flank the RACA-defined split of SF joint boundaries (Supplementary Table 2 and Supplementary Table 3). Because we were only able to test 76% of the putatively chimeric SOAPdenovo scaffolds, we mapped short- and long-insert size read libraries to the SOAPdenovo assembly to establish a minimum physical coverage of reads that mapped across the SF joint intervals, following previous publications [18]. By comparing the PCR results and the read mapping coverage, we established 158x as the minimum physical coverage that allowed differentiation of scaffolds that were likely to be chimeric from those that were likely to be authentic (Supplementary Table 2).

Comment 2. Pg 7 Line 32: The fragmentary X chromosome assembly is only mentioned in the abstract, but it represents a major limitation of this assembly version. A reason why this chromosome was not successfully scaffolded should be listed here or in the previous sections.

Response. We agree with the reviewer that the fragmentary assembly of chromosome X might be an issue for some researchers. It is known that chromosome X in Cetartiodactyla species is highly rearranged (Proskuryakova et al., 2017), and therefore, using RACA in this clade would not improve much the contiguity in this chromosome. This fact, combined with the high fragmentation already present in the SOAPdenovo+Chicago assembly, where chromosome X was assembled into 66 scaffolds, while giraffe chromosome 3, of a similar size, was assembled into 19 scaffolds, explains why chromosome X is represented in 10 fragments in the final assembly. We have included this explanation in the manuscript, and it now reads: “The final genome assembly comprised PCFs placed on 14 giraffe autosomes and 10 chromosome X fragments (Table 1). Because chromosome X in Cetartiodactyls (including giraffe, cattle, and pigs) has been highly rearranged during evolution [19], tools such as RACA, that use a reference-assisted assembly approach, will have limited success in increasing the contiguity of the assembly of sex chromosomes in the Cetartiodactyl clade.”

Comment 3. Table1: The listed assembly lengths vary considerably. It would be helpful to list the percentages of gap sequence in each assembly iteration.

Response. A new row in Table 1 has been added showing the percentage of gap sequence in each assembly.

Comment 4. Figure3: If one were to believe the BUSCO scores, the original assembly scaffolds (SOAPdenovo) were the "most complete" version of the assembly and subsequent scaffolding actually removed single copy genes from the assembly. This is a known issue with BUSCO evaluation, but it deserves mentioning in the results and discussion. Confirming that BUSCO single copy genes were deleted by RACA or Chicago edits would be important to report.

Response. As the reviewer pointed and it has been previously established, comparing different BUSCO runs on different versions of genome assemblies might produce inconsistent results (as shown by several issues reported in the BUSCO github page, f.e. https://gitlab.com/ezlab/busco/issues/94 and in Waterhouse et al., 2017). Only 34 BUSCO single copy genes found in the SOAPdenovo assembly were reported missing in the Final assembly. After looking at the responses from the authors in their github account, we re-ran BUSCO by modifying the hardcoded parameter of “maximum candidate region size of a contig” from 70 to 35% and found that all the inconsistencies

disappeared. Since we modified the BUSCO code, we would prefer to not report these findings because they will not be reproducible by other researchers. As the reviewer suggested, we included an explanation of the discrepancies between the different genome assemblies in the main text. It now reads:
“Although comparing BUSCO results on different versions of genome assemblies might be inappropriate due to difference in parameter estimations [23], we found a high agreement between genome assemblies, with only 34 BUSCO single copy genes present in the SOAPdenovo assembly reported missing in the final assembly, while 42 BUSCO genes reported as fragmented and an additional 14 reported as missing in the SOAPdenovo assembly were labelled as complete in the final assembly. Overall, approximately 95% of the core mammalian gene set was complete in the SOAPdenovo and SOAPdenovo + Chicago assemblies; SOAPdenovo + RACA included 94% of the mammalian gene set, while the final chromosome-level assembly contained 95% complete BUSCO genes, similar to other reference-quality ruminant assemblies (94% for cattle ARS-UCD1.2 and goat ARS1).”

Comment 5. References: Citations to the manuscripts that accompanied the release of the cattle and goat reference genomes are missing.

Response. We have added the references to these manuscripts.

Reviewer #4:
The manuscript describes assembly of a genome of an important mammal, the giraffe, whose extraordinary physical features have interested evolutionary biologists for more than centuries. The assembly presented here fills the gaps that were previously unaddressed by Agaba et al and contribute significantly new resource to the analysis and understanding of mammalian evolution and specifically to giraffe biology.
The authors use a set of complementary sequencing approaches to generate huge amounts of sequence data, and combined these with appropriate sequence assembly tools to arrive at a reference genome of the giraffe that is comparable to those of cattle and goats (ARS1), furthermore chromosome evolution is ascertained with direct observation with FISH analysis. The paper is reasonably well written and organised, however, there are two sections that can be improved;

Comment 1. The section on genome evolution only highlights phylogenetic relationships of selected ruminants and can be perhaps bolstered by moving the results of synthetic comparison between cattle and giraffe to this section.

Response.  Although we agree with the reviewer that a more in-depth analysis of the giraffe genome will lead to new insights into its biology, we decided to publicly release the genome as soon as possible to allow the rest of the community access it. As such, and following GigaSicence Data Note style format, only a short description of the assembly should be included. Even though we believe that synteny comparisons between giraffe and other species will provide insights into the biology of this and other species, in this particular work we used these comparisons to assess and assemble the genome at chromosome-level. Therefore, we would prefer to leave the paragraph about FISH and pair-wise alignment results in the assembly section.

Comment 2. The manuscript appears to end abruptly, and so I suggest that authors should consider adding a section with conclusion. Some of the elements for such a section are already in the manuscript page 7 line 28 - 36.

Response. We thank the reviewer for this suggestion. We have included a new section in the manuscript, entitled “Conclusions”. It now reads:
“Herein, we report a de novo chromosome-scale genome assembly for Masai giraffe using a combination of sequencing and assembly methodologies aided by physical mapping of 153 BACs onto giraffe metaphase chromosomes. Gene and repeat annotation of the assembly identified a similar number of genes and transposable elements as found in other ruminant species. Following the example of the sable antelope [42] and the California condor [43], the new giraffe genome assembly will foster research into conservation of this charismatic species, serving as a foundation for characterizing the genetic diversity of wild and captive populations. Furthermore,

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| | the high quality, chromosome-scale assembly described in this report contributes to the goals of the Genome 10K Project [24] and the Earth BioGenome Project [25].” |
| **Additional Information:** | |
| **Question** | **Response** |
| Are you submitting this manuscript to a special series or article collection? | No |
| **Experimental design and statistics**<br><br>Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends.<br><br>Have you included all the information requested in your manuscript? | Yes |
| **Resources**<br><br>A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible.<br><br>Have you included the information requested as detailed in our Minimum Standards Reporting Checklist? | Yes |
| **Availability of data and materials**<br><br>All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” | No |

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| section of your manuscript.<br><br>Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist? | |
| If not, please give reasons for any omissions below.<br><br>as follow-up to "**Availability of data and materials**<br><br>All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript.<br><br>Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?<br><br>" | Genome annotations, phylogenetic tree and BUSCO summaries will be uploaded to GigaDB |

# An integrated chromosome-scale genome assembly of the Masai Giraffe (*Giraffa camelopardalis tippelskirchi*)

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## Abstract

**Background.** The Masai giraffe (*Giraffa camelopardalis tippelskirchi*) is the largest-bodied giraffe and the world's tallest terrestrial animal. With its extreme size and height, the giraffe's unique anatomical and physiological adaptations have long been of interest to diverse research fields. Giraffes are also critical to ecosystems of sub-Saharan Africa, with their long neck serving as a conduit to food sources not shared by other herbivores. Although the genome of a Masai giraffe has been sequenced, the assembly was highly fragmented and unsuitable for the analysis of chromosome evolution. Herein we report an improved giraffe genome assembly to facilitate evolutionary analysis of the giraffe and other ruminant genomes. **Findings.** Using SOAPdenovo2 and 170 Gbp of Illumina paired-end and mate-pair reads we generated a 2.6 Gbp female Masai giraffe genome assembly, with a scaffold N50 of 3 Mbp. The incorporation of 114.6 Gbp of Chicago library sequencing data resulted in a HiRise SOAPdenovo + Chicago assembly with an N50 of 48 Mbp and containing 95% of expected genes according to BUSCO analysis. Using the Reference-Assisted Chromosome Assembly tool, we were able to order and orient scaffolds into 42 predicted chromosome fragments (PCFs). Using fluorescence in situ hybridization we placed 153 cattle BACs onto giraffe metaphase spreads to assess and assign the PCFs on 14 giraffe autosomes and the X chromosome. In this assembly, 21,621 protein-coding genes were identified using both *de novo* and homology-based predictions. **Conclusions.** We have produced the first chromosome-scale genome assembly for a Giraffidae species. This assembly provides a valuable resource for the study of artiodactyl evolution and for understanding the molecular basis of the unique adaptive traits of giraffes. In addition, the assembly will provide a powerful resource to assist conservation efforts of Masai giraffe, whose population size has declined by 52% in recent years.

**Keywords (3-10 words):** giraffe, *Giraffa camelopardalis tippelskirchi*, assembly, annotation, ruminant

## Background information

Giraffes (*Giraffa*) are a genus of even-toed ungulate mammals comprising four species [1]. They are members of the family Giraffidae, which also includes the okapi (*Okapia johnstoni*). The Masai giraffe (also known as Kilimanjaro giraffe; *Giraffa camelopardalis tippelskirchi*; Figure 1) is native to East Africa and distributed throughout Tanzania and Kenya [2]. Masai giraffes are not only the largest-bodied giraffes [3] but also the tallest terrestrial animals. Giraffes present several distinctive

anatomical characteristics, such as their long neck and legs, horn-like ossicones and coat patterns, which together with their unique cardiovascular and musculoskeletal adaptations have interested researchers in many fields [3-6].

The giraffe genome comprises 15 pairs of chromosomes (2n = 30) that are believed to have originated by multiple Robertsonian fusions from the pecoran ancestral karyotype (2n = 58) [7, 8]. In 2016, Agaba and colleagues generated the first genome sequence of a female Masai giraffe and compared it with the genome sequence of an okapi [9]. This study identified candidate genes and pathways involved in the giraffes' unique skeletal and cardiovascular adaptations [9]. The reported genome was fragmented, which hinders its use for studies of overall genome architecture and evolution. Missing and fragmented genes also limit the utility of the assembly for study of the genetic basis of the giraffe's unique adaptations. Here we report a chromosome-scale assembly of a female Masai giraffe genome sequenced *de novo*. This assembly will facilitate studies of ruminant genome evolution and will be a powerful resource for further elucidation of the genetic basis for the giraffe's characteristic features. Furthermore, having another Masai giraffe genome sequence will assist conservation efforts for this species, whose population has declined by more than 52% in recent decades [2, 10].

## Data description

**Library construction, sequencing, and filtering**

Genomic DNA was extracted from a fibroblast cell culture of a female Masai giraffe using the DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Isolated genomic DNA was then used to construct twelve sequencing libraries, four short-insert (170, 250, 500, and 800 bp) and eight long-insert size (2, 5, 10, and 20 Kbp), following Illumina (San Diego, CA, USA) standard protocols. Using a whole-genome shotgun sequencing strategy on the Illumina Hiseq 2000 platform, we generated 296.23 Gbp of raw sequencing data with 100 bp or 50 bp paired-end sequencing for the short-insert or long-insert size libraries, respectively (Supplementary Table 1). To improve read quality, low-quality bases from both ends of the reads were trimmed, duplicated reads and those with more than 5% of uncalled ("N") bases were removed. A total of 171.09 Gbp of filtered read data were used for genome assembly (Supplementary Table 1).

Two Chicago libraries were generated by Dovetail Genomics (Santa Cruz, CA) as previously described [11]. Briefly, high-molecular-weight DNA was assembled into chromatin *in vitro*, chemically cross-linked and digested by restriction enzymes. The resulting digestion overhangs were filled in with a biotinylated nucleotide, and the chromatin was incubated in a proximity-ligation reaction. The cross-links were then reversed, and the DNA purified from the chromatin. These libraries were sequenced

in one flow-cell lane using the Illumina HiSeq 4000 platform, resulting in the generation of ~385 million read pairs or 114.60 Gbp of sequence data (Supplementary Table 1).

**Evaluation of genome size**

The Masai giraffe genome size was estimated by k-mer analysis. A k-mer refers to an artificial sequence division of K nucleotides iteratively from sequencing reads. A raw sequence read with L bp contains (L-K+1) different k-mers of length K bp. K-mer frequencies can be calculated from the genome sequence reads and typically follow a Poisson distribution when plotted against the sequence depth gradient. The genome size, G, can then be calculated from the formula G=K_num/K_depth, where the K_num is the total number of k-mers, and K_depth denotes the depth of coverage of the k-mer with the highest frequency. For giraffe, at K=17, K_num was 75,710,429,964 and the K_depth was 30. Therefore, we estimated the genome size of *Giraffa camelopardalis tippelskirchi* to be 2.5 Gbp, comparable to the C-value of 2.7 and 2.9 reported for reticulated giraffe (*Giraffa camelopardalis reticulata*) [12]. All the filtered Illumina sequencing reads provided approximately 68.44x mean coverage of the genome, while the Chicago libraries' reads presented an estimated genome coverage of 88.41x.

**Genome assembly**

We applied SOAPdenovo (version 2.04) with default parameters to construct contigs and scaffolds as described previously [13]. All reads were aligned against each other to produce contigs which were further assembled in scaffolds using the paired-end information. The generated Masai giraffe genome assembly was 2.55 Gbp long, including 76.82 Mbp (3%) of unknown bases ("Ns"). The contig and scaffold N50 lengths were 21.78 Kbp and 3.00 Mbp, respectively (Table 1). To assess the assembly quality, approximately 90 Gbp (representing 35.6x genome coverage) high-quality short-insert size reads were aligned to the SOAPdenovo assembly using BWA (with parameters of -t 1 -l). A total of 98.9% reads could be mapped and covered 98.9% of the assembly, excluding gaps. Approximately 92% of these reads were properly paired, having an expected insert size associated with the libraries of origin.

To increase the contiguity of the assembly we used the HiRise2.1 scaffolder [11] and sequence information from the Chicago libraries and SOAPdenovo assembly as inputs. The SOAPdenovo + Chicago assembly introduced a total of 56 breaks in 54 SOAPdenovo scaffolds, and formed 3,200 new scaffold joints, resulting in an increased scaffold N50 length of 57.20 Mbp (Table 1).

*Evaluation of the SOAPdenovo genome assembly and PCR verification of putatively chimeric scaffolds*

To identify putatively chimeric scaffolds, we utilized the Masai giraffe SOAPdenovo genome assembly to obtain predicted chromosome fragments (PCFs) using Reference-Assisted Chromosome Assembly (RACA) software [14]. The RACA tool uses a combination of comparative information and sequencing data to order and orient scaffolds of target species and generate PCFs. The cattle (*Bos taurus*, bosTau6) and human (*Homo sapiens*, hg19) genome assemblies were used as a reference and outgroup, respectively, and all Illumina paired-end and mate-pair libraries were included in the RACA assembly. The read libraries were aligned to the SOAPdenovo scaffolds using Bowtie2 [15]. The cattle-giraffe and cattle-human pairwise alignments were performed using lastZ and UCSC Kent utilities [16], as previously described [14, 17]. The RACA software was used at a minimum resolution of 150 Kbp for syntenic fragment (SF) detection. Only SOAPdenovo scaffolds >10 Kbp were used as input for RACA, comprising 95% of the assembly length.

After an initial run of RACA with default parameters, we tested the structure of 32/41 (76%) RACA-split SF adjacencies corresponding to 40 SOAPdenovo scaffolds flagged as putatively chimeric. Chimerism was evaluated using PCR amplification of Masai giraffe DNA with primers that flank ~~across~~ the RACA-defined split of SF joint boundaries ~~with giraffe genomic DNA~~ (Supplementary Table 2 and Supplementary Table 3). Because we were only able to test 76% of the putatively chimeric SOAPdenovo scaffolds, we mapped short- and long-insert size read libraries to the SOAPdenovo ~~genome~~assembly to establish a minimum physical coverage of reads that mapped across the SF joint intervals, following previous publications [18]. By comparing the PCR results and the read mapping coverage, ~~On the basis of results obtained using PCR and mapping of short- and long-insert size read libraries,~~ we established 158x as the minimum physical coverage ~~of reads mapped across the SF joint intervals~~ that allowed ~~us to~~ differentiation ~~e~~ of ~~between~~ ~~separation of~~ scaffolds that were likely to be chimeric from those that were likely to be authentic (Supplementary Table 2). This threshold was used to update the parameters of a second round of RACA (stage 2 RACA), which resulted in the generation of 47 PCFs, of which 13 were homologous to complete cattle chromosomes. The stage 2 RACA assembly had an N50 length of 85.22 Mbp. This assembly comprised 1,283 SOAPdenovo scaffolds, representing 93% of the original SOAPdenovo assembly, of which 33 were split by RACA, and two were manually split as they had been shown to be chimeric by PCR (Table 1). These results indicate the power of comparative information for improving assembly contiguity and for identifying problematic regions in *de novo* assemblies.

*Evaluation of the HiRise SOAPdenovo + Chicago assembly*

More than 94% of the joints introduced in the SOAPdenovo + Chicago assembly were concordant with the RACA assembly, 4% were inconsistent between the two assemblies, and 1% represented

extra adjacencies with intervening scaffolds located at the ends of PCFs. Among the 54 SOAPdenovo scaffolds broken in the SOAPdenovo + Chicago assembly, 26 were also broken in the RACA assembly. Among the remaining 28 scaffolds, five were not included in PCFs because they were under the 150 Kbp SF resolution set in the RACA tool; 16 were broken in the Chicago assembly, with one of the fragments below SF resolution, and seven scaffolds were broken in the SOAPdenovo + Chicago assembly and intact in the RACA assembly (SOAPdenovo scaffolds 82, 813, 816, 849, 906, 940, and 995). Additionally, among the 16 SOAPdenovo scaffolds PCR-verified to be chimeric, 13 were also broken in the SOAPdenovo + Chicago assembly. The remaining three chimeric joints, within SOAPdenovo scaffolds 181, 267, and 696 were manually split in the SOAPdenovo + Chicago assembly (scaffolds Sc_7219;HRSCAF=8761 and Sc_732785;HRSCAF=735706). The final SOAPdenovo + Chicago genome assembly comprises 2.55 Gbp and has an N50 length of 57.20 Mbp (Table 1).

Comparison to cattle chromosomes identified five chromosomal fusions in the giraffe SOAPdenovo + Chicago assembly. Two of those fusions, (cattle chromosomes BTA1/BTA28 and BTA26/BTA28), were previously detected using cytogenetic approaches, and both locate on giraffe chromosome 2 [7, 8]. Finally, we ran RACA using the SOAPdenovo + Chicago scaffolds and cattle (bosTau6) and human (hg19) genomes as reference and outgroup, respectively. RACA produced 42 PCFs (Table 1), 20 of them representing complete cattle chromosomes, a substantial improvement over the SOAPdenovo + RACA assembly.

*Evaluation of SOAPdenovo + Chicago + RACA assembly and scaffold placement into chromosomes using FISH*

In order to assess and map the SOAPdenovo + Chicago + RACA PCFs onto giraffe chromosomes, we performed fluorescence *in situ* hybridization (FISH) of cattle bacterial artificial chromosomes (BACs) from the CHORI-240 library (http://www.chori.org/bacpac) with giraffe metaphase spreads (Figure 2) following previous publications [19]. Briefly, giraffe fibroblast cells were incubated at 37°C and 5% $CO_2$ in Alpha MEM (Gibco) supplemented with 15% Fetal Bovine Serum (Gibco), 5% AmnioMAX-II (Gibco) and antibiotics (ampicillin 100 µg/ml, penicillin 100 µg/ml, amphotericin B 2.5 µg/ml). Metaphases were obtained by adding colcemid (0.02 mg/ml) and EtBr (1.5 mg/ml) to actively dividing cultures. Hypotonic treatment was performed with KCl (3 mM) and sodium citrate (0.7 mM) for 20 min at 37°C and followed by fixation with 3:1 methanol-glacial acetic acid fixative. BAC DNA was isolated using a plasmid DNA isolation kit (Biosilica, Novosibirsk, Russia) and amplified using whole genome amplification (GenomePlex Whole Genome Amplification Kit, Sigma). Labeling of BAC DNA was performed using the GenomePlex WGA Reamplification Kit (Sigma) by incorporating biotin- 16-

dUTP (Roche) or digoxigenin-dUTP (Roche). Two color FISH experiments on G-banded metaphase chromosomes were performed as described previously [19].

BAC clone coordinates for cattle (bosTau6) assembly were downloaded from NCBI CloneDB [20] and converted to coordinates in the giraffe SOAPdenovo + Chicago + RACA PCFs using the UCSC Genome Browser LiftOver tool [21]. A total of 153 BACs were successfully mapped to the giraffe assembly and were retained for the following analysis. To evaluate the 146 scaffold joints introduced by RACA, a reliability score was further calculated considering four components: (i) the relative positions of the BACs in giraffe metaphase spreads compared to the PCFs (Figure 2), (ii) if the joint was supported by sequence reads from Chicago libraries, (iii) physical coverage of Illumina pair-end reads, and (iv) comparative syntenic information. Different weights were given to each component of the score, ranging from 10% for the comparative syntenic information to 40% for the physical map using BAC data (Supplementary Table 43). Only those joints with a reliability score >30% were considered as authentic, indicating that at least FISH or Chicago library read support was present. More than 89% (N=130) of the adjacencies had FISH and/or Chicago support, while six (4%) adjacencies had syntenic support only (Supplementary Figure 1). The final genome assembly comprised PCFs placed on 14 giraffe autosomes and 10 chromosome X fragments (Table 1). Because chromosome X in Cetartiodactyls~~a species~~ (including giraffe, cattle, and pigs) has been highly rearranged during evolution [19], tools such as RACA, that ~~-~~use~~ing~~ a reference-assisted assembly approach, will have limited success in increasing the contiguity of the assembly of sex chromosomes in the Cetartiodactyl clade~~a clade~~.

*Completeness evaluation of genome assemblies using BUSCO*

We evaluated genome completeness using the Benchmarking Universal Single-Copy Orthologs (BUSCO; version 3.0; [22]) software. Although comparing BUSCO results~~uns~~ on different versions of genome assemblies might ~~produce inconsistent results~~be inappropriate due to difference in parameter estimations [23], we found a high agreement between genome assemblies, with only 34 BUSCO single copy genes present in the SOAPdenovo assembly reported missing in the final assembly, while 42 BUSCO genes reported as fragmented and an additional 14 reported as missing in the SOAPdenovo assembly were labelled as complete in the final assembly. Overall, approximately 95% of the core mammalian gene set was complete in the SOAPdenovo and SOAPdenovo + Chicago assemblies; SOAPdenovo + RACA included 9~~4~~3% of the mammalian gene set, while the final chromosome-level assembly contained 95% complete BUSCO genes, similar to other reference-quality ruminant assemblies (94% for cattle ARS-UCD1.2 and goat ARS1). In comparison, the Masai giraffe genome assembly reported by Agaba and colleagues [9] included 87% of BUSCO genes (Figure 3).

These results show that the genome assemblies we generated are of high completeness and accuracy, and a significant improvement over the genome assembly currently available for Masai giraffe. ~~Furthermore, the high quality, chromosome scale assembly described in this report contributes to the goals of the Genome 10K Project~~ [24]~~ and the Earth BioGenome Project~~ [25]~~.~~

**Genome annotation**

To annotate transposable elements (TEs) in the Masai giraffe genome, we started by predicting TEs by homology to RepBase sequences using RepeatProteinMask and RepeatMasker [26] with default parameters. Results from both types of software were combined to produce a non-redundant final set of TEs. Approximately 40% of the Masai giraffe's genome is comprised of TEs, with LINEs being the most frequent group (24%, Supplementary Table 6~~4~~).

The remainder of the SOAPdenovo genome assembly was annotated using both homology-based and *de novo* methods. For the homology-based prediction, human, mouse, cow, and horse proteins were downloaded from Ensembl (release 64) and mapped onto the genome using tblastn. The homologous genome sequences were aligned against the matching proteins using Genewise [27] to define gene models. For *de novo* prediction, Augustus [28], Genscan [29], and SNAP [30] were applied to predict coding genes as described in Zhang et al. 2018 [31]. Finally, homology-based and *de novo* derived gene sets were merged to form a comprehensive and non-redundant reference gene set using GLEAN [32]. We obtained a reference gene set that contained 21,621 genes (Supplementary Table 7~~5~~).

To assign functions to the newly annotated genes in the Masai giraffe genome we aligned them to SwissProt database using blastp with an (E)-value cutoff of 1 $e^{-5}$. A total of 18,910 genes (87.46% of the total annotated genes) had a Swissprot match. Publicly available databases (including Pfam, PRINTS, PROSITE, ProDom, and SMART) were used to annotate motifs and domains in the gene sequences using InterPro, producing a total of 16,137 genes annotated with domain information (74.64%). By searching the KEGG database using a best hit for each gene, 9,087 genes were mapped to a known pathway (42.03% of the genes). Finally, we assigned a gene ontology term to 12,263 genes, representing 56.72% of the full gene set. Overall, 18,955 genes (87.67%) had at least one functional annotation (Supplementary Table 8~~6~~).

**Genome evolution**

The position of the Giraffidae family in the Ruminantia has been highly debated, with some studies using mitochondrial DNA or SNPchip data suggesting that Giraffidae are an outgroup to Bovidae and Cervidae [33, 34], while palaeontological and biochemical evidence suggested that

Giraffidae and Cervidae are sister taxa [35, 36]. To shed light on the giraffe phylogeny, we first used the TreeFam methodology [37] to define gene families in eight mammalian genomes (cattle, sheep, gemsbok, yak, giraffe, Pere David's deer, horse, and human) using newly defined or available gene annotations. We applied the same pipeline and parameters as described by Kim and co-workers [38]. A total of 16,148 gene families, of which 1,327 are single-copy orthologous families, were obtained. Concatenated protein sequence alignments of single-copy orthologous families were used as input for building the tree, with the JTT+gamma model, using PhyMLv3.3 [39]. Branch reliability was assessed by 1,000 bootstrap replicates. Finally, PAML mcmctree [40] was used to determine divergence times with the approximate likelihood calculation method and data from TimeTree [41]. The resulting tree suggests that Giraffidae are a sister taxon to the Cervidae, diverging ~21.5 million year ago (Figure 4); however, further studies using more deer species and other ruminants, such as pronghorn, as well as other methodologies to detect orthologous genes, will be needed to clarify the ruminant phylogeny.

**Conclusions**

~~In this manuscript~~Herein, ~~-~~we report~~ed~~ a de novo chromosome-~~level~~scale genome assembly for Masai ~~the~~ giraffe using a combination of sequencing and assembly~~ing~~ methodologies ~~and~~ aided by physical~~ly~~ mapping of 153 BACs onto~~into~~ giraffe metaphase chromosomes. Gene and repeat annotation of the ~~genome, made publicly available,~~ assembly identified a ~~showed~~ similar number of genes and transposable elements as found in other ruminant species. Following the example of the sable antelope [42] and the California condor [43], t~~T~~he new giraffe genome assembly will foster research into conservation of this charismatic species, serving as a foundation for characterizing the genetic diversity ~~status~~ of wild and captive populations~~, following the example of the sable antelope [42]~~(~~Kloepfi et al 2019~~) and the California condor [43]~~(~~Primmer CR 2009~~)~~. Furthermore, the high quality, chromosome-~~-~~scale assembly described in this report contributes to the goals of the Genome 10K Project [24] and the Earth BioGenome Project [25].

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## List of abbreviations

BUSCO: Benchmarking Universal Single-Copy Orthologs; PCF: Predicted Chromosome Fragment; RACA: Reference-Assisted Chromosome Assembly; TE: Transposable Element.

## Availability of supporting data

The raw sequence data have been deposited in the Short Read Archive (SRA) under accession numbers SRR7503131, SRR7503132, SRR7503129, SRR7503130, SRR7503127, SRR7503128, SRR7503125, SRR7503126, SRR7503158, SRR7503157, SRR7503156, SRR7503155. The SOAPdenovo + Chicago

assembly is also available in NCBI under accession number RAWU00000000. Annotations and chromosome reconstructions are available in the *GigaScience* database doi: 10.5524/100590 [44].

## Competing interests

The authors declare that they have no competing interests.


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## Author contributions

M.F. generated the SOAPdenovo + RACA assembly, evaluated the assemblies, and co-wrote the manuscript. I.D. performed PCR verifications and ran the adjusted parameters SOAPdenovo + RACA assembly. Q.L. and Y.Z. assembled the sequencing reads with SOAPdenovo and annotated the genome. J.D. performed paired-end read mapping and co-wrote the manuscript. A.P., A.K., and A.S.G. performed FISH on giraffe chromosomes. L.G.C. and O.A.R. prepared cell cultures and extracted DNA. G.Z. supervised SOAPdenovo assembly and gene annotation. J.K. and J.M. assisted in RACA assemblies. D.M.L. and H.A.L. supervised the project and revised the manuscript.


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## References


1. Fennessy J, Bidon T, Reuss F, Kumar V, Elkan P, Nilsson MA, et al. Multi-locus Analyses Reveal Four Giraffe Species Instead of One. Current biology : CB. 2016;26 18:2543-9. doi:10.1016/j.cub.2016.07.036.
2. Muller Z, Bercovitch F, Brand R, Brown D, Brown M, Bolger D, et al. Giraffa camelopardalis. The IUCN Red List of Threatened Species 2016. 2016.
3. Dagg AI. Giraffe: Biology, Behaviour and Conservation. Cambridge University Press; 2014.

4. Solounias N. The remarkable anatomy of the giraffe's neck. Journal of Zoology. 1999;247 2:257-68.
5. Estes R. The Behavior Guide to African Mammals: Including Hoofed Mammals, Carnivores, Primates. University of California Press; 1991.
6. Nowak RM. Walker's Mammals of the World. Johns Hopkins University Press; 1999.
7. Huang L, Nesterenko A, Nie W, Wang J, Su W, Graphodatsky AS, et al. Karyotype evolution of giraffes (Giraffa camelopardalis) revealed by cross-species chromosome painting with Chinese muntjac (Muntiacus reevesi) and human (Homo sapiens) paints. Cytogenetic and genome research. 2008;122 2:132-8. doi:10.1159/000163090.
8. Cernohorska H, Kubickova S, Kopecna O, Kulemzina AI, Perelman PL, Elder FF, et al. Molecular cytogenetic insights to the phylogenetic affinities of the giraffe (Giraffa camelopardalis) and pronghorn (Antilocapra americana). Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology. 2013;21 5:447-60. doi:10.1007/s10577-013-9361-0.
9. Agaba M, Ishengoma E, Miller WC, McGrath BC, Hudson CN, Bedoya Reina OC, et al. Giraffe genome sequence reveals clues to its unique morphology and physiology. Nature communications. 2016;7:11519. doi:10.1038/ncomms11519.
10. Bolger DT, Ogutu JO, Strauss M, Lee DE, Fennessy J and Brown D. Masai giraffe (Giraffa camelopardalis tippelskirchi) conservation status report. IUCN/SSC Giraffe and Okapi Specialist Group. 2015.
11. Putnam NH, O'Connell BL, Stites JC, Rice BJ, Blanchette M, Calef R, et al. Chromosome-scale shotgun assembly using an in vitro method for long-range linkage. Genome Res. 2016;26 3:342-50. doi:10.1101/gr.193474.115.
12. 2017. http://www.genomesize.com.
13. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience. 2012;1 1:18. doi:10.1186/2047-217x-1-18.
14. Kim J, Larkin DM, Cai Q, Cai Q, Zhang Y, Ge R-L, et al. Reference-assisted chromosome assembly. Proc Natl Acad Sci U S A. 2013;110 5:1785-90. doi:10.1073/pnas.1220349110.
15. Langmead B and Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9 4:357-9. doi:10.1038/nmeth.1923.
16. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. Genome Res. 2002;12 6:996-1006. doi:10.1101/gr.229102. Article published online before print in May 2002.
17. Damas J, O'Connor R, Farre M, Lenis VP, Martell HJ, Mandawala A, et al. Upgrading short read animal genome assemblies to chromosome level using comparative genomics and a universal probe set. Genome Res. 2016; doi:10.1101/gr.213660.116.
18. Ruvinskiy D, Larkin DM and Farré M. A Near Chromosome Assembly of the Dromedary Camel Genome. Frontiers in Genetics. 2019;10 32 doi:10.3389/fgene.2019.00032.
19. Proskuryakova AA, Kulemzina AI, Perelman PL, Makunin AI, Larkin DM, Farré M, et al. X Chromosome Evolution in Cetartiodactyla. Genes. 2017;8 9:216. doi:10.3390/genes8090216.
20. Schneider VA, Chen HC, Clausen C, Meric PA, Zhou Z, Bouk N, et al. Clone DB: an integrated NCBI resource for clone-associated data. Nucleic acids research. 2013;41 Database issue:D1070-8. doi:10.1093/nar/gks1164.

21. Karolchik D, Baertsch R, Diekhans M, Furey TS, Hinrichs A, Lu YT, et al. The UCSC Genome Browser Database. Nucleic acids research. 2003;31 doi:10.1093/nar/gkg129.
22. Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV and Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31 19:3210-2. doi:10.1093/bioinformatics/btv351.
23. Waterhouse RM, Seppey M, Simao FA, Manni M, Ioannidis P, Klioutchnikov G, et al. BUSCO applications from quality assessments to gene prediction and phylogenomics. Mol Biol Evol. 2017; doi:10.1093/molbev/msx319.
24. Koepfli K-P, Benedict Paten, Scientists tGKCo and O'Brien SJ. The Genome 10K Project: A Way Forward. Annual Review of Animal Biosciences. 2015;3 1:57-111. doi:doi:10.1146/annurev-animal-090414-014900.
25. Lewin HA, Robinson GE, Kress WJ, Baker WJ, Coddington J, Crandall KA, et al. Earth BioGenome Project: Sequencing life for the future of life. Proceedings of the National Academy of Sciences. 2018; doi:10.1073/pnas.1720115115.
26. Tarailo-Graovac M and Chen N. Using RepeatMasker to identify repetitive elements in genomic sequences. Curr Protoc Bioinformatics. 2009;Chapter 4:Unit 4.10. doi:10.1002/0471250953.bi0410s25.
27. Birney E, Clamp M and Durbin R. GeneWise and Genomewise. Genome Res. 2004;14 5:988-95. doi:10.1101/gr.1865504.
28. Stanke M, Keller O, Gunduz I, Hayes A, Waack S and Morgenstern B. AUGUSTUS: ab initio prediction of alternative transcripts. Nucleic Acids Res. 2006;34 Web Server issue:W435-9. doi:10.1093/nar/gkl200.
29. Burge C and Karlin S. Prediction of complete gene structures in human genomic DNA. J Mol Biol. 1997;268 1:78-94. doi:10.1006/jmbi.1997.0951.
30. Korf I. Gene finding in novel genomes. BMC Bioinformatics. 2004;5:59. doi:10.1186/1471-2105-5-59.
31. Zhang C, Chen L, Zhou Y, Wang K, Chemnick LG, Ryder OA, et al. Draft genome of the milu (Elaphurus davidianus). GigaScience. 2018;7 2:gix130-gix. doi:10.1093/gigascience/gix130.
32. Elsik CG, Mackey AJ, Reese JT, Milshina NV, Roos DS and Weinstock GM. Creating a honey bee consensus gene set. Genome Biol. 2007;8 1:R13. doi:10.1186/gb-2007-8-1-r13.
33. Hassanin A, Delsuc F, Ropiquet A, Hammer C, Jansen van Vuuren B, Matthee C, et al. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes rendus biologies. 2012;335 1:32-50. doi:10.1016/j.crvi.2011.11.002.
34. Decker JE, Pires JC, Conant GC, McKay SD, Heaton MP, Chen K, et al. Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics. Proceedings of the National Academy of Sciences. 2009;106 44:18644-9. doi:10.1073/pnas.0904691106.
35. Mitchell G and Skinner JD. On the origin, evolution and phylogeny of giraffes Giraffa camelopardalis. Transactions of the Royal Society of South Africa. 2003;58 1:51-73. doi:10.1080/00359190309519935.
36. Irwin DM, Kocher TD and Wilson AC. Evolution of the cytochrome b gene of mammals. Journal of molecular evolution. 1991;32 2:128-44.
37. Li H, Coghlan A, Ruan J, Coin LJ, Heriche JK, Osmotherly L, et al. TreeFam: a curated database of phylogenetic trees of animal gene families. Nucleic acids research. 2006;34 Database issue:D572-80. doi:10.1093/nar/gkj118.

38. Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L, et al. Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature. 2011;479 7372:223-7. doi:10.1038/nature10533.
39. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W and Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59 3:307-21. doi:10.1093/sysbio/syq010.
40. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol. 2007;24 8:1586-91. doi:10.1093/molbev/msm088.
41. Hedges SB, Dudley J and Kumar S. TimeTree: a public knowledge-base of divergence times among organisms. Bioinformatics (Oxford, England). 2006;22 23:2971-2. doi:10.1093/bioinformatics/btl505.
42. Koepfli K-P, Tamazian G, Wildt D, Dobrynin P, Kim C, Frandsen PB, et al. Whole Genome Sequencing and Re-sequencing of the Sable Antelope (Hippotragus niger): A Resource for Monitoring Diversity in ex Situ and in Situ Populations. G3: Genes|Genomes|Genetics. 2019:g3.400084.2019. doi:10.1534/g3.119.400084.
43. Primmer CR. From conservation genetics to conservation genomics. Annals of the New York Academy of Sciences. 2009;1162:357-68. doi:10.1111/j.1749-6632.2009.04444.x.
44. Farré M; Li Q; Darolti I; Zhou Y; Damas J; Proskuryakova AA; Kulemzina AI; Chemnick LG; Kim J; Ryder OA; Ma J; Graphodatsky AS; Zhang G; Larkin DM; Lewin HA (2019): Supporting data for "An integrated chromosome-scale genome assembly of the Masai Giraffe (Giraffa camelopardalis tippelskirchi)" GigaScience Database. http://dx.doi.org/10.5524/100590

**Table 1. Assembly statistics of the *Giraffa camelopardalis tippelskirchi* genome.**

| | ASM165123* | SOAPdenovo | SOAPdenovo + Chicago | SOAPdenovo + RACA | SOAPdenovo + Chicago + RACA | FINAL assembly |
|---|---|---|---|---|---|---|
| **Total length (Mbp)** | 2,705.07 | 2,551.62 | 2,554.82 | 2,391.72 | 2,425.09 | 2,437.09 |
| **N50 (Mbp)** | 0.21 | 3.00 | 57.20 | 85.22 | 88.36 | 177.94 |
| **No. scaffolds/PCFs** | 513,177 | 739,028 | 735,884 | 47 | 42 | 24 |
| **Gap sequence (%)** | 3.48 | 3.01 | 3.13 | 3.06 | 3.22 | 3.69 |
| **No. input scaffolds/PCFs broken** | -- | -- | 54 | 35 | 16 | 0 |

***Agaba et al., 2016.**

**Figure 1. A representative adult female Masai giraffe (*Giraffa camelopardalis tippelskirchi*) in the Masai Mara national park, Kenya.** Picture taken by Bjørn Christian Tørrissen, licence CC BY-SA 3.0.

**Figure 2. Syntenic relationships between giraffe and cattle genomes.** (A) Circos plot showing syntenic relationships between cattle autosomes (labelled as BTA) and giraffe chromosomes. Chromosomes are colored based on cattle homologies. Ribbons inside the plot show syntenic relationships, while lines inside each ribbon indicate inversions. (B) Placement of cattle BACs onto the giraffe karyotype. The first column of numbers on the right of each pair of giraffe chromosomes correspond to cattle (BTA) chromosomes, while the second column locates the cattle BAC IDs hybridized to giraffe chromosomes. (C) Giraffe chromosome 14 showing homologous synteny blocks (HSBs) between giraffe and cattle. SOAPdenovo and SOAPdenovo + Chicago scaffolds are also displayed. Blue blocks indicate positive (+) orientation of tracks compared with the giraffe chromosome while red blocks, negative (−) orientation. Numbers inside each block represent cattle chromosomes or giraffe scaffold IDs. BTA: *Bos taurus*, cattle. Images of all giraffe chromosomes could be found in Supplementary Fig. 1.

**Figure 3. Benchmarking of genome completeness for the four giraffe assemblies using BUSCO ~~assessment results~~.** The BUSCO dataset of the mammalia_odb9 including 4,104 ~~BUSCO~~genes~~s~~ was used to assess the completeness of the four giraffe genome assemblies, as well as the previously published giraffe genome (ASM165123v1~~,~~ [9]). The newly released cattle (ARS−UCD1.2, GCA_002263795.2) and goat (ARS1, GCA_001704415.1) assemblies are included for comparison. ~~-~~

**Figure 4. Phylogenetic relationships of the giraffe.** Phylogenetic tree constructed with orthologous genes. Divergence times were extracted from the TimeTree database for calibration. Blue bars indicate the estimated divergence times in millions of years, and red circle indicates the calibration time.

Figure 1

Click here to access/download;Figure;Figure1.JPG

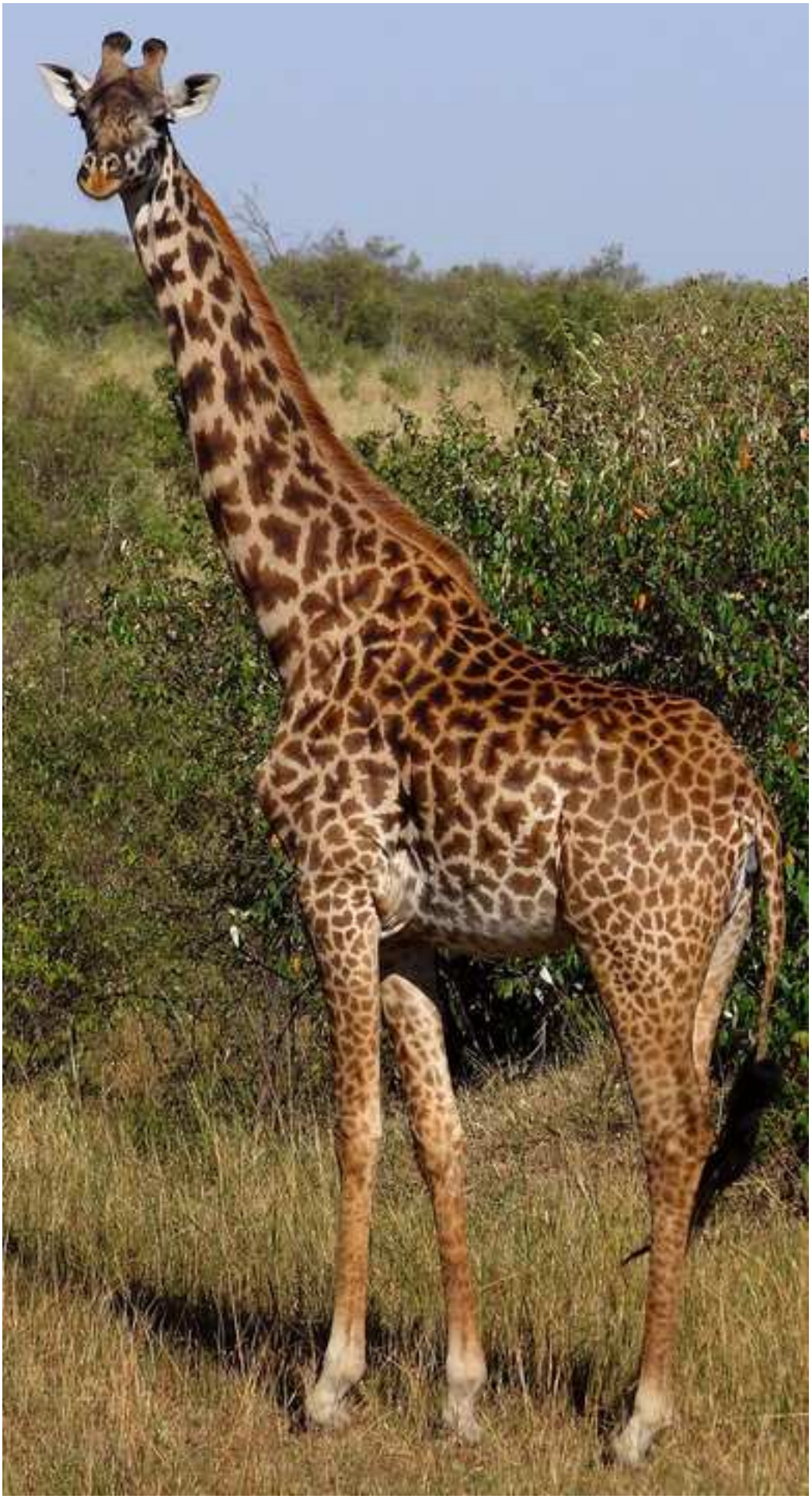


Figure 2



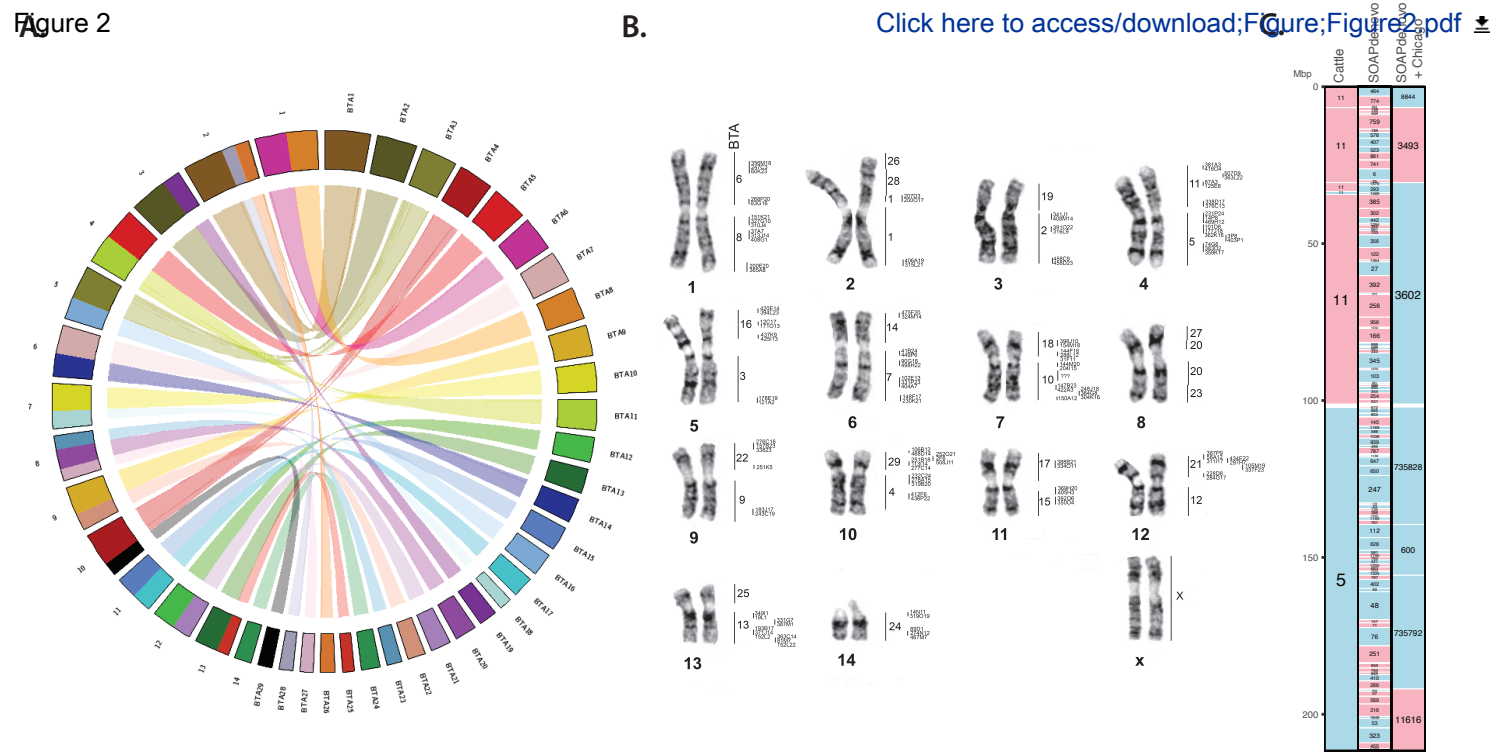


Figure 3



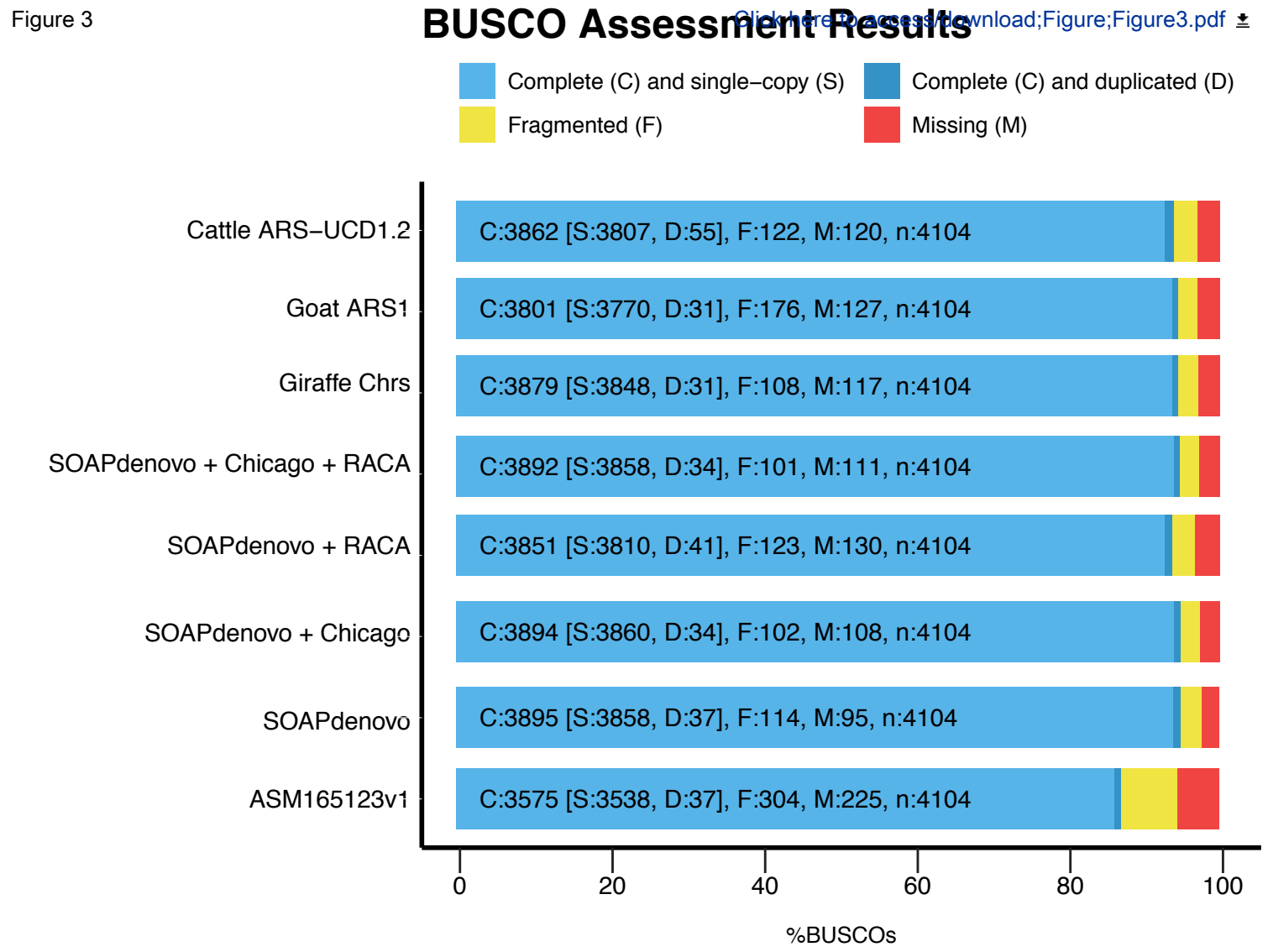
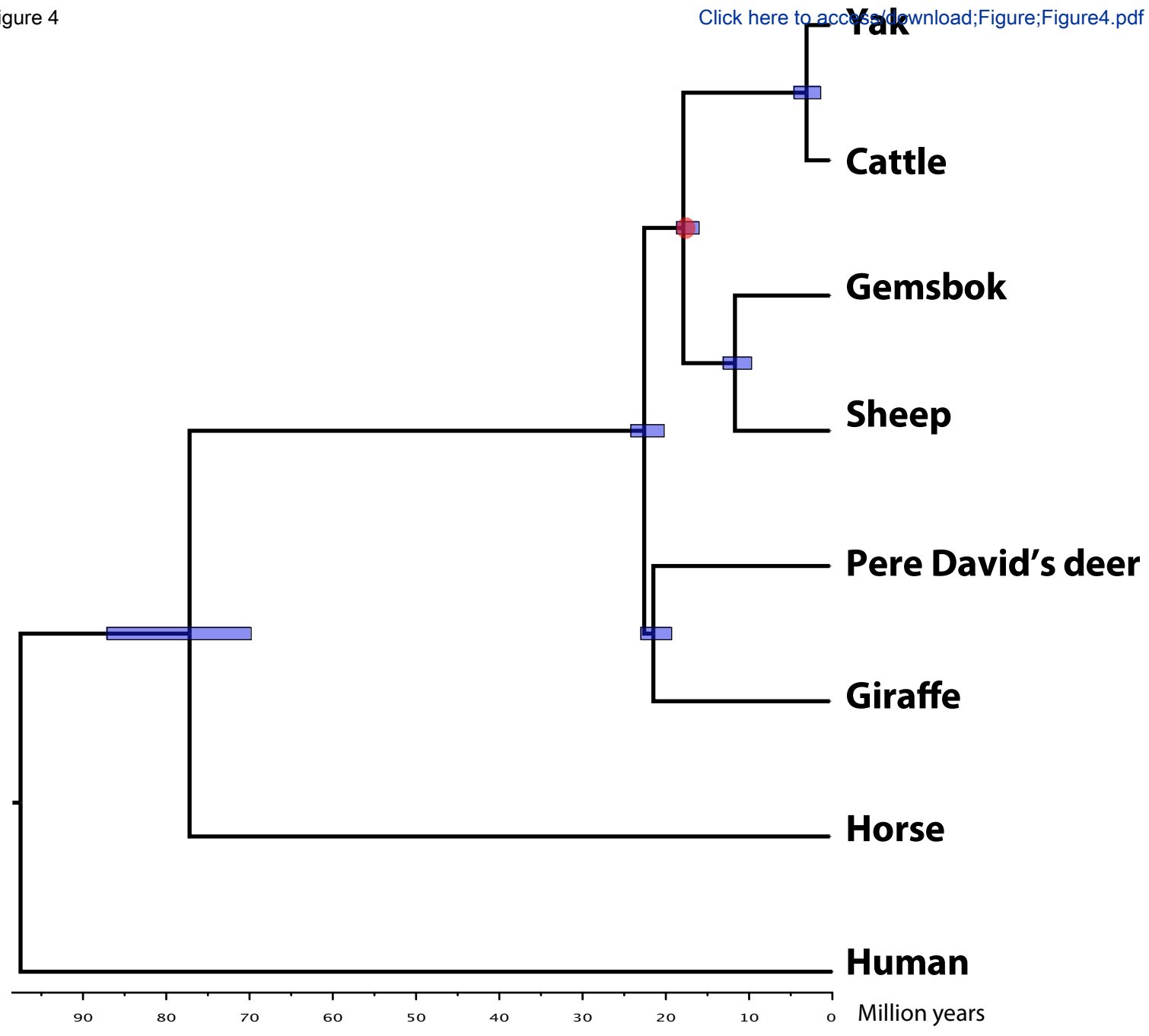


Figure 4





Supplementary Figure 1