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Supplemental Information

Whole-genome analysis of giraffe supports four distinct species

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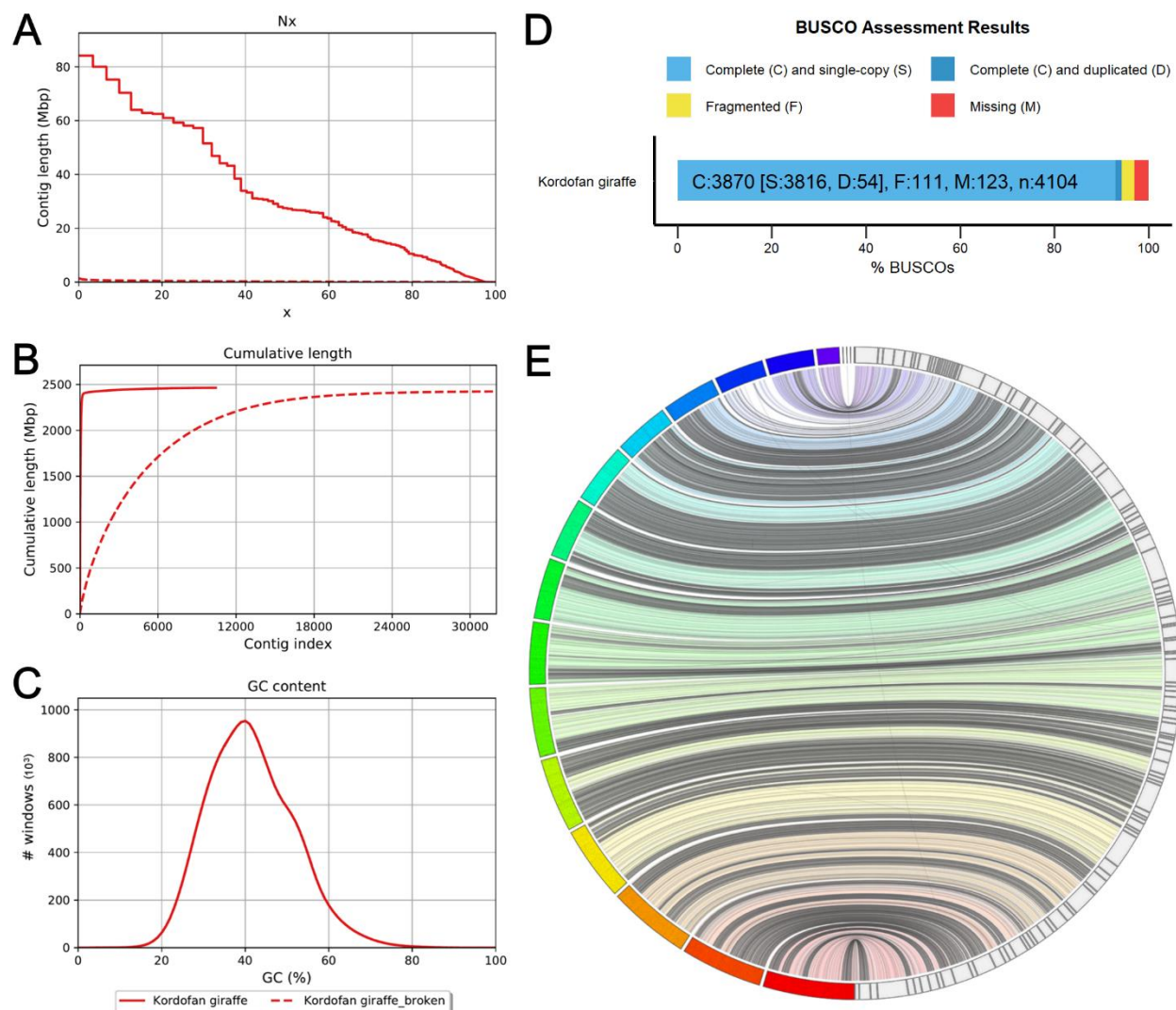


Figure S1. Quality assessment of the Kordofan giraffe genome assembly. Related to STAR Methods.

(A–C) Genome assembly continuity statistics measured as Nx (A) and cumulative length (B), and GC content (C). Plots include values calculated for contigs (dashed line) and scaffolds (solid line). (D) Assessment of genome completeness based on a total of 4,104 mammalian BUSCOs. (E) Genome consistency plot for the alignment of the Kordofan giraffe assembly against the nearly chromosome-length Masai giraffe *s. str.* assembly (<https://www.dnazoo.org/>). The Masai giraffe *s. str.* scaffolds are on the left, in color, and the Kordofan giraffe scaffolds on the right, in grey. The assemblies are largely consistent showing only a few split alignments. Only the largest scaffolds that are equal to 95% of the Kordofan giraffe genome assembly length are shown.

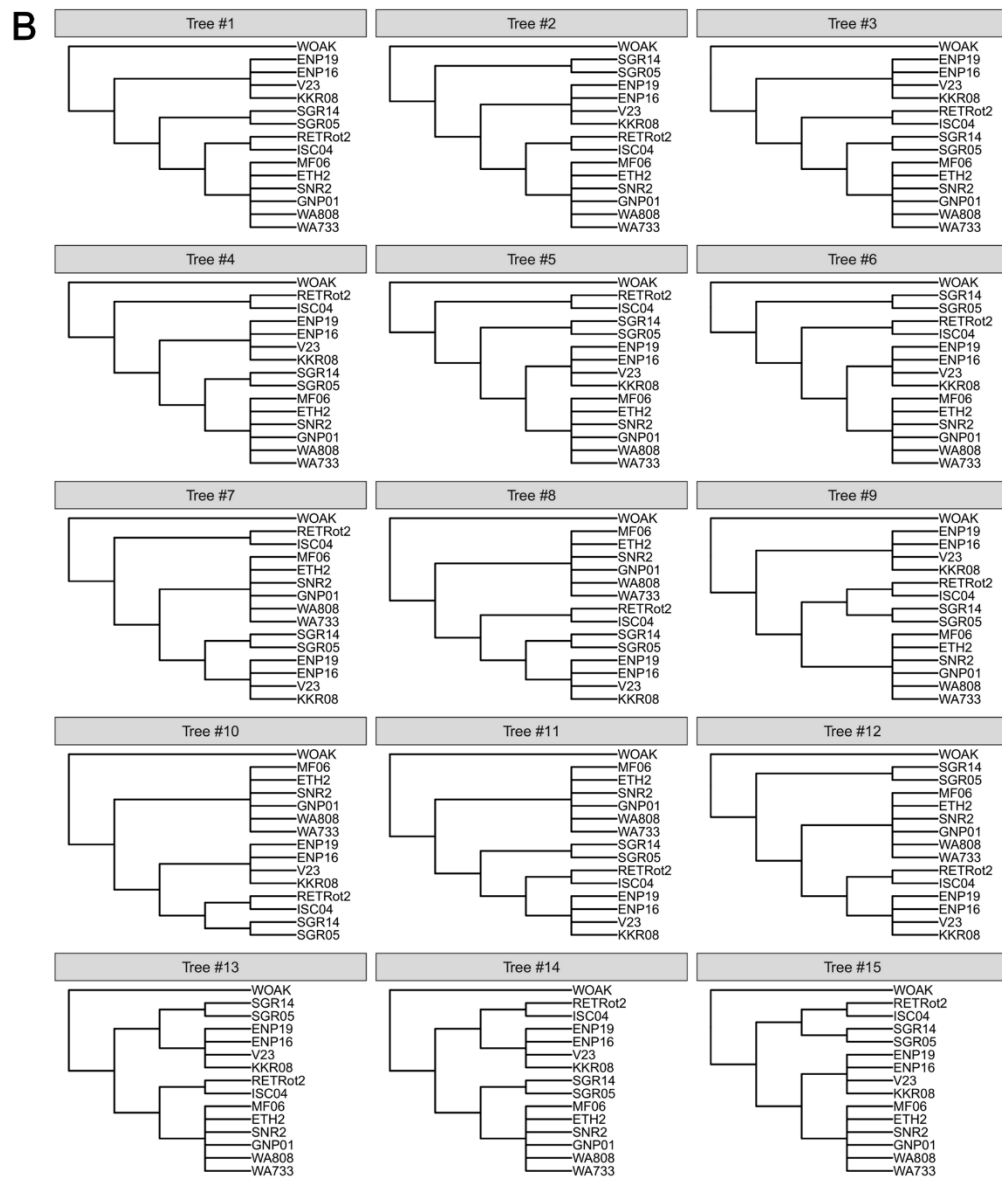
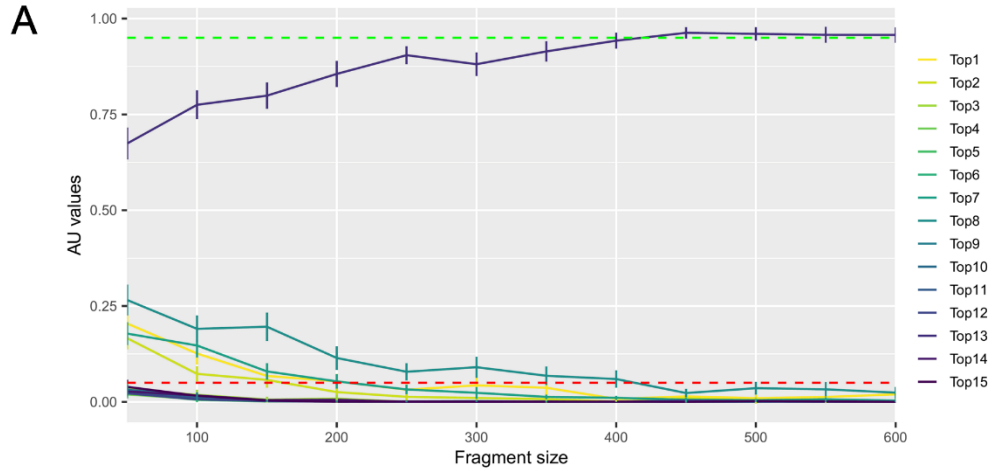


Figure S2. Approximately unbiased (AU) tree topology test for increasing GF sizes. Related to Figure 2 and STAR Methods. (A) AU test performed for fragment sizes of up to 600 kbp in steps of 50 kbp, with 200 randomly sampled GFs per fragment size. The AU test evaluates if the data can statistically reject ($p_{AU} < 0.05$) alternative tree topologies. Dashed lines mark p_{AU} values of 0.05 (red) and 0.95 (green). Topology 13 was the only one that could not be rejected at fragment sizes ≥ 450 kb. (B) The 15 possible tree topologies for the relationship between the four species of giraffe that were evaluated by the AU test. Each tree includes two randomly sampled individuals per (sub)species. The okapi was used as an outgroup.

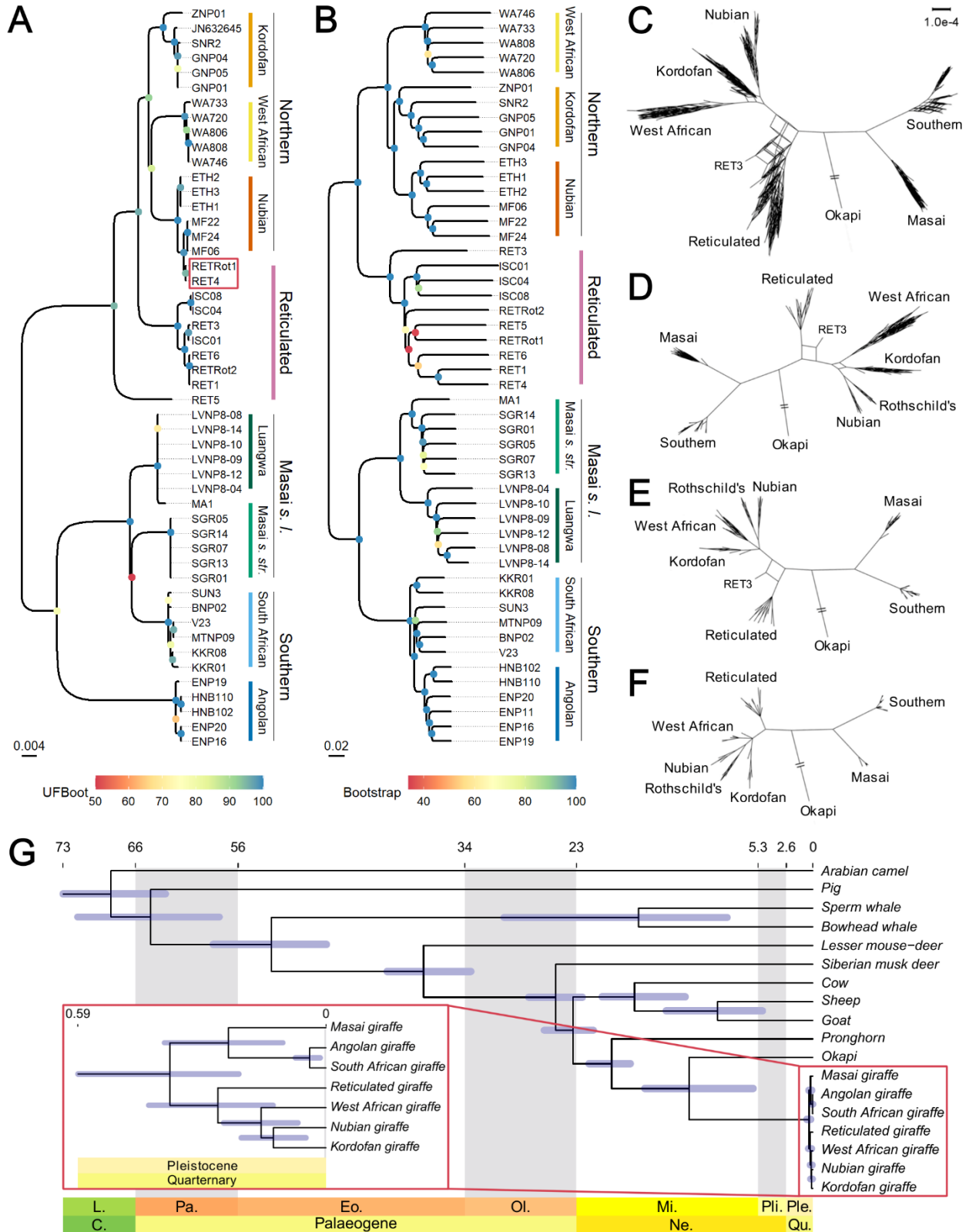


Figure S3. Mitochondrial and nuclear phylogenomics of giraffe and divergence times among Cetartiodactyla. Related to Figure 2. (A) Maximum likelihood phylogeny based on 13 mitochondrial protein-coding genes of 50 giraffe individuals. The okapi was used as an outgroup (not shown). Colored node circles indicate ultrafast bootstrap support. Individuals formed clades corresponding to their respective (sub)species with high support (> 99). Different from the nuclear tree is the grouping of South African and Masai giraffe *s. l.* and the placement of the West African giraffe as sister to the Nubian giraffe, although with low support (85). The red frame shows potential reticulated \times Nubian giraffe hybrids. (B) Neighbor-joining tree based on genotype likelihoods of genome-wide SNPs. The okapi was used as an outgroup (not shown). Colored node circles indicate support values based on 1,000 bootstrapped genetic distance matrices with replacement blocks of 500 SNPs. (C–F) Consensus network from 1,068 maximum likelihood GF trees at 5% (C), 10% (D), 15% (E), and 20% (F) minimum thresholds for conflicting edges. The four species of giraffe are discriminated at all thresholds. Reticulations in the network increase at thresholds $\leq 15\%$ indicating phylogenetic discordance. The placement of individual RET3 in reticulations between the northern and the reticulated giraffe at thresholds $\leq 15\%$ suggests that it is a hybrid. (G) Divergence time tree of Cetartiodactyla estimated from 2,127 orthologous sequences (1,496,715 bp). Giraffe and okapi diverged ~ 12 Ma ago. Northern and reticulated giraffe split from southern and Masai giraffe ~ 370 ka ago. The divergence between southern and Masai giraffe occurred at ~ 260 ka ago followed by the split between northern and reticulated giraffe at ~ 230 ka ago. Node bars represent the 95% highest posterior density (HPD) intervals. A list of the 95% HPD intervals for node ages and the four calibration points used for dating the tree are shown in Table S4.

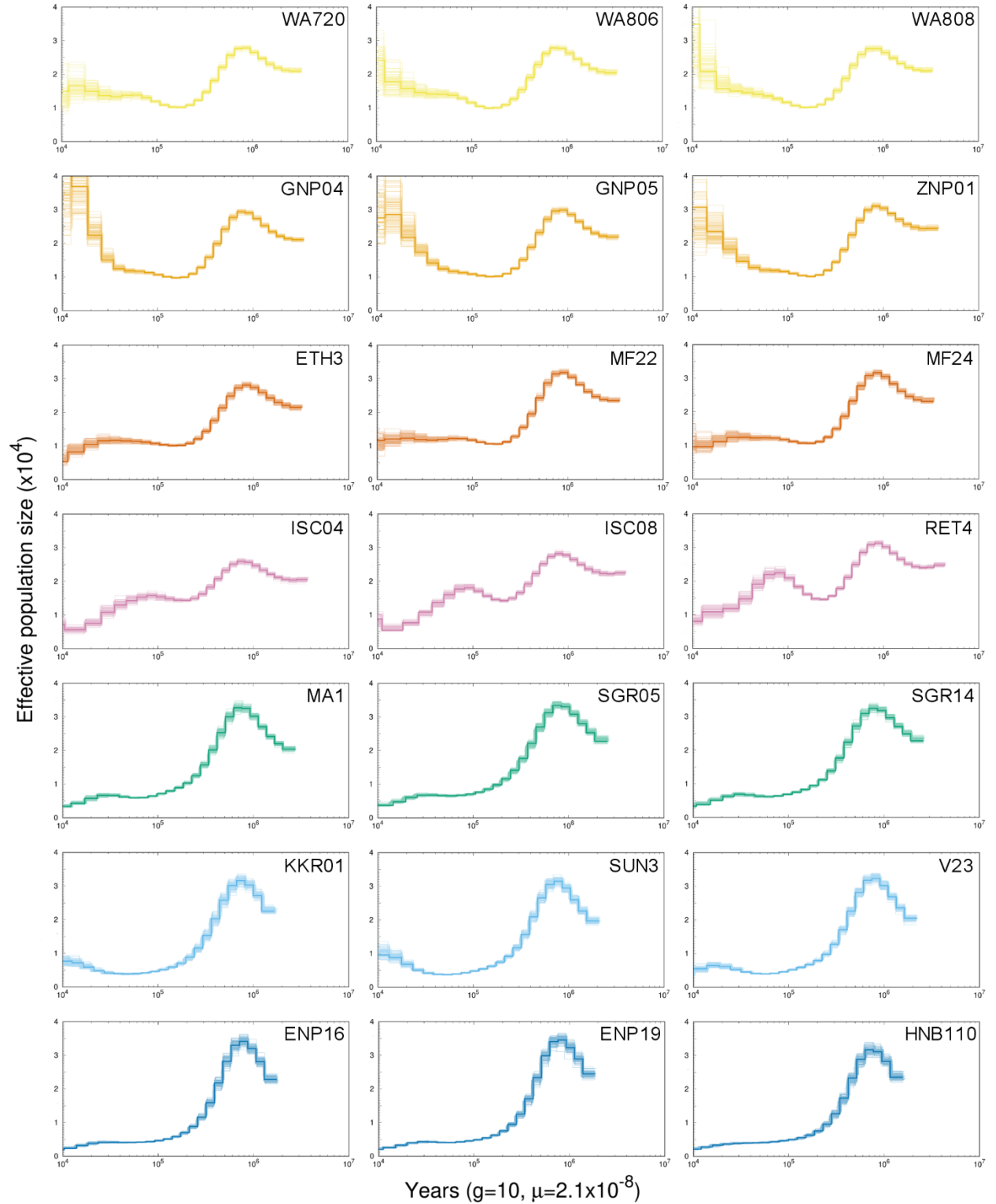


Figure S4. Demographic history of each giraffe individual with 100 bootstrap replicates. Related to Figure 3. Changes in N_e through time calculated by PSMC for the three individuals with the best sequencing

coverage per (sub)species. Sample names are given in each panel. Colors follow (sub)species affiliation as in Figure 3. Bootstrap replicates (light colored lines) show little variation in the data, except for West African and Kordofan giraffe individuals towards the more recent past indicating a potential artefact of the method. μ : mutation rate; g: generation time.

Family	Count	Length (bp)	Assembly length (%)
DNA	336,027	59,522,517	2.40
LINEs	1,257,542	633,147,039	25.69
LTRs	403,541	120,965,926	4.90
RC	1,819	304,773	0.01
SINEs	1,824,520	271,747,368	11.02
Unclassified	3,218	502,692	0.02
Low complexity	76,018	3,749,788	0.15
Satellites	4,234	1,293,195	0.06
Simple repeats	458,733	19,728,782	0.80
Small RNAs	5,975	505,536	0.03
Total	4,371,627	1,111,467,616	45.11

Table S1. Summary of the repetitive elements found in the Kordofan giraffe genome assembly.

Related to Figure 1 and STAR Methods. The number (count), combined lengths, and percentage of the genome assembly length are shown for each repeat family.

Sample	Origin	Taxonomy [S1]	Taxonomy [S2]	Total reads (10 ⁶)	Mapped (%)	Dup. (%)	Ins. size (bp)	Depth (×)	
								Raw	Clean
WA720	Koure, Niger	<i>Giraffa camelopardalis peralta</i>	<i>Giraffa camelopardalis peralta</i>	399.1	98.8	22.3	278	22	17
WA733	Koure, Niger	<i>Giraffa camelopardalis peralta</i>	<i>Giraffa camelopardalis peralta</i>	299.1	97.8	15.1	312	16	13
WA746	Koure, Niger	<i>Giraffa camelopardalis peralta</i>	<i>Giraffa camelopardalis peralta</i>	257.4	98.4	12.4	285	14	11
WA806	Koure, Niger	<i>Giraffa camelopardalis peralta</i>	<i>Giraffa camelopardalis peralta</i>	378.8	99.8	20.9	282	21	16
WA808	Koure, Niger	<i>Giraffa camelopardalis peralta</i>	<i>Giraffa camelopardalis peralta</i>	335.1	99.2	14.4	305	18	16
GNP01	Garamba NP, DR Congo	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	328.0	99.7	16.0	305	18	15
GNP04	Garamba NP, DR Congo	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	364.7	99.7	15.7	317	20	17
GNP05	Garamba NP, DR Congo	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	404.3	99.7	15.9	292	22	18
PLA01 ^a	Zoo Planckendael, Belgium	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	818.7	n/a	n/a	n/a	n/a	n/a
SNR2	Shambe NP, South Sudan	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	340.1	99.7	16.0	304	18	15
ZNP01 ^b	Zakouma NP, Chad	<i>Giraffa camelopardalis antiquorum</i>	<i>Giraffa camelopardalis antiquorum</i>	476.6	99.6	2.1	474	22	21
ETH1	Gambella NP, Ethiopia	<i>Giraffa camelopardalis camelopardalis</i>	<i>Giraffa camelopardalis camelopardalis</i>	371.8	99.8	13.5	289	19	15
ETH2	Gambella NP, Ethiopia	<i>Giraffa camelopardalis camelopardalis</i>	<i>Giraffa camelopardalis camelopardalis</i>	334.2	99.7	16.2	301	18	14
ETH3	Gambella NP, Ethiopia	<i>Giraffa camelopardalis camelopardalis</i>	<i>Giraffa camelopardalis camelopardalis</i>	337.3	99.8	15.4	300	19	15
MF06	Murchison Falls NP, Uganda	<i>Giraffa camelopardalis rothschildi</i>	<i>Giraffa camelopardalis camelopardalis</i>	309.9	99.0	13.1	283	17	14
MF22	Murchison Falls NP, Uganda	<i>Giraffa camelopardalis rothschildi</i>	<i>Giraffa camelopardalis camelopardalis</i>	522.5	99.8	18.9	320	29	24
MF24	Murchison Falls NP, Uganda	<i>Giraffa camelopardalis rothschildi</i>	<i>Giraffa camelopardalis camelopardalis</i>	466.3	99.8	9.7	319	26	23
RET1	Zoo Nürnberg, Germany	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	398.7	99.2	13.4	311	22	19
RET3	Zoo Nürnberg, Germany	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	287.0	99.7	12.5	286	15	13
RET4	Zoo Nürnberg, Germany	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	455.5	99.6	12.1	325	26	23
RET5	Zoo Nürnberg, Germany	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	411.4	99.6	14.4	318	23	19
RET6	Zoo Nürnberg, Germany	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	390.4	99.6	13.2	289	20	16
RETRot1	Zoo Rotterdam, the Netherlands	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	437.5	99.0	19.7	300	24	19
RETRot2	Zoo Rotterdam, the Netherlands	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	396.8	99.8	16.1	320	22	18
ISC01	Ishaqbini Conservancy, Kenya	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	306.3	57.7	20.8	247	7	6
ISC04	Ishaqbini Conservancy, Kenya	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	311.6	99.5	17.2	281	17	14
ISC08	Ishaqbini Conservancy, Kenya	<i>Giraffa camelopardalis reticulata</i>	<i>Giraffa reticulata</i>	391.7	99.8	13.5	288	20	17
LVNP8-04	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	234.3	99.7	17.8	278	11	8
LVNP8-08	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	312.1	99.7	20.2	290	17	12
LVNP8-09	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	381.3	99.7	20.6	296	18	12
LVNP8-10	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	322.0	99.7	26.6	287	14	9
LVNP8-12	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	305.1	98.3	19.7	293	16	11
LVNP8-14	Luangwa Valley NP, Zambia	<i>Giraffa camelopardalis thornicrofti</i>	<i>Giraffa tippelskirchi</i>	368.0	99.8	14.5	320	21	15
MA1 ^c	Masai Mara National Reserve, Kenya	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	415.7	98.8	0.8	515	22	21

Sample	Origin	Taxonomy [S1]	Taxonomy [S2]	Total reads (10 ⁶)	Mapped (%)	Dup. (%)	Ins. size (bp)	Depth (x)	
								Raw	Clean
SGR01	Selous Game Reserve, Tanzania	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	315.7	99.6	12.1	321	18	15
SGR05	Selous Game Reserve, Tanzania	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	504.7	96.5	14.1	315	27	23
SGR07	Selous Game Reserve, Tanzania	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	378.0	98.0	22.9	280	21	15
SGR13	Selous Game Reserve, Tanzania	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	410.3	99.1	17.5	317	23	18
SGR14	Selous Game Reserve, Tanzania	<i>Giraffa camelopardalis tippelskirchi</i>	<i>Giraffa tippelskirchi</i>	542.2	99.5	11.8	329	31	25
BNP02	Bwabwata NP, Namibia	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	295.6	99.7	22.3	289	16	12
KKR01	Khamab Kalahari Reserve, South Africa	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	335.6	99.5	15.0	310	19	16
KKR08	Khamab Kalahari Reserve, South Africa	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	316.0	99.0	12.9	304	17	14
MTNP09	Mosi-oa-Tunya NP, Zambia	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	326.5	99.4	21.7	279	18	14
SUN3	Sun Hotel, Livingstone, Zambia	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	320.5	97.9	13.9	313	18	15
V23	Vumbura Concession, Botswana	<i>Giraffa camelopardalis giraffa</i>	<i>Giraffa giraffa giraffa</i>	348.4	99.6	14.8	320	19	16
ENP11	Etosha NP, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	442.7	99.2	12.1	395	16	13
ENP16	Etosha NP, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	372.0	98.8	12.7	331	21	18
ENP19	Etosha NP, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	418.8	99.7	16.3	319	24	19
ENP20	Etosha NP, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	339.9	99.7	14.1	329	19	16
HNB102	Hoanib River Catchment, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	362.7	99.7	24.0	302	20	15
HNB110	Hoanib River Catchment, Namibia	<i>Giraffa camelopardalis angolensis</i>	<i>Giraffa giraffa angolensis</i>	340.0	99.7	14.4	322	19	16
WOAK ^d	White Oak Conservation Center	<i>Okapia johnstoni</i>		413.3	94.6	6.3	530	20	19

^a used for *de novo* genome assembly; ^b accession: ERR1248124; ^c accession: SRR3218456; ^d accessions: SRR3217625 and SRR3217884.

Table S2. Sample information and mapping statistics. Related to Figure 1 and STAR Methods. Sample name, place of origin, taxonomic classification, total number of reads, percentage of reads mapped to the Kordofan giraffe genome assembly, percentage of duplicated reads, median insert size, and depth of coverage (before and after cleaning the BAM files) are shown per individual.

Common name	Scientific name	Accession	Source
Arabian camel	<i>Camelus dromedarius</i>	GCA_000803125.2	Ensembl
Pig	<i>Sus scrofa</i>	GCA_000003025.6	Ensembl
Sperm whale	<i>Physeter catodon</i>	GCA_002837175.2	Ensembl
Bowhead whale	<i>Balaena mysticetus</i>		Bowhead Whale Genome Resource
Lesser mouse-deer	<i>Tragulus kanchil</i>		Ruminant Genome Database
Siberian musk deer	<i>Moschus moschiferus</i>	GCA_004024705.2	Ensembl
Cow	<i>Bos taurus</i>	GCA_002263795.2	Ensembl
Goat	<i>Capra hircus</i>	GCA_001704415.1	Ensembl
Sheep	<i>Ovis aries</i>	GCA_000298735.1	Ensembl
Pronghorn	<i>Antilocapra americana</i>		Ruminant Genome Database
Okapi	<i>Okapia johnstoni</i>		Ruminant Genome Database
West African giraffe	<i>Giraffa camelopardalis peralta</i>		This study; sample WA808.
Kordofan giraffe	<i>G. c. antiquorum</i>		This study; sample GNP05.
Nubian giraffe	<i>G. c. camelopardalis</i>		This study; sample MF24.
Reticulated giraffe	<i>G. reticulata</i>		This study; sample ISC04.
Masai giraffe	<i>G. tippelskirchi</i>		This study; sample SGR05.
South African giraffe	<i>G. giraffa giraffa</i>		This study; sample V23.
Angolan giraffe	<i>G. g. angolensis</i>		This study; sample ENP16.

Table S3. List of Cetartiodactyla species used for orthology search and divergence time analysis. Related to STAR Methods. Common names, scientific names, accession numbers, and source databases of downloaded sequences.

Tree node	Node ages (Ma)		Calibrated nodes	
	Mean	95% HPD	Age (Ma)	Refs.
Cetartiodactyla	68.39	63.08–73.05	63–73	[S3]
Pig + Cetruminantia	64.52	57.84–71.62		
Cetruminantia	52.76	47.39–58.44	47–58.75	[S4]
Cetacea	16.96	8.35–30.09		
Ruminantia	37.92	33.31–41.48	33–41.25	[S5]
Pecora	25.05	22.46–27.69		
Bovidae + Pronghorn/Giraffidae	23.37	21.38–26.17		
Bovidae	17.37	12.43–20.54		
Caprinae	9.28	4.89–14.29		
Pronghorn + Giraffidae	19.60	17.78–21.81	18–22.5	[S6]
Giraffidae	12.07	5.81–16.37		
<i>Giraffa</i>	0.37	0.21–0.59		
Northern giraffe + reticulated giraffe	0.26	0.12–0.42		
West African giraffe + Kordofan/Nubian giraffe	0.15	0.07–0.24		
Kordofan giraffe + Nubian giraffe	0.12	0.05–0.20		
Masai giraffe + southern giraffe	0.23	0.10–0.38		
South African giraffe + Angolan giraffe	0.04	0.01–0.07		

Table S4. List of the 95% highest posterior density (HPD) intervals obtained for splits within Cetartiodactyla and the four calibration points used for the divergence time analysis. Related to Figure S3 and STAR Methods. Node age estimates are in million years ago (Ma).

SUPPLEMENTAL REFERENCES

- S1. Dagg, A.I. (1971). *Giraffa camelopardalis*. Mamm. Species, 1–8.
- S2. Fennessy, J., Bidon, T., Reuss, F., Kumar, V., Elkan, P., Nilsson, M.A., Vamberger, M., Fritz, U., and Janke, A. (2016). Multi-locus analyses reveal four giraffe species instead of one. Curr. Biol. 26, 2543–2549.
- S3. Gatesy, J. (2009). Whales and even-toed ungulates (Cetartiodactyla). In The Timetree of Life, S. B. Hedges and S. Kumar, eds. (New York, NY: Oxford University Press), pp. 511–515.
- S4. Gingerich, P.D. (2001). Origin of whales from early artiodactyls: Hands and feet of Eocene Protocetidae from Pakistan. Science 293, 2239–2242.
- S5. Metais, G., Chaimanee, Y., Jaeger, J.-J., and Ducrocq, S. (2001). New remains of primitive ruminants from Thailand: Evidence of the early evolution of the Ruminantia in Asia. Zool. Scr. 30, 231–248.
- S6. Sánchez, I.M., Cantalapiedra, J.L., Ríos, M., Quiralte, V., and Morales, J. (2015). Systematics and evolution of the Miocene three-horned palaeomerycid ruminants (Mammalia, Cetartiodactyla). PLoS One 10, e0143034.