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First insights into past biodiversity of giraffes based on mitochondrial sequences from museum specimens

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[6] urn:lsid:zoobank.org:author:7754B40E-DEB5-4133-B91E-0C0341D2211D
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This paper is dedicated to the memory of our colleague and friend, Dr. med.vet. Jacques Rigoulet, who passed away on 18 May 2020.

Abstract. Intensified exploration of sub-Saharan Africa during the 18th and 19th centuries led to many newly described giraffe subspecies. Several populations described at that time are now extinct, which is problematic for a full understanding of giraffe taxonomy. In this study, we provide mitochondrial sequences for 41 giraffes, including 19 museum specimens of high importance to resolve giraffe taxonomy, such as Zarafa from Sennar and two giraffes from Abyssinia (subspecies *camelopardalis*), three of the first southern individuals collected by Levaillant and Delalande (subspecies *capensis*),

topotypes of the former subspecies *congoensis* and *cottoni*, and giraffes from an extinct population in Senegal. Our phylogeographic analysis shows that no representative of the nominate subspecies *camelopardalis* was included in previous molecular studies, as Zarafa and two other specimens assigned to this taxon are characterized by a divergent haplogroup, that the former subspecies *congoensis* and *cottoni* should be treated as synonyms of *antiquorum*, and that the subspecies *angolensis* and *capensis* should be synonymized with *giraffa*, whereas the subspecies *wardi* should be rehabilitated. In addition, we found evidence for the existence of a previously unknown subspecies from Senegal (newly described in this study), which is now extinct. Based on these results, we propose a new classification of giraffes recognizing three species and 10 subspecies. According to our molecular dating estimates, the divergence among these taxa has been promoted by Pleistocene climatic changes resulting in either savannah expansion or the development of hydrographical networks (Zambezi, Nile, Lake Chad, Lake Victoria).

Introduction

The Europeans saw for the first time a living giraffe in Roman times when Julius Caesar returned with a menagerie of exotic animals from Africa in 46 BC. The Romans named this extraordinary animal '*Cameleopard*', due to its head and tail like a camel and coat like a leopard (Pliny the Elder AD 64 translated by Bostock & Riley 1855). Based on the illustration of a living giraffe made by Belon du Mans (1553) and descriptions provided by subsequent authors (Ray 1653; Hasselquist 1757), Linnaeus (1758) classified the giraffe as *Cervus camelopardalis* Linnaeus, 1758 (type locality: Sennar and Ethiopia), because its horns were supposed to be similar to deer antlers. A few years later, Brisson (1762) emphasised the permanence of the horns and assigned giraffes to their own genus *Giraffa* Brisson, 1762. However, neither Brisson nor Linnaeus had ever seen a living giraffe.

During the years 1780–1785, the French explorer François Levaillant undertook two expeditions in the Cape of Good Hope (western part of present South Africa). During his second journey (1783–1785), he encountered several giraffes close to the Orange River (Levaillant 1797), of which he sent a skin to the Muséum national d'histoire naturelle (MNHN) in Paris (illustrated by von Schreber 1784) and a skeleton to the collection of the governor of Netherlands, Guillaume V, in The Hague, which was transferred to the MNHN in 1795 (Schickh 1828). Pierre-Antoine Delalande (1822) enriched the giraffe collection of the MNHN with three additional skulls and a skin from giraffes of the Cape Colony.

In 1824, two young giraffes were caught "eight to ten days' caravan south of Sennaar" (Salze 1827), the former capital of the Sennar region located in present Sudan. The giraffes were offered by Mehmet Ali, the Pasha of Egypt, to Charles X, King of France, and George IV, King of the United Kingdom (Allin 1999). Only the giraffe of Charles X, later named Zarafa, reached Europe in good health, entering France via Marseille in 1826 (Allin 1999). After some weeks of quarantine, Zarafa continued its journey by foot towards Paris, accompanied by Étienne Geoffroy Saint-Hilaire (Geoffroy Saint-Hilaire 1827). It lived 18 years at the Ménagerie du Jardin des Plantes of the MNHN (Rigoulet 2012). By comparing Zarafa with southern giraffes collected by Levaillant (1797) and Delalande (1822), Geoffroy Saint-Hilaire was the first zoologist to distinguish two giraffe species: *Giraffa camelopardalis* (Linnaeus, 1758) from Sennar and Ethiopia and the "Girafe du Cap" from the Cape Region (Geoffroy Saint-Hilaire 1827). Some years later, Lesson (1842) classified the southern species under the name *Giraffa capensis* (Lesson, 1842). With the exploration of Africa during the 19th century, many giraffes were collected

for European natural history museums, which led to the description of several subspecies, primarily based on differences in coat pattern and colouration (see Table 1). Lydekker (1914) recognized two giraffe species and 14 subspecies: *Giraffa camelopardalis*, represented by six subspecies in northern sub-Saharan Africa (*G. c. camelopardalis*, *G. c. antiquorum* (Jardine, 1835), *G. c. congoensis* Lydekker, 1903, *G. c. cottoni* Lydekker, 1904, *G. c. peralta* Thomas, 1898 and *G. c. rothschildi* Lydekker, 1903), two subspecies in East Africa (*G. c. thornicrofti* Lydekker, 1911 and *G. c. tippelskirchi* Matschie, 1898) and four subspecies in southern Africa (*G. c. angolensis* Lydekker, 1903, *G. c. capensis* (Lesson, 1842), *G. c. infumata* Noack, 1908 and *G. c. wardi* Lydekker, 1904); and *Giraffa reticulata* Thomas, 1901, including two subspecies from Kenya (*G. r. reticulata* de Winton, 1899 and *G. r. nigrescens* Lydekker, 1911). This classification was adopted by most scientists over the next decades, until Dagg (1962) proposed a new classification recognizing only the single species *Giraffa camelopardalis*, including 13 subspecies (see Fig. 1A). Ansell (1968) agreed with the existence of a single species, but recognized only nine subspecies: *G. c. camelopardalis*, *G. c. angolensis*, *G. c. antiquorum*, *G. c. peralta*, *G. c. giraffa* (Boddaert, 1784), *G. c. reticulata*, *G. c. rothschildi*, *G. c. thornicrofti* and *G. c. tippelskirchi* (Fig. 1B, see Table 1 for synonymized subspecies). This view was adopted (sometimes with minor changes) in most subsequent classifications (e.g., Dagg & Foster 1976; Wilson & Mittermeier 2011; Ciofolo & Le Pendu 2013; Muller *et al.* 2018).

A mitochondrial fragment, covering the cytochrome *b* gene (*Cytb*), tRNA genes for Threonine and Proline, and the 5′ region of the control region (CR) (length = 1764 base pairs (bp)), was sequenced for giraffes of seven of the nine recognized subspecies (n = 23; *G. c. camelopardalis* and *G. c. thornicrofti* missing) in the molecular study of Hassanin *et al.* (2007). Their phylogenetic analyses have indicated that northern giraffes constitute a monophyletic group, distinct from that of southern giraffes. Brown *et al.* (2007) analysed two mitochondrial fragments (cytochrome *b*, tRNA genes for Threonine and Proline, and 5′ region of the CR; length = 1705 bp) and 14 nuclear microsatellite loci for many wild
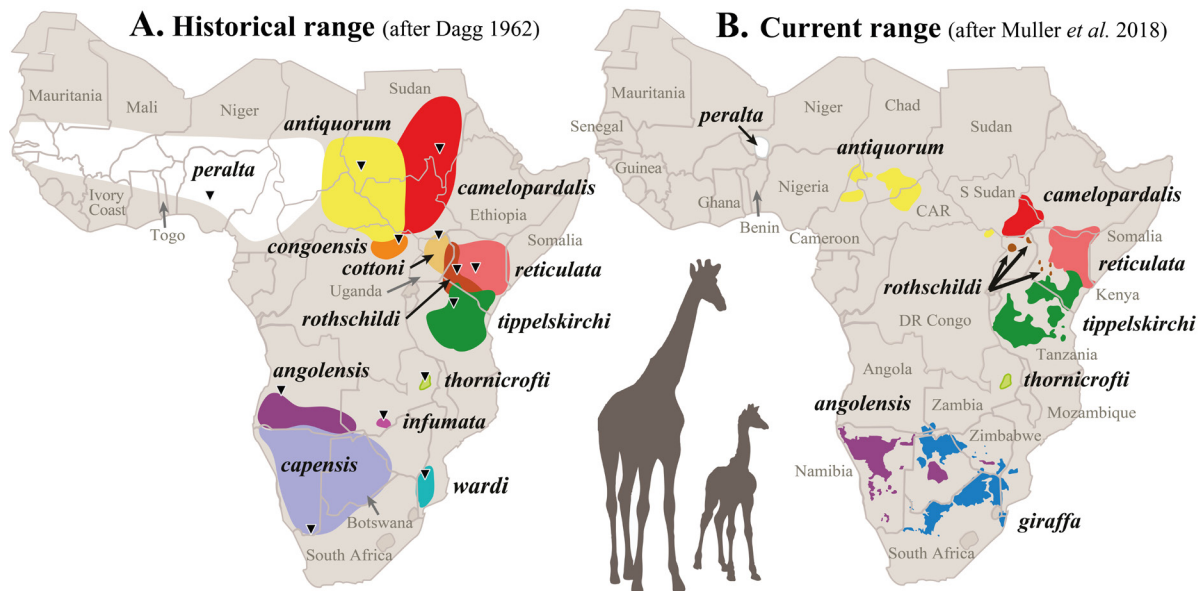
Fig. 1. Distribution range of giraffe subspecies. **A**. Within historic times (after Dagg 1962). **B**. At present (after Muller *et al.* 2018). The subspecies are distinguished by different colours on both maps, whereby the assignment of colours for the nine currently recognized subspecies (B) was modified from https://giraffeconservation.org/giraffe-species/. The type locality for each subspecies is indicated by a triangle in map A and detailed in Table 1.

Table 1. Currently accepted giraffe subspecies (Muller *et al.* 2018) with their synonyms (modified after Shorrocks 2016).

Subspecies	Description	Type specimen	Type locality	Synonyms
camelopardalis	Linnaeus 1758	Living giraffe illustrated by Belon du Mans (1553), never deposited in a museum collection.	Sennar (Sudan) and Ethiopia	*Camelopardalis biturigum* Duvernoy, 1844 *Camelopardalis aethiopica* Ogilby, 1837 *Giraffa camelopardalis typica* Bryden, 1899
giraffa	Boddaert 1784	Specimens of the Prince of Orange Museum (The Hague) and of Vosmaer (1787) (Museum Leiden), Netherlands.	Cape of Good Hope, South Africa	*Camelopardalis capensis* Lesson, 1842 *Camelopardalis australis* Swainson, 1835 *Camelopardalis maculata* Weinland, 1863 *Giraffa camelopardalis wardi* Lydekker, 1904
antiquorum	Jardine 1835	SMF-498: unspec. type and SMF-497: paratype	South of Darfour, Sudan	*Giraffa camelopardalis senaariensis* Trouessart, 1898 *Giraffa camelopardalis congoensis* Lydekker, 1903
peralta	Thomas 1898	NHMUK-1898.2.18.1	Lokoja junction Niger and Benue rivers, Nigeria	–
tippelskirchi	Matschie 1898	ZMB-084951 (syntype); second specimen might be considered lost	Lake Eyasi, Tanzania	*Giraffa schillingsi* Matschie, 1898
reticulata	de Winton 1899	NHMUK-18971.30.1	Loroghi Mountains, Kenya	*Giraffa hagenbecki* Knottnerus-Meyer, 1910 *Giraffa reticulata nigrescens* Lydekker, 1911 *Giraffa camelopardalis australis* Rhoads, 1896
rothschildi	Lydekker 1903	NHMUK-1903.4.15.1	Guasin-gisha Plateau east of Mount Elgon, Kenya	*Giraffa camelopardalis cottoni* Lydekker, 1904
angolensis	Lydekker 1903	NHMUK- 1939.480	Cunene River, Angola	*Giraffa camelopardalis infumata* Noack, 1908
thornicrofti	Lydekker 1911	NHMUK-1910.10.17.1	Petauke district, Zambia	–

individuals (n = 266 and 381, respectively) from six subspecies (*G. c. camelopardalis*, *G. c. antiquorum* and *G. c. thornicrofti* missing). They suggested the existence of at least six species, corresponding to *Giraffa angolensis*, *G. giraffa*, *G. peralta*, *G. reticulata*, *G. rothschildi* and *G. tippelskirchi.* Bock *et al.* (2014) analysed a similar mitochondrial fragment of 102 giraffes (cytochrome *b* + 5′ region of the CR; length = 1555 bp) and proposed to synonymize the two subspecies *G. c. thornicrofti* and *G. c. tippelskirchi*, because they have very similar mitochondrial haplotypes. Fennessy *et al.* (2016) and Winter *et al.* (2018b) recognized four giraffe species based on the analyses of a mitochondrial fragment (cytochrome *b* + 5′ region of the CR; length = 1556 bp) and several nuclear introns (7 and 21, respectively): *G. camelopardalis* (northern giraffe), *G. giraffa* (southern giraffe), *G. reticulata* (reticulated giraffe) and *G. tippelskirchi* (Masai giraffe). More recently, Petzold & Hassanin (2020) re-examined all available molecular data using different methods of species delimitation that rely on different species concepts, i.e., multispecies coalescent (Genealogical species concept, Baum & Shaw 1995), population genetic (Genetic species concept, Baker & Bradley 2006) and phylogenetic (Phylogenetic species concept, Donoghue 1985) methods. Their results provided strong support for the distinction of three species only, corresponding to *G. camelopardalis* (including *reticulata*), *G. giraffa* and *G. tippelskirchi*. This species classification is the one adopted herein. For convenience, we will use the following subspecies names: *G. camelopardalis camelopardalis*, *G. c. antiquorum*, *G. c. congoensis*, *G. c. cottoni*, *G. c. peralta*, *G. c. reticulata*, *G. c. rothschildi*, *G. giraffa giraffa*, *G. g. angolensis*, *G. g. capensis*, *G. g. wardi*, *G. tippelskirchi tippelskirchi* and *G. t. thornicrofti*.

The present study provides further insights on the genetic diversity among giraffe subspecies. It is important to note that most subspecies previously identified using phenotypic features were found monophyletic with mitochondrial data, i.e., *G. c. antiquorum*, *G. c. peralta*, *G. c. rothschildi*, *G. g. giraffa*, *G. g. angolensis*, and *G. t. thornicrofti*. This does not mean that subspecies are fully isolated taxa, as gene flow between subspecies can be maintained through dispersing males, at least occasionally. However, the philopatry of females is apparently at the origin of some morphological characteristics. In this perspective, we report herein the first mitochondrial sequences for several key museum specimens. These include the famous giraffe Zarafa from Sennar, which is assumed to belong to the type population of *G. c. camelopardalis*, two giraffes from Abyssinia, several historical specimens collected by Levaillant and Delalande, which were described as *G. g. capensis* by Lesson (1842), as well as topotypes of the subspecies *G. c. congoensis* and *G. c. cottoni*, and two giraffes collected in Senegal during the 19th century (see Material and methods for more details). Our three main objectives were: (1) to compare for the first time past giraffe populations with current biodiversity; (2) to include in the phylogenetic analyses some subspecies described in former classifications in order to re-evaluate their taxonomic status; and (3) to estimate divergence times among giraffes in order to provide a more comprehensive phylogeographic scenario.

Material and methods

Museum specimens

Bone, tooth, faeces or skin samples were obtained from 41 giraffes, comprising samples from 19 museum specimens (Table 2, illustrated in Fig. 2, sample localities shown in Fig. 3). Among the most important samples are three specimens of the subspecies *G. g. capensis* (Lesson, 1842) collected by Levaillant (1797) and Delalande (1822), the first southern taxon investigated by the scientific community, with populations formerly found north of the Orange River (Levaillant 1797; see Fig. 3), a topotype of the subspecies *G. c. cottoni* Lydekker, 1904 collected by Powell-Cotton in the Lado enclave (left bank of the White Nile) in northwest Uganda and South Sudan, five specimens from the Haut-Uele Province in Democratic Republic of the Congo, which may be representatives of the type population of the subspecies *G. c. congoensis* described from the type locality Dungu (Lydekker 1903), two giraffes collected in Bakel (Senegal) by Girardin in 1830, which according to Dagg (1962) may represent the most western distribution point of *G. c. peralta* (Fig. 1A), and three specimens from the type locality of the nominate subspecies *G. c. camelopardalis*, i.e., the famous Zarafa from Sennar and two giraffes from Abyssinia (Ethiopia).
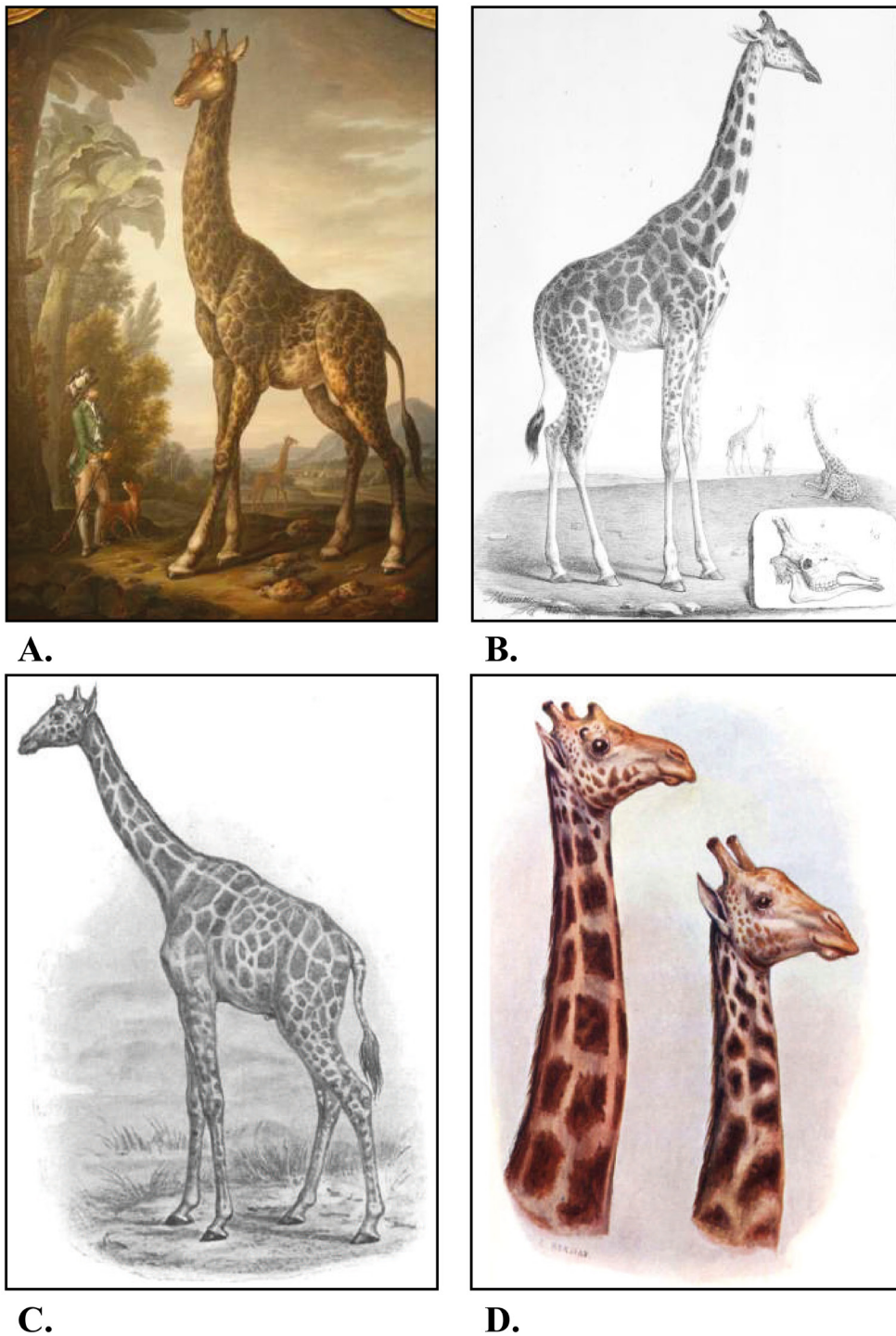
Fig. 2. Illustrations of historical giraffe specimens. **A**. The 'Giraffe of Levaillant', anonymous painting made in the late 18th century and early 19th century, exhibited in 'hôtel de Magny', Jardin des Plantes in Paris (France). **B**. The 'Giraffe from Sennaar', representing a lithography of Zarafa (MNHN-1845-211) and the skull of a giraffe from the Cape region (Geoffroy Saint-Hilaire 1827). **C**. Drawing of the holotype of *Giraffa camelopardalis congoensis* Lydekker, 1903 (RMCA-452), housed in the Royal Museum of Central Africa, Tervuren (Belgium) (Lydekker 1904). **D**. Head drawings of the holotypes of *G. c. cottoni* Lydekker, 1904 (NHMUK-1904.1.21.1, left) and *G. g. wardi* Lydekker, 1904 (NHMUK-1903.11.18.1, right) (Lydekker 1914).

Table 2. Museum specimens sequenced in this study (subspecies assignations have been made by morphological characters or distribution range). DRC = Democratic Republic of the Congo; SA = South Africa.

Voucher	Subspecies	Collector, Date	Locality	N° Accession
IRSNB-IG19076	?	Unknown, 1953	Anglo-Egyptian Sudan (Sudan / South Sudan)	MT542052
MNHN-A10753	*peralta*?	Gérardin, 1830	Bakel, Senegal	MT542037
MNHN-A10617	*peralta*?	Gérardin, 1830	Bakel, Senegal	MT542038
MNHN-1896-45	*capensis*	Delalande, 1818–1820	Cape of Good Hope (SA)	MT542039
MNHN-A10749	*capensis*	Delalande, 1818–1820	Cape of Good Hope (SA)	MT542040
MNHN-A7977	*capensis*	Levaillant, 1783–1785	Cape of Good Hope (SA)	MT542041
MNHN-1845-211	*camelopardalis*	Mouker Bey, 1824	Sennar (Sudan)	MT542042
MNHN-A8012	*camelopardalis*	Clot Bey, 1843	Abyssinia (Ethiopia)	MT542043
MNHN-1913-523	*tippelskirchi*?	Babault, 1912–1913	Kenya	MT542054
MHNT-1996.121.2	*camelopardalis*	Unknown, 1843	Abyssinia (Ethiopia)	MT542044
RMCA-21645M	*antiquorum*	Huese, 1953	Sarh, Chad	MT542046
RMCA-25672M	*congoensis*	Poll, 1959	Gangala, DRC	MT542047
RMCA-25673M	*congoensis*	Poll, 1959	Gangala, DRC	MT542048
RMCA-83.006-M0553	*congoensis*	Colyn, 1946	Garamba Park, DRC	MT542049
RMCA-3748M	*congoensis*	De Calonne, 1914	Kapili, DRC	MT542050
RMCA-767M	*cottoni*	Powell-Cotton, 1908	Lado enclave, northwest Uganda and South Sudan	MT542051
RMCA-5956M	*congoensis*	Pilette, 1923	North-East Uele, DRC	MT542053
RMCA-2128M	*tippelskirchi*	Bayer, 1913	Serengeti-Mara, Kenya	MT542055
ZMB-48222	?	Unknown	Dikoa, Nigeria	MT542045

Repositories

IRSNB = Institut royal des Sciences naturelles de Belgique, Brussels, Belgium
NHMUK = Natural History Museum London, United Kingdom
MHNT = Muséum d'histoire naturelle de Toulouse, France
MNHN = Muséum national d'histoire naturelle, Paris, France
RMCA = Royal Museum of Central Africa, Tervuren, Belgium
SMF = Senckenberg Museum Frankfurt, Germany
ZMB = Museum für Naturkunde Berlin, Germany

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from 30–50 mg of bone or teeth powder for each sample, using two commercial kits (PrepFiler BTA Forensic DNA Extraction and QIAamp DNA Micro). In agreement with the protocol provided by Rohland & Hofreiter (2007), DNA extractions from museum samples were performed in a room dedicated to ancient DNA procedure, and multiple negative controls were used during DNA extractions and PCR reactions. We also extracted DNA from 22 extant giraffes from

Cameroon, Chad, Kenya, South Africa and zoos (Beauval in France and Al Ain in the United Arab Emirates) (Supplementary file 1) using 0.2 g per faecal sample, following the instructions of the QIAamp DNA Stool Mini Kit (QIAGEN).

A mitochondrial DNA (mtDNA) fragment, covering the complete cytochrome *b* gene, two tRNA genes (Threonine and Proline) and the 5′ part of the control region (D-Loop), was amplified by polymerase chain reaction (PCR) using 15 primer sets, which generate overlapping fragments (detailed in Supplementary file 2). Amplifications were performed on a total volume of 19 μl, comprising 10 μl of SYBR® Green Supermix (BioRAD), 6.8 μl H_2O, 0.6 μl of each primer (10 μM) and 1 μl of giraffe DNA per sample, using a CFX Connect Real-Time PCR Detection System thermocycler under the following conditions: 4 min at 94°C, followed by 94°C for 30 s, then 1 min at 45–55°C followed by 1 min at 72°C, ending with a single extension of 72°C for 7 min (40 cycles). The PCR products were sent to Eurofins Genomics (Ebersberg, Germany) for forward and reverse direction Sanger sequencing. The electropherograms were edited and assembled to a reference sequence using Sequencher ver. 5.1. (http://www.genecodes.com). All mitochondrial sequences generated for this study were deposited in GenBank (see accession numbers in Table 2 and Supplementary file 1).

Haplotype network

The 41 newly generated sequences were aligned in Geneious R10 (Biomatters, Auckland, New Zealand) with published mitochondrial sequences available in NCBI. The final alignment of 548 giraffe samples (length = 1742 bp) was used to construct a median-joining network (Bandelt *et al.* 1999) with PopArt 1.7 (Leigh & Bryant 2015). Since PopArt does not consider sites with missing data, the two regions corresponding to tRNA-Thr and tRNA-Pro genes, and the last 35 bp of the D-loop were removed from our alignment because they were not sequenced in most previous studies (Brown *et al.* 2007; Fennessy *et al.* 2013, 2016; Bock *et al.* 2014; Winter *et al.* 2018a, 2018b).

Phylogenetic analyses based on mitochondrial and nuclear datasets

The nuclear DNA (nuDNA) dataset of 21 introns published by Fennessy *et al.* (2016) and Winter *et al.* (2018b) (accession numbers LT596685–LT598170, MG257969–MG262280) was used to select the 75 sequenced giraffes fulfilling the following three criteria: (1) all the 21 nuclear introns are available; (2) each individual represents a unique nuclear haplotype; and (3) the mitochondrial fragment, including the cytochrome *b* gene and 5′ region of the control region, is also available (individuals in compliance with the criteria are listed in Supplementary file 3). Heterozygous sites (double peaks) were coded according to the IUPAC code. The mtDNA dataset contains the same 75 individuals and the 13 new haplotypes detected for past populations in this study (see museum specimens indicated in Fig. 4A). Three outgroup species were used to root the giraffe tree: *Bos taurus* Linnaeus, 1758, *Ovis canadensis* Shaw, 1804 and *Okapia johnstoni* Sclater, 1901 (for more details, see Petzold & Hassanin 2020).

The nuDNA dataset (named nuDNA-78T; length = 17 275 bp) and mtDNA dataset (named mtDNA-91T; length = 1776 bp) were analysed with probabilistic methods, using the GTR+I+G substitution model, as selected in jModeltest ver. 2.1.10 (Darriba *et al.* 2012) with the Akaike information criterion. Bayesian inferences were conducted in MrBayes ver. 3.2.6 (Ronquist *et al.* 2012) by calculating the posterior probabilities (PP) after 10^7 Metropolis-coupled MCMC generations, with tree sampling every 1000 generations and a burn-in of 25%. Maximum Likelihood (ML) analyses were performed with PhyML ver. 3.1 (Guindon *et al.* 2010) and Bootstrap percentages (BP) were calculated after 1000 replicates.

Multispecies coalescent analyses of the nuclear dataset

The phased alleles of the 21 introns of the nuDNA-78T dataset (see above) were analysed under BEAST ver. 2.4.8 (Bouckaert *et al.* 2014) to infer a multispecies coalescent (MSC) tree phylogeny. As in Petzold & Hassanin (2020), the two alleles of the 21 introns were assigned at the level of individuals,

i.e., for each of the 75 giraffes and the three outgroup taxa. For each of the 21 introns, the substitution model was selected in jModeltest: K80 for *OTOF*; K80+I for *RASSF4;* F81 for *C1orf74*, *NOTCH2*, *SOS1* and *UBN2*; F81+G for *ACP5*; HKY for *CCT2*, *COL5A2*, *CTAGE5*, *CWF19L1*, *DDX1*, *DHX36*, *MACF1*, *NUP155*, *PLCE1* and *USP54*; HKY+I for *IGF2B1*, *RFC5* and *SAP130*; HKY+G for *USP33*. Analyses were run under the Yule species tree prior for 10^9 generations, sampling trees every 20 000 steps.

The .log files were analysed with Tracer ver. 1.7 (Rambaut *et al.* 2018) to verify the convergence of model parameters (effective sample size (ESS) >200). The species tree was summarised as a Maximum Clade Credibility tree in TreeAnnotator ver. 1.10 (Rambaut & Drummond 2007) discarding 50% as burn-in and subsequently displayed with Figtree ver. 1.4.4 (http://tree.bio.ed.ac.uk/software/).

Estimation of mitochondrial and nuclear divergence times

Divergence times among giraffe subspecies were estimated with the nuDNA-78T and mtDNA-91T datasets using the Bayesian approach implemented in BEAST ver. 1.8.4 (Drummond *et al.* 2012) under the GTR+G+I model and assuming a relaxed clock following a lognormal distribution. The Birth–Death speciation process was specified as tree prior and the most recent common ancestor (MRCA) of *Giraffa* was used as a calibration point, set at 1.1 ± 0.1 Mya in agreement with the molecular study of Hassanin *et al.* (2012) and the fossil record (Harris 1991). Analyses were run with random starting seeds for 10^8 generations, sampling trees every 10 000 steps. Tracer ver. 1.7 (Rambaut *et al.* 2018) was used to visualise the posterior distribution to assess the convergence of model parameters (effective sample size (ESS) >200). The chronograms were summarised in TreeAnnotator ver. 1.10 (Rambaut & Drummond 2007) discarding 25% as burn-in and subsequently displayed with Figtree ver. 1.4.4 (http://tree.bio.ed.ac.uk/software/).

Mitochondrial and nuclear pairwise distances

The 88 giraffe sequences of the mtDNA-91T dataset were analysed in PAUP* ver. 4.0b10 (Swofford 2003) to calculate nucleotide pairwise distances (p-distances) within and among haplogroups revealed in the haplotype network of Fig. 3. Nucleotide pairwise distances were also calculated for the 75 giraffe sequences of the nuDNA-78T dataset.

Abbreviations

BP	=	bootstrap percentages
CR	=	control region
DRC	=	Democratic Republic of the Congo
ES	=	exclusive synapomorphies
ESS	=	effective sample size
ML	=	maximum likelihood
MRCA	=	most recent common ancestor
MSC	=	multispecies coalescent
PP	=	posterior probabilities
SA	=	South Africa

Results

Haplotype network

The haplotype network built from the mtDNA sequences of 548 giraffes is shown in Fig. 3. The network reveals three divergent geographic haplogroups separated by more than 30 mutations, named northern (N), southeastern (SE) and southwestern (SW). The northern and southeastern haplogroups can be further divided into subgroups that are separated by at least 10 mutations.
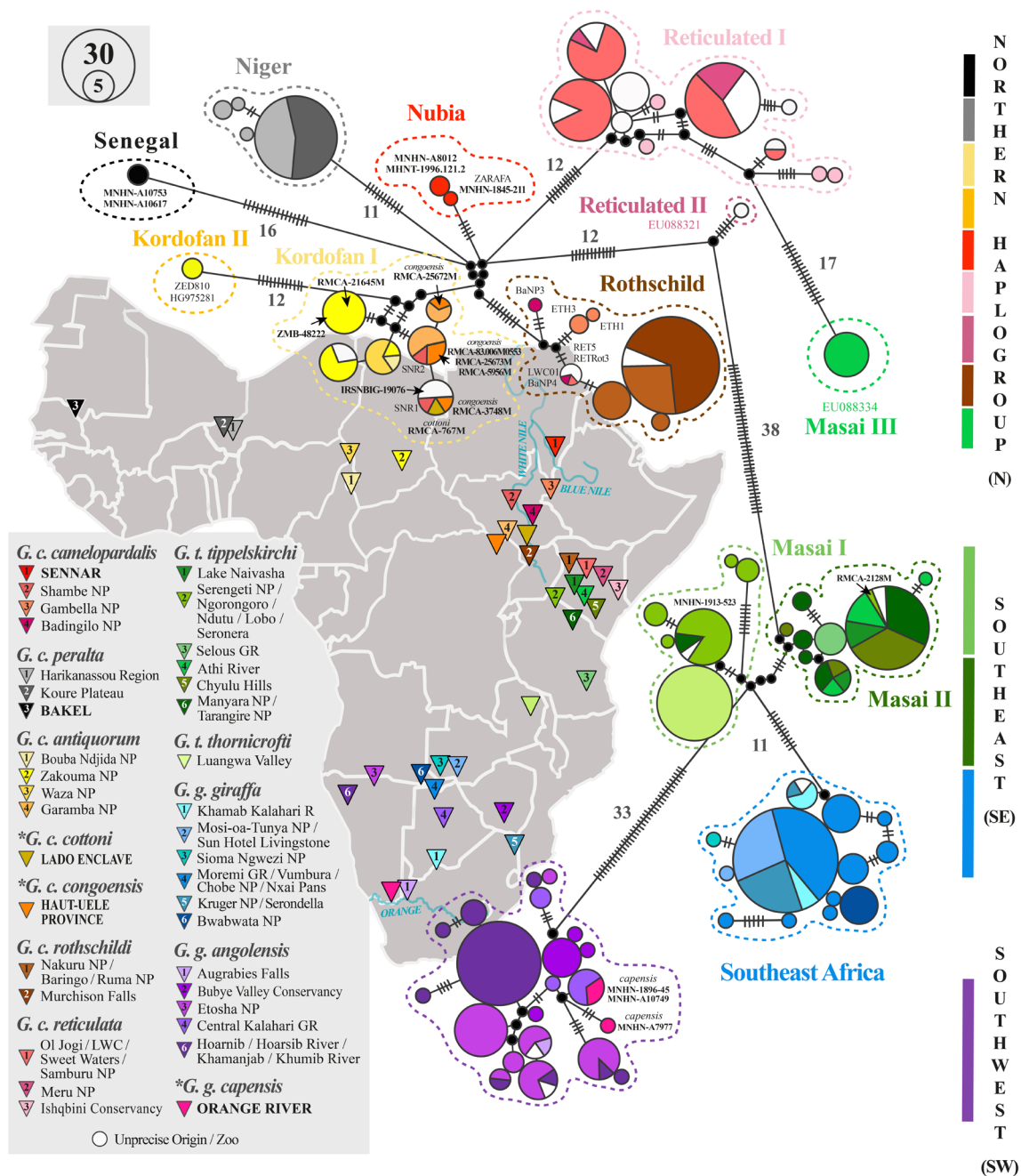
Fig. 3. Median-joining network of mitochondrial haplotypes. The network was constructed in PopART 1.7 (Leigh & Bryant 2015) based on the mitochondrial sequences of 548 giraffes. The number of mutations between haplotypes is indicated by perpendicular lines on the branches and is specified if greater than 10. The size of the circles is proportional to the number of individuals sharing a certain haplotype with colours assigned by subspecies. The sample locations are indicated by triangles in the map and highlighted in bold capital letters for museum specimens. The subspecies marked with an asterisk represent formerly recognized subspecies, which were synonymized in recent classifications (e.g., Shorrocks 2016). Historical key specimens are highlighted by the respective abbreviation of the museum and the catalogue number.

The northern (N) haplogroup includes all giraffes from northern sub-Saharan Africa belonging to the subspecies *G. c. camelopardalis*, *G. c. antiquorum*, *G. c. peralta*, *G. c. reticulata* and *G. c. rothschildi.* Within this group, several subspecies do not form clusters, but nine haplogroups separated by at least 10 mutations can be defined: (1) the haplogroup named 'Senegal' is represented by two giraffes collected in Senegal in the 19th century (MNHN-A10753, MNHN-A10617); (2) the haplogroup named 'Niger' comprises the four haplotypes detected in 44 individuals of the subspecies *G. c. peralta*; (3) the haplogroup named 'Nubia' includes three museum specimens of the subspecies *G. c. camelopardalis* collected during the 19th century, i.e., Zarafa from Sennar (MNHN-1845-211) and the two giraffes from Abyssinia (MNHN-A8012 and MHNT-1996.121.2); (4) the haplogroup named 'Kordofan I' contains 28 extant giraffes of the subspecies *G. c. antiquorum* from Cameroon (Bouba Njida NP, Waza NP), Chad (Zakouma NP) and the DRC (Garamba NP), as well as two giraffes assigned to the subspecies *G. c. camelopardalis* (SNR1, SNR2) by Fennessy *et al.* (2016) from the Shambe Nature Reserve in South Sudan, three museum specimens collected in southern Chad (RMCA-21645M; locality: Sarh), Sudan (IRSNB-IG19076) and northeastern Nigeria (ZMB-48222; locality: Dikoa), as well as all the five museum specimens assigned to the former subspecies *G. c. congoensis* (RMCA-25672M, RMCA-25673M, RMCA-3748M, RMCA-5956M, RMCA-83.006-M0553) and the single individual of *G. c. cottoni* (RMCA-767M); (5) the haplogroup named 'Kordofan II' includes two individuals of the subspecies *G. c. antiquorum* from Zakouma NP (Chad) sequenced by two independent teams (HG97528: Bock *et al.* 2014; ZED810: this study); (6) the haplogroup named 'Rothschild' comprises all 75 giraffes assigned to the subspecies *G. c. rothschildi* (three haplotypes), as well as three reticulated giraffes (subspecies *G. c. reticulata*) (LWC01 from central Kenya; RET5 and RETRot3 from a German zoo), and five individuals assigned to the subspecies *G. c. camelopardalis* by Fennessy *et al.* (2016) (BaNP3, BaNP4, ETH1, ETH2, ETH3); (7) the haplogroup named 'Reticulated I' includes 11 haplotypes detected in 66 individuals of the subspecies *G. c. reticulata* from Kenya and European zoos; (8) the haplogroup named 'Reticulated II' is only represented by a single haplotype (EU088321) sequenced by Brown *et al.* (2007) for one giraffe from central Kenya; and (9) the haplogroup named 'Masai III' is only represented by a single haplotype (EU088334) sequenced by Brown *et al.* (2007) for nine individuals of the subspecies *G. t. tippelskirchi* from the Athi River (Kenya).

The southeastern (SE) haplogroup comprises giraffes from southeastern Africa belonging to the subspecies *G. g. giraffa*, *G. t. tippelskirchi* and *G. t. thornicrofti.* Three subgroups are separated by more than 10 mutations: (1) the haplogroup named 'Southeast Africa' is represented by 13 haplotypes and 83 individuals found exclusively among members of the subspecies *G. g. giraffa*; (2) the haplogroup named 'Masai I' includes the single haplotype shared by all 34 representatives of the subspecies *G. t. thornicrofti* and three haplotypes of *G. t. tippelskirchi* giraffes, comprising 24 extant individuals sampled in northern Tanzania (Serengeti National Park (NP); Manyara NP) and a museum specimen (MNHN-1913-523) collected in Kenya; and (3) the haplogroup named 'Masai II' includes eight haplotypes of the subspecies *G. t. tippelskirchi* detected in 58 extant individuals collected over the whole sampling area and a museum specimen (RMCA-2128M) sampled in the Serengeti-Mara in southern Kenya.

The southwestern (SW) haplogroup contains only giraffes from southwestern Africa, i.e., the 21 haplotypes of 95 individuals assigned to the subspecies *G. g. angolensis* and the three specimens of the former subspecies *G. g. capensis* collected by Levaillant (1797) and Delalande (1822) from the Cape Region: one represents a formerly unknown haplotype (MNHN-A7977), and the two others (MNHN-1896-45, MNHN-A10749) are identical with haplotypes of giraffes currently found in the Central Kalahari Game Reserve (Botswana).

Comparison between mtDNA and nuDNA phylogenetic trees

The Bayesian trees, ML trees and BEAST chronograms reconstructed from the mtDNA-91T and nuDNA-78T datasets are available in the Supplementary files 4, 5, 6, 7 and 8. Note that the northern haplogroups

'Reticulated II' and 'Masai II' (Fig. 3) were excluded from the analyses because nuclear introns are not available. In Fig. 4, the chronogram inferred from the mtDNA-91T dataset (Fig. 4A) is compared to the MSC species-tree reconstructed from the nuDNA-78T dataset (Fig. 4B). The divergence times estimated with the nuDNA-78T dataset under BEAST were reported for the main nodes of the MSC species-tree.

The monophyly of *Giraffa* obtained maximal support ($PP_{Bayes/BEAST} = 1$; BP = 100) in all analyses, and the genus can be diagnosed by 65 exclusive synapomorphies (ES) in the mtDNA-91T dataset and 164 ES in the nuDNA-78T dataset.

All phylogenetic analyses of the mtDNA-91T dataset show a dichotomy separating northern giraffes (*G. camelopardalis*) ($PP_{Bayes/BEAST} = 1$; BP = 98) from southern giraffes (*G. giraffa* + *G. tippelskirchi*) ($PP_{Bayes} = 0.98$; $PP_{BEAST} = 0.99$; BP = 77). Molecular dating estimates calculated from the mtDNA-91T
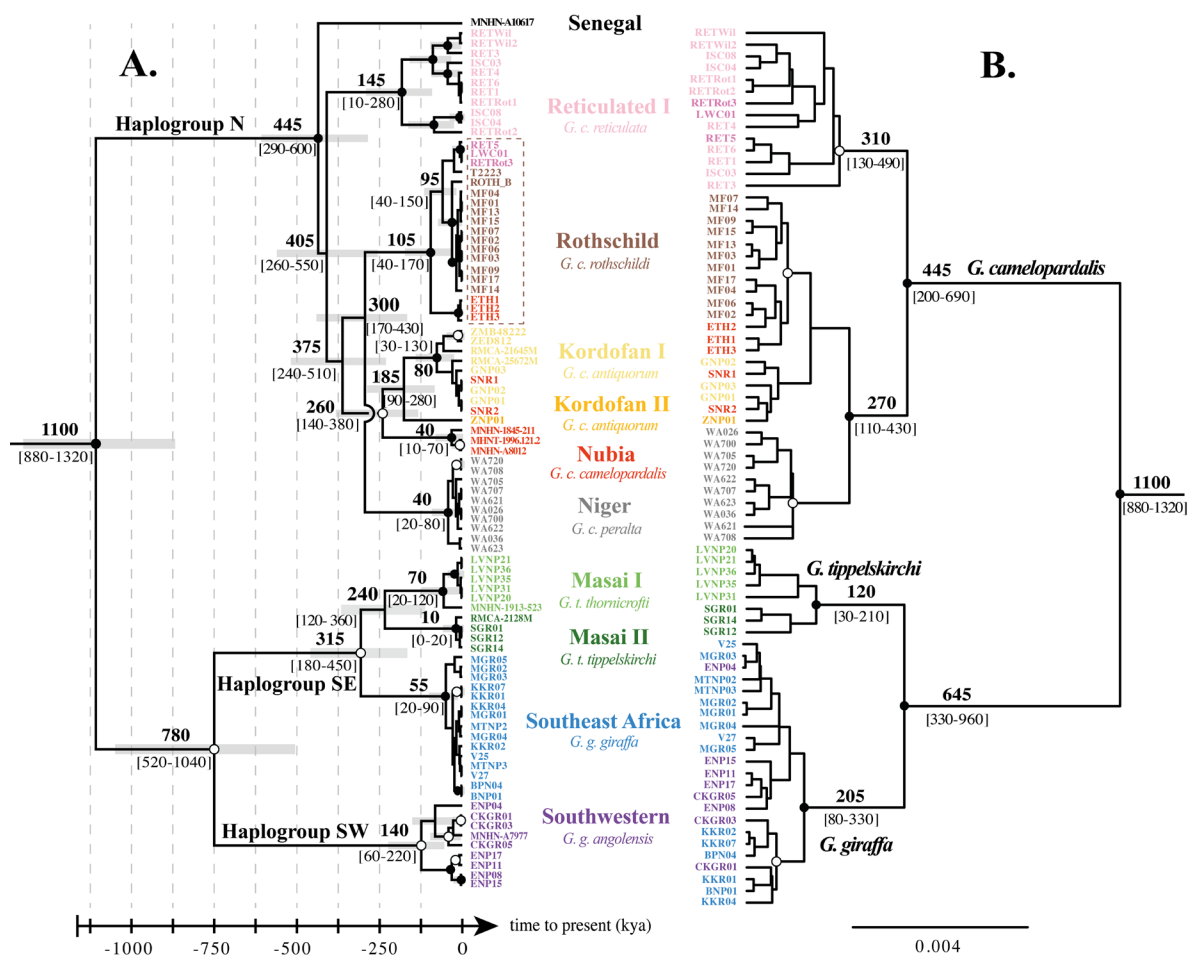

Fig. 4. Comparison of mitochondrial and nuclear divergence time estimates. **A.** Chronogram inferred from the mtDNA dataset using BEAST ver. 1.8.4 (Drummond *et al.* 2012). The mean divergence times are reported on the nodes, and the horizontal grey bars show 95% confidence intervals. **B.** Phylogram reconstructed from the multispecies coalescent analysis of the nuDNA dataset using *BEAST ver. 2.4.8 (Bouckaert *et al.* 2014). Divergence times estimated in BEAST ver. 1.8.4 (Drummond *et al.* 2012) are reported on the nodes to allow comparison with the mtDNA chronogram (Supplementary file 8). Nodes with a white circle were supported by $PP_{BEAST} \geq 0.95$, whereas nodes with a black circle were supported by both $PP_{BEAST} \geq 0.95$ and $BP_{ML} \geq 80$.

dataset suggest 780 ± 260 kya for the MRCA of southern giraffes and 445 ± 155 kya for the MRCA of northern giraffes. All mitochondrial haplogroups identified in the network of Fig. 3 are found to be monophyletic and can be diagnosed by several ES (Supplementary file 9). The haplogroup N ($PP_{Bayes/BEAST} = 1$; BP = 98; six ES) contains seven subgroups corresponding to 'Nubia' ($PP_{Bayes/BEAST} = 1$; BP = 99), 'Kordofan I' ($PP_{Bayes/BEAST} = 1$; BP = 80; one ES), its sister-group, i.e., 'Kordofan II' ($PP_{Bayes} = 0.97$; $PP_{BEAST} = 0.81$; BP = 48; two ES), 'Niger' ($PP_{Bayes/BEAST} = 1$; BP = 100; three ES), 'Rothschild' ($PP_{Bayes/BEAST} = 1$; BP = 99; one ES), 'Reticulated I' ($PP_{Bayes/BEAST} = 1$; BP = 100; two ES) and 'Senegal', which is represented by a single haplotype (five ES). Southern giraffes can be further separated in two main geographic haplogroups, SE and SW. The MRCA of haplogroup SE was dated at 315 ± 135 kya ($PP_{Bayes} = 0.99$; $PP_{BEAST} = 1$; BP = 77; one ES). It comprises the three subgroups 'Southeast Africa' ($PP_{Bayes/BEAST} = 1$; BP = 99; two ES), 'Masai I' ($PP_{Bayes/BEAST} = 1$; BP = 92; three ES) and 'Masai II' ($PP_{Bayes/BEAST} = 1$; BP = 98; one ES). The MRCA of haplogroup SW was dated at 140 ± 80 kya. It was highly supported in the BEAST analysis ($PP_{BEAST} = 1$), but less supported in the two other analyses ($PP_{Bayes} = 0.44$; BP = 36). It can be diagnosed by three ES.

In agreement with the mtDNA analyses, all nuDNA analyses support the separation of northern giraffes (*G. camelopardalis* = haplogroup N) ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 100; eight ES) from southern giraffes (*G. giraffa* + *G. tippelskirchi*) ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 56; four ES) (see Supplementary file 10 for ES). The MRCA of *G. camelopardalis* was dated at 445 ± 245 kya. The nuDNA-78T dataset does not provide strong signals for relationships within haplogroup N. However, the subspecies *G. c. reticulata* is found to be monophyletic ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 38), as it includes all individuals of the mitochondrial haplogroup 'Reticulated I', as well as the three individuals of the mitochondrial haplogroup 'Rothschild' morphologically assigned to *G. c. reticulata* (RET5, RETRot3, LWC01). Other subspecies of *G. camelopardalis* are grouped together ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 96). Within this group, the subspecies *G. c. peralta* (= mitochondrial haplogroup 'Niger') is found monophyletic in the ML tree (BP = 66) and MSC species-tree ($PP_{*BEAST} = 0.98$), but polyphyletic in other Bayesian trees, as two individuals (WA026, WA708) cluster with individuals of the subspecies *G. c. antiquorum* and *G. c. rothschildi* ($PP_{BEAST} = 0.63$; $PP_{Bayes} = 0.59$). In the MSC species-tree (Fig. 4B), the subspecies *G. c. rothschildi* can be considered as monophyletic ($PP_{*BEAST} = 1$) if three giraffes from western Ethiopia (ETH1–3), geographically assigned to *G. c. camelopardalis* in a previous study, are included into this taxon. Similarly, the subspecies *G. c. antiquorum* can be considered as monophyletic ($PP_{*BEAST} = 0.92$) if two giraffes from Shambe NP – South Sudan (SNR1 and SNR2), previously assigned to *G. c. camelopardalis*, are included into this taxon. The MRCA of southern giraffes was dated at 645 ± 315 kya. This group can be further divided into two taxa corresponding to the species *G. giraffa* ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 100; 14 ES) and *G. tippelskirchi* ($PP_{Bayes/BEAST/*BEAST} = 1$; BP = 100; seven ES). The species *G. giraffa* includes individuals from the mitochondrial haplogroups 'SW' and 'Southeast Africa'. The MRCA of *G. giraffa* was dated at 205 ± 125 kya. The species *G. tippelskirchi* contains individuals of the mitochondrial haplogroups 'Masai I' and 'Masai II', which are found to be reciprocally monophyletic in the BEAST trees, but with low support ($PP_{BEAST/*BEAST} = 0.45/0.53$ and $PP_{BEAST/*BEAST} = 0.74/0.53$, respectively). The MRCA of *G. tippelskirchi* was dated at 120 ± 90 kya.

Mitochondrial and nuclear pairwise distances

The mtDNA-91T and nuDNA-78T datasets were used to calculate nucleotide pairwise distances (Table 3 and Table 4, respectively).

The mean mitochondrial distances between the three main haplogroups N, SE and SW range from 2.80% (SE vs SW) to 4.51% (SE vs N). Within haplogroup N, mean distances between the seven subgroups are comprised between 0.96% ('Kordofan I' vs 'Nubia') and 1.99% ('Kordofan II' vs 'Senegal'). The genetic distances between the West African subgroups 'Niger' and 'Senegal' are comprised between 1.74% (WA708 vs MNHN-A10617) and 1.93% (WA036 vs MNHN-A10617). The distances between

Table 3. Minimum and maximum pairwise distances (in %), as well as mean distance (between brackets), calculated using the mtDNA-91T dataset both within and between haplogroups (Fig. 3). **Boldface** = maximal intrapopulational variation.

Taxa	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.
I. Nubia	**0.17**										
II. Senegal	1.38 – 1.5 (1.44)	**0**									
III. Niger	1.09 – 1.47 (1.24)	1.74 – 1.93 (1.82)	**0.26**								
IV. Kordofan I	0.75 – 1.14 (0.96)	1.67 – 1.78 (1.7)	1.28 – 1.67 (1.41)	**0.52**							
V. Kordofan II	1.22 – 1.41 (1.32)	1.99	1.67 – 1.86 (1.75)	0.9 – 1.15 (0.98)	**0**						
VI. Rothschild	1.15 – 1.73 (1.35)	1.49 – 2 (1.68)	1.21 – 1.67 (1.35)	1.21 – 1.67 (1.48)	1.48 – 1.67 (1.58)	**0.58**					
VII. Reticulated I	1.54 – 1.99 (1.69)	1.55 – 1.87 (1.75)	1.48 – 2.19 (1.74)	1.48 – 2.12 (1.68)	1.8 – 2.06 (1.89)	1.35 – 1.99 (1.60)	**1.16**				
VIII. Masai I	3.75 – 3.87 (3.82)	4.13 – 4.13 (4.13)	3.99 – 4.13 (4.05)	3.68 – 3.93 (3.80)	4.12 – 4.12 (4.12)	3.81 – 4.26 (4.01)	3.81 – 4.07 (4.00)	**0**			
IX. Masai II	3.73 – 3.99 (3.85)	4.08 – 4.26 (4.17)	3.92 – 4.25 (4.04)	3.50 – 3.91 (3.74)	4.05 – 4.24 (4.11)	3.85 – 4.37 (4.03)	3.86 – 4.31 (4.03)	0.31 – 0.94 (076)	**0.98**		
X. Southeast Africa	4.12 – 4.31 (4.21)	4.26 – 4.45 (4.34)	4.31 – 4.69 (4.46)	4.18 – 4.63 (4.32)	4.44 – 4.63 (4.51)	4.18 – 4.63 (4.35)	4.12 – 4.57 (4.37)	1.35 – 1.54 (1.43)	1.29 – 1.56 (1.40)	**0.32**	
XI. Southwestern	3.13 – 3.73 (3.52)	3.49 – 4.12 (3.95)	3.53 – 4.11 (3.80)	3.07 – 3.86 (3.58)	3.66 – 4.11 (3.91)	3.54 – 4.31 (3.94)	3.73 – 4.24 (3.95)	2.64 – 2.9 (2.78)	2.48 – 3.09 (2.80)	3.02 – 3.41 (3.16)	**0.65**

Table 4. Minimum and maximum pairwise distances (in %), as well as mean distance (between brackets), calculated using the nuDNA-78T dataset both within and between haplogroups (Fig. 3). **Boldface** = maximal intrapopulational variation.

Taxa	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
I. Niger	**0.09**								
II. Kordofan I	0.03 – 0.11 (0.06)	**0.04**							
III. Kordofan II	0.08 – 0.12 (0.1)	0.04 – 0.05 (0.04)	**0**						
IV. Rothschild	0.04 – 0.21 (0.08)	0.02 – 0.17 (0.07)	0.05 – 0.2 (0.09)	**0.2**					
V. Reticulated I	0.05 – 0.27 (0.15)	0.02 – 0.21 (0.14)	0.08 – 0.23 (0.18)	0 – 0.25 (0.13)	**0.12**				
VI. Masai I	0.40 – 0.47 (0.44)	0.40 – 0.49 (0.44)	0.46 – 0.48 (0.47)	0.38 – 0.5 (0.44)	0.32 – 0.47 (0.41)	**0**			
VII. Masai II	0.4 – 0.46 (0.44)	0.44 – 0.47 (0.45)	0.46 – 0.46 (0.46)	0.38 – 0.49 (0.44)	0.32 – 0.46 (0.40)	0.02 – 0.06 (0.04)	**0.04**		
VIII. Southeast Africa	0.44 – 0.56 (0.51)	0.46 – 0.58 (0.51)	0.52 – 0.57 (0.54)	0.44 – 0.59 (0.51)	0.36 – 0.54 (0.46)	0.28 – 0.4 (0.35)	0.28 – 0.38 (0.34)	**0.08**	
IX. Southwestern	0.43 – 0.57 (0.51)	0.45 – 0.59 (0.52)	0.5 – 0.59 (0.55)	0.42 – 0.6 (0.52)	0.35 – 0.55 (0.47)	0.27 – 0.41 (0.34)	0.27 – 0.39 (0.34)	0 – 0.12 (0.05)	**0.14**

museum specimens of *G. c. camelopardalis* (subgroup 'Nubia') and the five individuals previously assigned to *G. c. camelopardalis* (Fennessy *et al.* 2016; Winter *et al.* 2018b) range from 0.89% (MNHN-1845-211 vs SNR1) to 1.73% (MNHN-A8012 vs ETH1). The three reticulated giraffes (RET5, RETRot3, LWC01) from the mitochondrial subgroup 'Rothschild' are highly divergent from reticulated giraffes of the subgroup 'Reticulated I', i.e., between 1.35% (RETWil vs LWC01) and 1.67% (ISC08 vs RETRot3). Within haplogroup SE, similar distances were found between members of the three subgroups 'Masai I', 'Masai II' and 'Southeast Africa': from 0.31% (MNHN-1913-523 vs LVNP20) to 0.94% (RMCA-2128M vs LVNP36) for 'Masai I' vs 'Masai II'; from 1.35% (KKR01 vs LVNP20) to 1.54% (BNP03 vs LVNP36) for 'Masai I' vs 'Southeast Africa'; from 1.29% (KKR01 vs SGR01) to 1.56% (MGR04 vs RMCA-2128M) for 'Masai II' vs 'Southeast Africa'. Within haplogroup SW, the distances range from 0% (ENP11 vs ENP08) to 0.65% (ENP11 vs MNHN-A7977).

The mean nuclear distances between the three species of *Giraffa* are comprised between 0.35% (*G. giraffa* vs *G. tippelskirchi*) and 0.5% (*G. giraffa* and *G. camelopardalis*). Within *G. camelopardalis*, minimal distances between subspecies range from 0.01% between the subspecies *G. c. antiquorum* and *G. c. rothschildi* (GNP01 vs ETH3) to 0.03% between *G. c. antiquorum* and *G. c. peralta* (SNR1 vs WA036), between *G. c. reticulata* and *G. c. rothschildi* (RET3 vs ETH2) or between *G. c. peralta* and *G. c. rothschildi* (WA036 vs MF03). The highest distances between subspecies range from 0.1% between *G. c. antiquorum* and *G. c. peralta* (ZNP01 vs WA621) or between *G. c. peralta* and *G. c. rothschildi* (WA708 vs MF07) to 0.2% between *G. c. reticulata* and *G. c. rothschildi* (ISC04 vs WA708), between *G. c. peralta* and *G. c. reticulata* (WA708 vs ISC04) or between *G. c. antiquorum* and *G. c. reticulata* (ZNP01 vs ISC04). Within *G. tippelskirchi*, distances between the two subgroups Masai I and Masai II range from 0.02% (LVNP36 vs SGR14) to 0.06% (LVNP31 vs SGR01). Within *G. giraffa*, intraspecific distances range from 0% (ENP04 vs ENP11) to 0.12% (CKGR03 vs ENP08).

Discussion

Diversification of giraffes during the Pleistocene epoch

The first fossil remains related to extant giraffes date back to 1 Mya and were found in West Turkana (East Africa) (Harris 1991), which is in agreement with molecular dating estimates of the MRCA of *Giraffa* based on complete mitochondrial genomes (Hassanin *et al.* 2012: 1.1 Mya). This corresponds to one of the most arid periods of the Pleistocene epoch (de Menocal 2004), suggesting that the associated expansion of the savannah may have promoted the split between northern and southern giraffes.

Among southern giraffes, the subsequent divergence between the species *G. giraffa* and *G. tippelskirchi* took place at 645±315 kya with nuclear data or 780±260 kya with mtDNA data. Around 800 kya, the landscape was shaped by a strongly humid interglacial period, which caused in southwestern Africa the development of a complex hydrographical network nourished by the Okavango and the Zambezi Basin, culminating in the evolution of the Paleo-lake Makgadikgadi (Goudie 2005; Moore *et al.* 2012). These newly formed physical barriers may have impeded gene flow between giraffes separated by the Zambezi River, with *G. tippelskirchi* in the North and *G. giraffa* in the South.

The rapid diversification of the northern species *G. camelopardalis* into several subspecies was dated at 445±155 kya with the mtDNA dataset and 445±245 kya with the nuDNA dataset. These estimates fall into an unusually long interglacial period, which might have been associated with milder savannah conditions (Yin & Guo 2007), favouring the radiation of northern subspecies. The humid conditions led further to the development of a hydrographical network in Niger, with the rise of water levels of Mega Lake Chad, but also in East Africa, with the extension of the White Nile to the proportion of a Paleo-lake (Williams *et al.* 2003) and the origin of Lake Victoria at around 400 kya (Johnson *et al.* 2000). The

establishment of this drainage system in East Africa may have isolated (at least temporarily) several giraffe populations, favouring their subsequent diversification at the subspecies level.

The giraffes of the Nile: *G. c. camelopardalis*, *G. c. antiquorum* and *G. c. rothschildi*

Linnaeus (1758) described the species *Cervus camelopardalis* based on the description published by Belon du Mans (1553), who saw several living giraffes in the "castle of Cairo", Ray (1693), who refers to Belon du Mans, and Hasselquist (1757), who examined a skin and wrote that giraffes live "in the forests" of Sennar and Ethiopia (indicated as type localities by Linnaeus). Around 1750, the Sultanate of Sennar was located in the current Sudan and covered the regions surrounding the cities of Khartoum and Sennar in the northern parts of the White and Blue Niles. At that time, Ethiopia was smaller than today: bordering southeastern Sennar, it included the regions surrounding the city of Gondar and southern parts of the Blue Nile and its source, the Lake Tana. All first reports on giraffes were based on animals captured by Egyptians, including the giraffes offered to Frederick II (1245), Lorenzo de Medici (1487), and Charles X (1827), as well as the giraffes held captive in Cairo described by Belon du Mans (1553) and the skins studied by Hasselquist (1757). All these giraffes have been collected by exploring the southern regions close to the borders of the Blue and White Niles. Today, the countries crossed by the two main tributaries of the Nile are, from north to south, Sudan, Ethiopia, South Sudan, northeastern DRC and Uganda. In this area, Lydekker (1914) recognized the five following subspecies: *G. c. camelopardalis*, *G. c. antiquorum*, *G. c. congoensis*, *G. c. cottoni* and *G. c. rothschildi* (Fig. 1A). Several of these subspecies were, however, synonymized in subsequent classifications: *G. c. rothschildi* was treated as a synonym of *G. c. camelopardalis* (East 1998); *G. c. congoensis* was considered as the synonym of *G. c. camelopardalis* (Ansell 1968; Dagg 1971) or *G. c. antiquorum* (Ciofolo & Le Pendu 2013; Dagg 2014; Shorrocks 2016; Fennessy & Marais 2018); and *G. c. cottoni* was ranged into *G. c. rothschildi* (Ansell 1968). To better understand the taxonomy of giraffes, we included in this study several museum specimens of the 18th and 19th centuries collected within the type localities of the subspecies *G. c. camelopardalis*, *G. c. congoensis* and *G. c. cottoni*.

Fennessy *et al.* (2016) and Winter *et al.* (2018b) analysed seven giraffe samples supposed to belong to the nominate subspecies *G. c. camelopardalis* (Linnaeus 1758), because they were collected in South Sudan and Ethiopia, two countries where the subspecies is still possibly present according to the IUCN (Wube *et al.* 2018). These samples include SNR1 and SNR2, which were collected on the left bank of the White Nile in South Sudan (Shambe NP), BaNP3 and BaNP4, which were collected on the right bank of the White Nile in South Sudan (Bandingilo NP), and ETH1, ETH2 and ETH3, which were collected in western Ethiopia (Gambella NP), far from both White and Blue Nile rivers (see Figs 3, 5). Our network and phylogenetic analyses based on the mitochondrial sequences of giraffes (Figs 3, 4A) show that SNR giraffes belong to the haplogroup 'Kordofan I', whereas BaNP and ETH giraffes belong to the haplogroup 'Rothschild'. In agreement with mtDNA results, our analyses of nuclear introns confirm that SNR giraffes belong to the subspecies *G. c. antiquorum*, whereas ETH giraffes belong to the subspecies *G. c. rothschildi*. Based on similar phylogenetic results, Fennessy *et al.* (2016) have, however, concluded that the subspecies *G. c. rothschildi* should be synonymized with *G. c. camelopardalis*, as previously suggested by some authors (Kingdon 1997; East 1998). Our analyses of museum specimens, however, support another taxonomic interpretation. Indeed, the two mtDNA haplotypes sequenced from three specimens of the 19th century assigned to *G. c. camelopardalis* (haplogroup Nubia, Figs 3, 4) form a well-supported monophyletic group (PP = 1; BP = 99), distinct from all other haplogroups previously identified (nucleotide distance >0.75% from Kordofan I; 1.22% from Kordofan II; >1.15% from Rothschild): the first haplotype was obtained from the famous Zarafa (MNHN 1845-211), which was captured in 1824 near the mountains located south of the city of Sennar (Salze 1827), probably close to the boundary between Sudan and Ethiopia; the second haplotype was sequenced in two giraffes collected in 'Abyssinia' (Ethiopian Empire), which both arrived in France in 1843 (MNHN-A8012 and MNHT-1996.121.2). Zarafa was collected near the Blue Nile, but we do not know if it was captured on the left

bank or on the right bank of the river. During the 19th century, several explorers have mentioned the presence of many giraffes in the region between the Blue Nile and Tekezé/Atbara rivers in southeastern Sudan (eastern Sennar) and northern Ethiopia (Abyssinia) (Combes & Tamisier 1838; Baker 1880). Since the haplotype from Sennar is highly similar to the haplotype from Abyssinia (1 mutation), we suggest that the subspecies *G. c. camelopardalis* was endemic to this region. However, we cannot completely exclude that *G. c. camelopardalis* was also present in the region of western Sennar between the White Nile and Blue Nile, delimited in the south by the Sobat River in the west, followed in the east by the Baro River and Ethiopian Highlands (Fig. 5).

The taxonomic status of other subspecies related to the Nile was problematic. For instance, some authors have treated *G. c. rothschildi* as a synonym of *G. c. camelopardalis* (Ciofolo & Le Pendu 2013; Fennessy *et al.* 2016). However, our phylogeographic analyses support that *G. c. camelopardalis* and *G. c. rothschildi* are two distinct subspecies, and that populations of *G. c. rothschildi* are found in eastern South Sudan, western Ethiopia, northeastern Uganda and northwestern Kenya, a biogeographical region delimited by the White Nile river in the west, by the Sobat River (or alternatively the Blue Nile) in the north, Ethiopian Highlands in the east and a discontinuous barrier in the south, including Lake Victoria (Fig. 5). Our results therefore corroborate previous hypotheses that giraffes avoid to cross
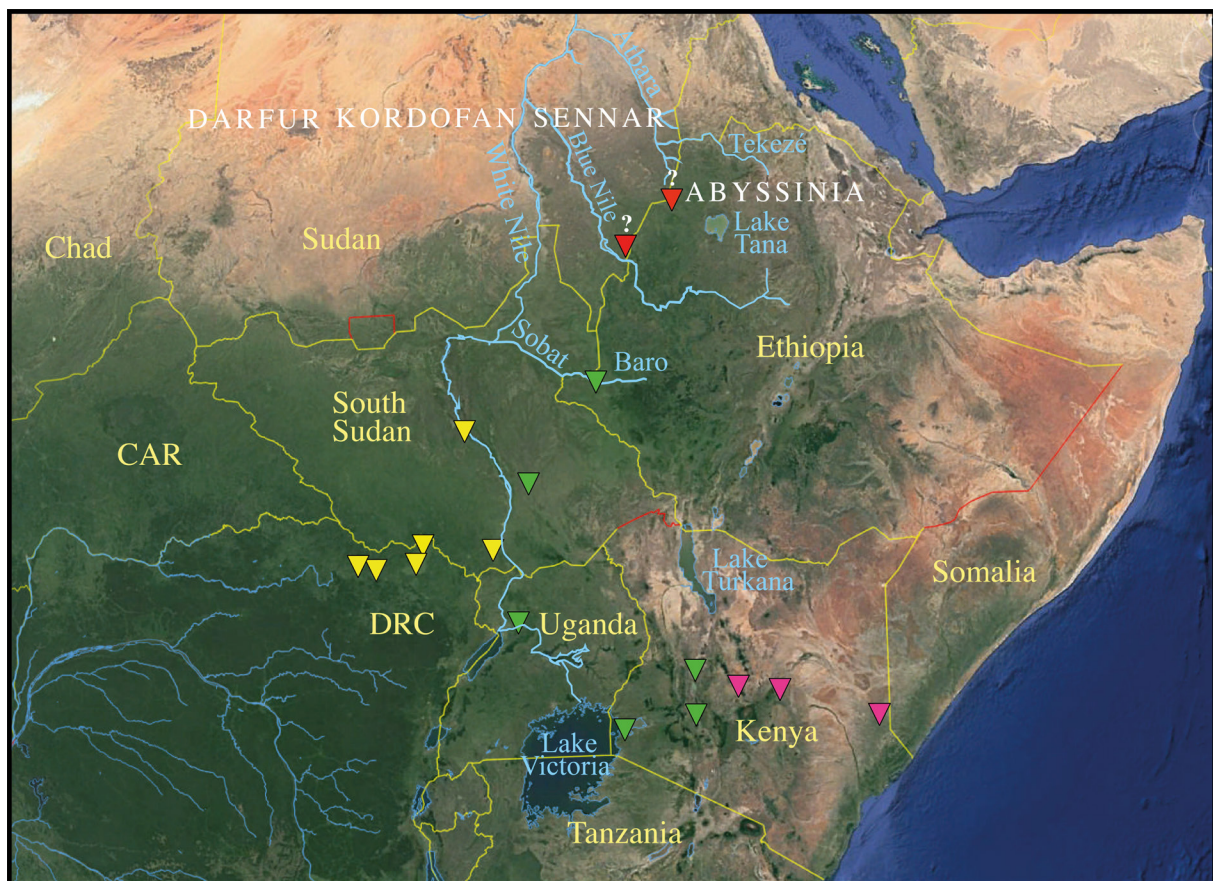

Fig. 5. Giraffe subspecies of the Nile region. The map (extracted from Google Earth; https://www.google.com/intl/de/earth/) shows the geographical barriers (rivers and mountains) that may have isolated (at least temporarily) the subspecies *Giraffa camelopardalis camelopardalis* (Linnaeus, 1758) (red), *G. c. antiquorum* (Jardine, 1835) (yellow), *G. c. rothschildi* Lydekker, 1903 (green) and *G. c. reticulata* de Winton, 1899 (magenta). The question mark refers to the uncertain geographic origin of Zarafa (left) and the two specimens from Abyssinia (right) (see Discussion for more details).

high mountains (Happold 1978; Dagg 2014) and large rivers (MacClintock 1973; Henderson & Naish 2010). This might concern in particular females living in nursery herds with their calves (Bercovitch & Berry 2010), which tend to avoid the potential risk of overcoming biogeographic barriers (Petzold & Hassanin 2020). In eastern central Kenya, populations of *G. c. rothschildi* and *G. c. reticulata* can be found in sympatry (Kingdon 1997) and field observations of intermediate phenotypes have suggested occasional gene flow between these two subspecies (Stott & Selsor 1981; Kingdon 1997). In the mtDNA tree, three individuals of *G. c. reticulata* (RET5, RETRot3, LWC01) cluster within the haplogroup 'Rothschild' (Fig. 4A), whereas they are related to other individuals of *G. c. reticulata* in the nuDNA tree (Fig. 4B). This mito-nuclear discordance suggests that a mitochondrial introgression occurred from *G. c. rothschildi* to *G. c. reticulata* at 96 ± 55 kya (Fig. 4B).

Our phylogeographic analyses based on mtDNA sequences suggest that all populations located on the left bank of the White Nile belong to the subspecies *G. c. antiquorum*. Indeed, the topotype specimens of *G. c. congoensis* from the Haut-Uele Province in northeastern DRC (RMCA-25672M, RMCA-25673M, RMCA-3748M, RMCA-5956M, RMCA-83.006-M0553) and *G. c. cottoni* from the Lado enclave in northwest Uganda and South Sudan (RMCA-767M) cluster within the haplogroup 'Kordofan I', suggesting that the two subspecies *G. c. congoensis* and *G. c. cottoni* should be synonymized with *G. c. antiquorum.*

A new subspecies from Senegal

In 1898, Lieutenant R.H. McCorquodale shot the first ever recorded giraffe in Nigeria (McCorquodale 1898; Fig. 1A). He sent the skull to Mr. Oldfield Thomas at the British Museum in London, who described the subspecies *G. c. peralta* based on the "elongation of the face […] with a very large spatulate nasal opening […] and a more vertically upright direction of the ossicones" compared to other northern and southern races (Thomas 1898). The type locality is indicated as "southeast of the junction of the Benue and Niger" (Thomas 1898). However, we agree with Lydekker (1904) that Lokoja (Nigeria), which is located in the V-shaped area to the north of the confluence of the Niger and Benue rivers, is a more probable type locality, because the Benue and Niger rivers may have acted as barriers against giraffe dispersal towards the south of Nigeria (Happold 1969).

During the 20th century, giraffes were then recorded in other countries of West Africa, such as Senegal, Gambia, Mali, Ghana, and Niger (Sidney 1965; Happold 1969, 1978; Nežerková *et al.* 2004). Consequently, the distribution of *G. c. peralta* was supposed to cover a large area from Senegal in the west towards the western part of the Central African Republic in the east (Fig. 1A; Dagg 1962; Happold 1969). However, populations have severely decreased over the last decades, because of poaching, habitat loss, increasing aridity (Dagg & Foster 1976) and outbreaks of rinderpest (Roeder *et al.* 2013), and the West African giraffe is now considered regionally extinct in Burkina Faso, Guinea, Mali, Mauritania and Senegal (Fennessy *et al.* 2018). The mitochondrial study of Hassanin *et al.* (2007) suggested that giraffes found in Cameroon, Chad and the Central African Republic rather belong to the subspecies *G. c. antiquorum*. At present, *G. c. peralta* is listed as endangered, only represented by 607 individuals in Niger (Zabeirou 2017).

Our results show that the past diversity of West African giraffes was greater than previously assumed, as the mtDNA haplotype sequenced from two museum specimens (MNHN-A10617 and MNHN-A10753) collected in Senegal (Bakel) by Girardin in 1830 (Malte-Brun & Malte-Brun 1839) is distinct from other subspecies of the haplogroup N by at least 1.38% and can be diagnosed by five ES (Supplementary file 9). We assume that the Sahara Desert in the north, rainforests in the south and the Niger River in the east may have acted as physical barriers limiting gene flow between giraffes from Senegal and those from Niger and Nigeria (*G. c. peralta*). Both differ considerably in the colouration and pattern of the coat. The Niger giraffe can be recognized by its pale almond coat covered with numerous light brown spots

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differing in shape and getting smaller on the hindquarters, whereas the beige coat of the Senegal giraffe (Fig. 6) shows large dark brown patches with a clear contour and almost uniform in size on neck, trunk and hindquarters. All these elements indicate therefore that giraffes from Senegal should be placed into their own subspecies that will be formally described below. Unfortunately, the subspecies is now extinct, as the last giraffes of Senegal were killed in the 1970's (Vincke *et al.* 2005).

Taxonomic status of the giraffes collected in the Cape of Good Hope during the 18th and 19th centuries

Boddaert (1784) was the first to describe a southern giraffe from the Cape of Good Hope under the name '*Camelopardalis giraffa*', based on specimens with no precise locality housed in the Prince of Orange Museum in The Hague and Naturalis museum in Leiden (Netherlands). At that time, the Cape of Good Hope, also known as the Cape colony, was a Dutch colony covering most of the present-day South Africa, excepting the northeastern regions (see map of Levaillant & de La Borde 1790). Levaillant (1797) and Delalande (1822) caught several giraffes in the vicinity of the Orange River (Fig. 3), which were assigned to the species *Giraffa capensis* by Lesson (1842) with the type locality "Cape of Good Hope". Some decades later, Lydekker (1904) recognized three subspecies of southern giraffes: *capensis* for giraffes from the 'Cape colony and adjacent districts'; *wardi* for giraffes from Northern Transvaal (present-day Limpopo and Pretoria, northeastern South Africa); and *angolensis* for giraffes from Angola. This taxonomic view was adopted by most zoologists over the next decades, until Ansell (1968) proposed a new classification with only two subspecies: *angolensis* Lydekker, 1903 for giraffes from Angola and *giraffa* (Boddaert, 1784) (Fig. 1B) for giraffes from South Africa, the latter including *capensis* Lesson, 1842 and *wardi* Lydekker, 1904 as synonyms.
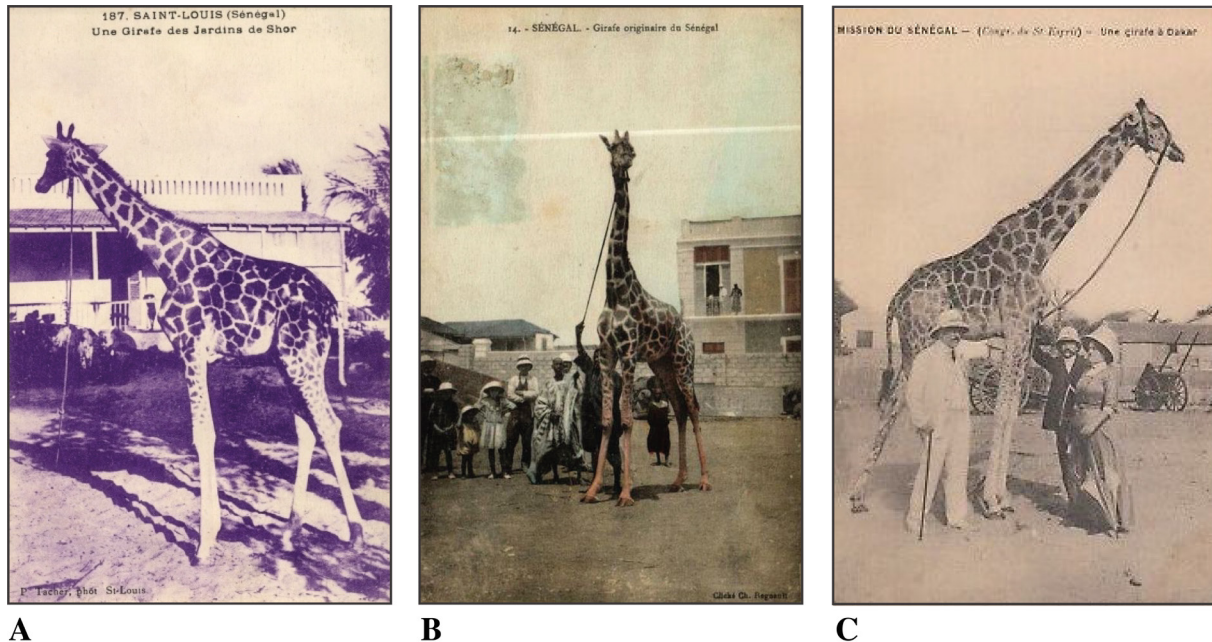

Fig. 6. Postcards of the Senegal giraffe at the beginning of the 20th century. **A**. "Une Girafe des Jardins de Shor", photo taken by P. Tacher in 1909 (https://oldthing.ch/AK-Saint-Louis-Une-Girafe-des-Jardins-de-Shor-Giraffe-im-Gehege-0033371252). **B**. "Girafe originaire du Sénégal" (https://www.ebay.fr/sch/Cartes-postales/914/i.html?cmd=Blend%7CBlend&_nkw=girafe). **C**. "Mission du Sénégal – girafe à Dakar" (https://www.picclickimg.com/d/400/pict/192836173148_/CPA-DAKAR--SENEGAL-MISSION-DU-SENEGAL-UNE.jpg).

Our mtDNA analyses of three 'Cape giraffes' collected by Levaillant (1797) and Delalande (1822) (MNHN-A7977, MNHN-1896-45, MNHN-A10749) show that the two haplotypes fall within the haplogroup SW, which also contains all haplotypes detected for the subspecies *G. g. angolensis*, including the southwestern giraffes from Angola and central Botswana, as well as an isolated population from Zimbabwe (Winter *et al.* 2018a). The haplogroup SW is genetically distinct by 3% from individuals of the haplogroup 'Southeast Africa', which includes giraffes from northeastern South Africa, southern Mozambique, northern Botswana, southern Zambia and southern Zimbabwe. The results indicate therefore that the subspecies *G. g. angolensis* and *G. g. capensis* should be synonymized with *G. g. giraffa* (giraffes of the Cape of Good Hope), whereas the subspecies *G. g. wardi* should be rehabilitated for southeastern giraffes.

Taxonomic treatment

The molecular investigation of key specimens of European museum collections provided the unique opportunity to reveal past giraffe biodiversity through the inclusion of (possibly) extinct populations. Our results have strong taxonomic implications for the classification of giraffes at the subspecies level. We propose the following revision of the classification of the genus *Giraffa* based on molecular (Petzold & Hassanin 2020; this study: see Supplementary files 9 and 10) and morphological diagnosis criteria (Lydekker 1914).

Class Mammalia Linnaeus, 1758
Order Artiodactyla Owen, 1848
Family Giraffidae Gray, 1821
Genus *Giraffa* Brisson, 1762

Giraffa camelopardalis (Linnaeus, 1758)

Diagnosis

Shanks white, presence of occipital horns, five ES in the *Cytb* gene: 186 A=>G, 288 G=>A, 333 A=>G, 597 C=>T, 924 C=>T; one ES in the CR: 462 A=>G; two ES in the *CTAGE5* intron: 570 T=>C, 705 C=>G; two ES in the *CWF19L1*intron: 263 T=>G, 264 T=>G; one ES in the *DDX1* intron: 268 dACAT; one ES in the *DHX36* intron: 50 iGTT; two ES in the *SOS1* intron: 103 T=>C, 118 G=>A.

Type material examined

   Neotype (here designated)
ETHIOPIA • 1 specimen (skin and complete skeleton); Abyssinia; MHNT-1996.121.2.

   Other specimens
ETHIOPIA • 1 specimen (skull and skeleton parts); Abyssinia; MNHN-A8012.

SUDAN • 1 specimen (skull), "Zarafa"; Sennar; MNHN-1845-211.

Distribution

Niger, Chad, Cameroon, Central African Republic, Democratic Republic of Congo, South Sudan (holotype), Uganda, Somalia, Ethiopia (neotype), Kenya.

Remarks

The holotype designation was based on a living giraffe illustrated by Belon du Mans (1553), which was not sampled for a museum collection. The neotype herein designated represents the most complete

specimen (skin and complete skeleton) and has been the first giraffe to be dissected, providing several anatomical drawings (see Joly & Lavocat 1845).

Giraffa camelopardalis camelopardalis (Linnaeus, 1758)

Camelopardalis aethiopicus Ogilby, 1837: 134.
Camelopardalis biturigum Duvernoy, 1844: 12, fig. 4.
Giraffa camelopardalis typica Bryden, 1899: 489, plate XIV.

Diagnosis

Front of the face sparsely and sides fully spotted, similar pattern to *reticulata* but with chestnut or sandy patches.

Past distribution

Probably extinct, former range between the Blue Nile and Tekezé/Atbara rivers in southeastern Sudan (eastern Sennar) and northern Ethiopia (Abyssinia).

Giraffa camelopardalis antiquorum (Jardine, 1835)

Giraffa camelopardalis sennaariensis Trouessart, 1898: p.902.
Giraffa camelopardalis congoensis Lydekker, 1903: 386.
Giraffa camelopardalis cottoni Lydekker, 1904: 207, fig. 1.

Diagnosis

Spots on the upper part of the fore-limbs and the thighs broken up in a number of very small and irregular ones.

Type material

Holotype
SUDAN • 1 ♂ (skull and tanned skin of a male giraffe); South of Darfur; SMF-498.

Paratype
SUDAN • 1 ♀ (skull and tanned skin of a female giraffe); South of Darfur; SMF-497.

Distribution

Cameroon, Chad, Central African Republic, Democratic Republic of Congo, South Sudan (holotype).

Remarks

The holotype designation was based on the information provided by Rüppel (1826), who collected two specimens in North Africa. Both specimens can be meanwhile found in the collection of the Senckenberg Museum Frankfurt (Germany) listed as SMF-498 (skull and tanned skin, male): unspecified type and SMF-497 (skull and tanned skin, female): paratype.

Giraffa camelopardalis peralta Thomas, 1898

Diagnosis

Elongated skull, large spatulate nasal opening, vertically upright direction of the ossicones, fawn-coloured patch below the ears, white sparsely spotted occipital region; two ES in the *Cytb* gene: 219 C=>T, 1080 C=>T, and one ES in the CR: 23 A=>G.

Type material

Holotype

NIGERIA • 1 specimen (skull and bones of right fore and left hind limb); Lokoja; NHMUK-1898.2.18.1.

Distribution

Niger.

Remarks

The type locality has originally been assigned to the junction between the Niger and Benue rivers in Nigeria, but it was corrected in this study to Lokoja (Nigeria) north of the confluence of the Niger and Benue rivers in accordance with Happold (1969).

Giraffa camelopardalis reticulata de Winton, 1899

Giraffa camelopardalis australis Rhoads, 1896: 518.
Giraffa hagenbecki Knottnerus-Meyer, 1910: 800.
Giraffa reticulata nigrescens Lydekker, 1911: 484.

Diagnosis

Deep liver-red colour with a coarse network of narrow white lines, one ES in the *Cytb* gene: 795 C=>T; one ES in the CR: 94 C=>T.

Type material

Holotype

KENYA • 1 specimen (skull, scalp and piece of neck skin); East of Loroghi mountains; NHMUK-1897.1.30.1.

Distribution

Southern Ethiopia, Kenya (holotype), Somalia.

Giraffa camelopardalis rothschildi Lydekker, 1903

Diagnosis

Lower parts of the legs pure white and unspotted, spots show a tendency to split up into stars, occipital pair of ossicones, one ES in the CR: 129 T=>C.

Type material

Holotype

KENYA • 1 specimen (mounted skin); Guasin-gisha Plateau east of Mount Elgon; NHMUK-1903.4.15.1.

Distribution

Western Ethiopia, Kenya (holotype), Uganda, South Sudan.

Giraffa camelopardalis senegalensis Petzold, Magnant & Hassanin, subsp. nov.
urn:lsid:zoobank.org:act:838C1DB1-59BA-49DD-B5AA-F5432F36B112

Diagnosis

Beige ground colour covered with dark brown spots following a reticulated pattern separated by narrow lines, skull features detailed in Blainville (1864), one ES in the *Cytb* gene: 732 A=>G; four ES in the CR: 92 dC, 95 A=>G, 359 C=>T, 463 A=>G.

Type material examined

 Holotype (here designated)
SENEGAL • 1 specimen (skeleton); Bakel; MNHN-A10617.

Past distribution

Probably extinct, former range extended over Senegal (holotype) and potentially Gambia, Mauritania and Mali.

Giraffa tippelskirchi Matschie, 1898

Diagnosis

Faint or strongly stellate form of the patches, absence of occipital horns, seven ES in the *UBN2* intron: 48 dA, 209 iCATAATATATTTAATATATTTAATATTTAATAA, 243 T=>A, 318 G=>C, 332 T=>G, 504 A=>C, 623 C=>T

Type material

 Lectotype (here designated)
TANZANIA • 1 specimen (skull and skin); Lake Eyasi; ZMB-084951.

Distribution

Kenya, Tanzania (lectotype), Zambia.

Remarks

Matschie (1898) mentions two different specimens as syntypes, but the second cannot be found in the collection catalogue and might be considered as lost.

Giraffa tippelskirchi tippelskirchi Matschie, 1898

Giraffa schillingsi Matschie, 1898: 79.

Diagnosis

Stellate formed spots, shanks olive-coloured and spotted down to the hoofs, anterior horn less developed, one ES in the *Cytb* gene: 1033 C=>T.

Distribution

Southern Kenya, Tanzania.

Giraffa tippelskirchi thornicrofti Lydekker, 1911

Diagnosis

Low and conical anterior horn, grey colour and scattered spotting of the sides of the face, fawn shanks, three ES in the CR: 39 A=>G, 272 T=>C, 336 T=>A; two ES in the *IGF2B1* intron: 60 C=>T, 304 G=>A.

Type material

   Holotype
ZAMBIA • 1 specimen (skin); Petauke district; NHMUK-1910.10.17.1.

Distribution

Luangwa Valley in Zambia.

Giraffa giraffa (Boddaert, 1784)

Diagnosis

Anterior horn rudimentary, shanks coloured and fully spotted, three ES in the *C1orf74* intron: 24 A=>T, 33 A=>G, 825 C=>G; three ES in the *DHX36* intron: 127 G=>A, 449 dAT, 498 A=>G; one ES in the *IGF2B1* intron: 45 G=>A; one ES in the *UBN2* intron: 386 dTCT; six ES in the *USP33* intron: 267 G=>A, 279 T=>C, 309 dAA, 538 G=>T, 805 A=>G, 928 G=>A.

Type material examined

   Neotype (here designated)
NAMIBIA • 1 specimen (mounted skeleton), "Giraffe of Levaillant"; North of the Orange river; MNHN-A7977.

Distribution

Angola, Botswana, Mozambique, Namibia (neotype), South Africa, Zambia, Zimbabwe.

Remarks

No concrete holotype specimen assigned, as specimens of the Prince of Orange Museum in The Hague and the giraffe of Vosmaer (1787) from the Naturalis museum in Leiden (Netherlands) are 'whereabouts unknown'.

Giraffa giraffa giraffa (Boddaert, 1784)

Camelopardalis australis Swainson, 1835: 284.
Camelopardalis capensis Lesson, 1842: 168.
Camelopardalis maculata Weinland, 1863: 205, fig. p. 206.
Giraffa camelopardalis angolensis Lydekker, 1903: 121, fig. p. 121.

Diagnosis

One ES in the *Cytb* gene: 798 T=>C; two ES in the CR: 130 iT, 170 A=>G.

Distribution

Angola, Botswana, Namibia, Zimbabwe.

Giraffa giraffa wardi Lydekker, 1904

Giraffa infumata Noack, 1903: 356.

Diagnosis

Irregular spots, spots on the side of the face restricted to the region below and behind the eyes, two ES in the *Cytb* gene: 634 C=>T, 705 A=>G.

Type material

Holotype
SOUTH AFRICA • 1 specimen (mounted head and neck); NHMUK-1903.11.18.1.

Distribution

Botswana, Mozambique, South Africa (holotype), Zambia, Zimbabwe.

Remarks

The holotype is given by Lydekker (1914) under the collection number NHMUK-1903.11.18.1, but this catalogue number leads to a fish specimen (*Pomatomus telescopus* Risso, 1810) on the collection website (https://data.nhm.ac.uk/dataset/collection-specimens/resource/05ff2255-c38a-40c9-b657-4ccb55ab2feb/record/3124756). However, two other specimens of *wardi* can be found in the museum collection (NHMUK-1903.11.17.1 and NHMUK-1903.11.17.3), one of which might be considered the neotype if the holotype cannot be found.

Acknowledgement

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Supplementary material

Supplementary file 1 List of 22 newly generated sequences for giraffes from current populations.

Supplementary file 2 List of primer combinations used for the amplification of historical DNA.

Supplementary file 3 Individual code, designation of subspecies and origin of the 75 giraffe samples sequenced for mitochondrial and nuclear markers.

Supplementary file 4 Bayesian tree from the analysis of the mtDNA-91T dataset.

Supplementary file 5 PhyML tree from the Maximum Likelihood analysis of the mtDNA-91T dataset.

Supplementary file 6 Bayesian tree from the analysis of the nuDNA-78T dataset.

Supplementary file 7 PhyML tree from the Maximum Likelihood analysis of the nuDNA-78T dataset.

Supplementary file 8 Comparison of mitochondrial and nuclear divergence time estimates.

Supplementary file 9 Exclusive synapomorphies characterizing giraffe taxa in the mtDNA-91T dataset.

Supplementary file 10 Exclusive synapomorphies characterizing giraffe taxa in the nuDNA-78T dataset.