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Taxonomy / Taxinomie

Mitochondrial DNA variability in *Giraffa camelopardalis*: consequences for taxonomy, phylogeography and conservation of giraffes in West and central Africa

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Abstract

The giraffe (*Giraffa camelopardalis*) still survives in four countries of West and central Africa. The populations of Niger and Cameroon are generally assigned to the subspecies *peralta*, but those of Chad and the Central African Republic are taxonomically problematic, as they are referred to as either *peralta*, or *antiquorum*, or *congoensis*. In this study, a mitochondrial fragment of 1765 nucleotide sites, covering the complete cytochrome *b* gene, three transfer RNAs and a large part of the control region, was sequenced to assess the relationships between several populations of giraffe. The phylogenetic analyses performed on the 12 identified haplotypes indicate that northern giraffes constitute a natural group, distinct from that of southern giraffes. Surprisingly, the giraffes of Niger are found to be more closely related to the giraffes of East Africa (subspecies *rothschildi* and *reticulata*) than to those of central Africa. We conclude therefore that the subspecies *peralta* contains only the Niger giraffes, whereas the subspecies *antiquorum* includes all populations living in Cameroon, Chad, the Central African Republic, and southwestern Sudan. We suggest that the ancestor of the Nigerian giraffe dispersed from East to North Africa during the Quaternary period and thereafter migrated to its current Sahelian distribution in West Africa, in response to the development of the Sahara desert. This hypothesis implies that Lake Mega-Chad acted as a strong geographical barrier during the Holocene, preventing any contact between the subspecies *peralta* and *antiquorum*. Our study has direct implications for conservation management, as we show that no subspecies *peralta* is represented in any European zoos, only in Niger, with a small population of less than 200 individuals. ***To cite this article: A. Hassanin et al., C. R. Biologies 330 (2007).***

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Résumé

Variabilité de l'ADN mitochondrial chez *Giraffa camelopardalis* : conséquences pour la taxinomie, la phylogéographie et la conservation des girafes en Afrique de l'Ouest et centrale. La girafe (*Giraffa camelopardalis*) subsiste encore à l'état sauvage dans quatre pays d'Afrique de l'Ouest et centrale. Alors que les populations du Niger et du Cameroun sont généralement classées dans la sous-espèce *peralta*, le statut taxinomique des girafes du Tchad et de la République centrafricaine est problématique, puisque ces populations peuvent être rattachées à trois sous-espèces différentes (*peralta, antiquorum*, ou *congoensis*). Lors de cette étude, un fragment mitochondrial de 1765 nucléotides, couvrant la totalité du gène du cytochrome b, trois ARN de transfert et une grande partie de la région de contrôle, a été séquencé afin d'évaluer les relations de parenté entre plusieurs populations de girafes. Les analyses phylogénétiques réalisées sur les 12 haplotypes identifiés indiquent que les girafes du Nord forment un groupe naturel, distinct de celui des girafes du Sud. De façon surprenante, la girafe du Niger apparaît plus proche des girafes d'Afrique de l'Est (sous-espèces *rothschildi* et *reticulata*) que de celles d'Afrique centrale. Du point de vue taxinomique, nos analyses montrent donc que la sous-espèce *peralta* correspond uniquement à la girafe du Niger, alors que la sous-espèce *antiquorum* regroupe toutes les populations vivant au Cameroun, au Tchad, en République centrafricaine et dans le Sud-Ouest du Soudan. Du point de vue phylogéographique, nos résultats suggèrent une dispersion de l'ancêtre de la girafe *peralta* à partir de l'Afrique de l'Est vers l'Afrique du Nord au cours de l'ère Quaternaire, suivie d'une migration vers le sud, pour finalement occuper sa distribution actuelle en Afrique de l'Ouest, et cela en réponse à l'extension du désert du Sahara. Cette hypothèse implique que le lac Méga-Tchad fut une importante barrière géographique durant l'Holocène, qui a empêché tout contact entre les sous-espèces *peralta* et *antiquorum*. Notre étude a des conséquences directes pour la conservation des girafes, puisque nous montrons que la sous-espèce *peralta* n'est malheureusement pas représentée dans les parcs zoologiques européens, et qu'elle est uniquement trouvée au Niger, où subsiste une petite population de moins de 200 individus. ***Pour citer cet article : A. Hassanin et al., C. R. Biologies 330 (2007).***
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Keywords: Giraffe; *Giraffa camelopardalis*; mtDNA; Taxonomy; Phylogeography; Conservation

Mots-clés : Girafe ; *Giraffa camelopardalis* ; ADNmt ; Taxinomie ; Phylogéographie ; Conservation

1. Introduction

The giraffe, *Giraffa camelopardalis* (Linnaeus, 1758), belongs to the family Giraffidae, which also contains the okapi, *Okapia johnstoni*, confined to the Ituri rainforests in the Northeast of the Democratic Republic of Congo. Although different in appearance and behaviour, giraffe and okapi share a number of common features, including a long neck, a long and dark-coloured tongue, bilobed lower canines, and skin-covered horns, called ossicones. The giraffe formerly roamed the majority of savannahs and open woodlands in Africa, particularly in areas with an abundance of *Acacia*, *Commiphora*, and *Terminalia* trees [1]. Within historic times, the giraffe disappeared from most countries in northern Africa, due to increasing aridity and expanding human pressures, including hunting and farming [2]. However, the species *G. camelopardalis* is currently listed as Lower Risk by the IUCN [3], because wild populations remain common locally in eastern and southern Africa.

The male and female giraffe both have a typical coat pattern, consisting of large, irregularly shaped, chestnut-brown to black patches separated from one another by a network of white or yellowish–white bands. Nine subspecies of giraffe have been recognized on the basis of distinctive regional differences in colour and pattern of the coat [2] (Fig. 1B): *angolensis* (Angolan giraffe), *antiquorum* (Kordofan giraffe), *camelopardalis* (Nubian giraffe), *giraffa* (Southern/South African giraffe), *peralta* (Nigerian/West African giraffe), *reticulata* (reticulated giraffe), *rothschildi* (Baringo/Rothschild's/Uganda giraffe), *thornicrofti* (Thornicroft's giraffe), and *tippelskirchi* (Masai giraffe). The criteria used for defining the various subspecies are however suspect; firstly, because important coat variations have been observed between individuals of the same population, and secondly, because there are areas of range overlap, resulting in hybridization commonly occurring between described subspecies, e.g., between *rothschildi* and *reticulata* in Laikipia in central Kenya [4]. As considerable uncertainty surrounds the validity and geographical limits of most of the subspecies described in Dagg and Foster [2], only six groups of populations have been defined by the IUCN [5] (Fig. 1A). Whereas the three subspecies (1) *reticulata*, (2) *thornicrofti*, and (3) *tippelskirchi* were accepted for the IUCN classification, three new groups were considered: (4) the western group, which includes *antiquorum* and *peralta*, (5) the Nubian/Rothschild group, which contains *camelopardalis* and *rothschildi*, and (6) the southern group, which is composed of *angolensis* and *giraffa*.

Populations of the western group have drastically decreased over the last decades due to habitat destruction and poaching. In West Africa, the giraffe was formerly

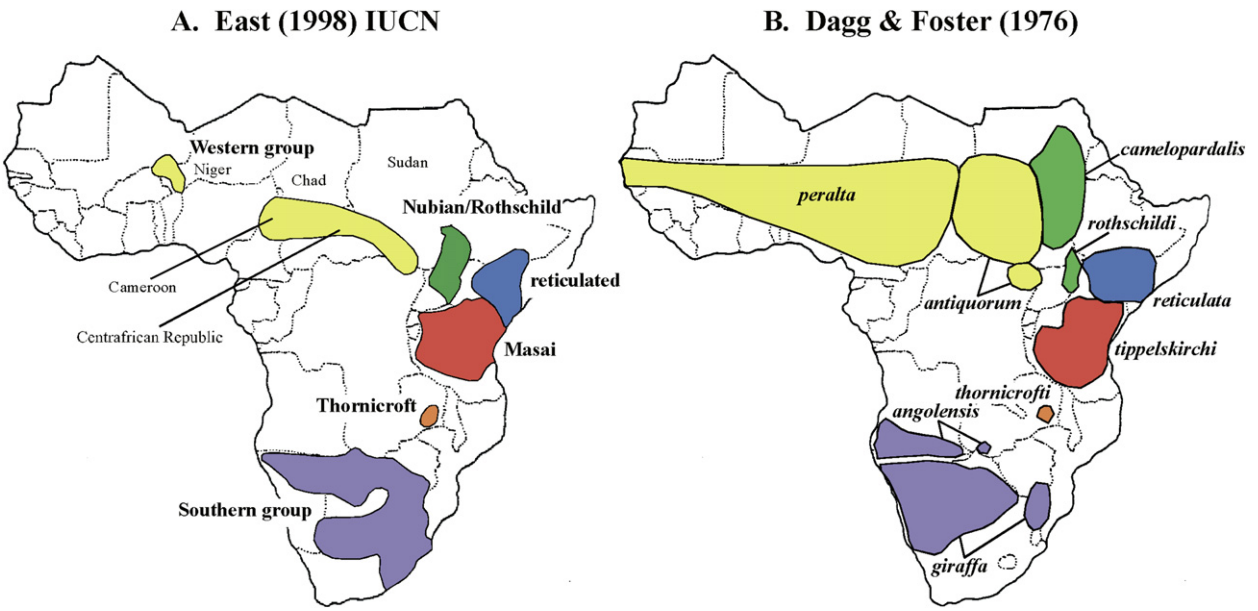

Fig. 1. Recent distribution of the giraffes at present (**A**, [5]) and within historic times (**B**, [2]). The colours indicate the six groups defined by the IUCN classification [5]. (For interpretation of the references to colour, the reader is referred to the web version of this article.)

found from Senegal to Lake Chad, but the only viable surviving population within this entire area is the small population of southwestern Niger (160–180 individuals, Omer Dovi, personal communication). The current attempt to protect Niger's remaining giraffes is a major international conservation priority [6]. The western giraffe survives in larger numbers in central Africa, but only the populations of northern Cameroon (Boubandjida, Benoue, and Waza National Parks) and Zakouma in Chad are reasonably secure [5]. Some urgent taxonomic research, based on a molecular approach, is therefore needed to clarify the status of these populations, because the absence of genetic information on the giraffe has led conservation agencies to employ management programmes based on poorly defined categories, which could reduce the long-term evolutionary potential of the species.

We report here the first molecular study dealing with the relationships among subspecies of *G. camelopardalis*. Our analyses are based on a mitochondrial DNA (mtDNA) fragment of 1765 nucleotides (nt), which covers the complete cytochrome *b* gene, three tRNA genes (Glu, Thr and Pro) and the 5′ region of the control region. The aims of this study were to (1) quantify the genetic variation between mitochondrial sequences of different populations of *G. camelopardalis*, (2) test the classifications proposed on the basis of coat variations, (3) determine the taxonomic status of the giraffes living in West and central Africa, and those held in captivity in European zoos, and (4) better understand the recent evolution of giraffes in Africa.

2. Material and methods

2.1. Samples used for DNA extraction

Our study includes 23 specimens of giraffe, representing all the nine subspecies of *G. camelopardalis* defined in Dagg and Foster [2], except the Thornicroft's giraffe (*thornicrofti*) from Zambia and the Nubian giraffe (*camelopardalis*) from eastern Sudan and western Eritrea (Table 1). The outgroup contains three species belonging to three different families of the suborder Ruminantia: *Bos grunniens* (Bovidae), *Muntiacus muntjak* (Cervidae), and *Okapia johnstoni* (Giraffidae), the closest living relative of *G. camelopardalis*.

Skin and blood samples were digested in CTAB (*hexade Cyl trimethylammonium bromide*) using the protocol detailed in Winnepenninckx et al. [7]. DNA was extracted from faecal samples using the method described in Porteous et al. [8]. DNA was purified in chloroform isoamyl alcohol and then precipitated with ethanol.

2.2. DNA amplification and sequencing

The mitochondrial DNA fragment selected for this study includes the 3′ end of the Glu-tRNA gene, the complete cytochrome *b* (*Cyb*) gene, the complete genes

Table 1
Origin of the tissues used for DNA analyses

Classifications						
East (1998)	**Dagg and Foster (1976)**	**Name / N°**	**Sex**	**Origin** (*wild, °zoo)	**Collector**	**Tissue**
Western group	***G.c. peralta***	PER1	M	* Kouré Plateau, Niger	Bertrand Chardonnet	Faeces
		PER2	F	* Kouré Plateau, Niger	Bertrand Chardonnet	Faeces
		PER3	F	* Kouré Plateau, Niger	Bertrand Chardonnet	Faeces
		DOSSO	?	* Niamey-Dosso Road, Niger	Céline Houssin	Faeces
		BOUBA	?	* Boubandjida National Park, Cameroon	Bertrand Chardonnet	Faeces
		WAZA1	M	* Waza National Park, Cameroon	Bertrand Chardonnet	Skin
		WAZA2	?	* Waza National Park, Cameroon	Bertrand Chardonnet	Faeces
	G.c. peralta	Uma / ZA4124	F	° Vincennes Zoo, France	Alexis Lécu	Blood
		Rafiki / ZA4128	M	° Vincennes Zoo, France	Anne-Laure Gourmand	Faeces
	G.c. peralta x antiquorum	Valere / M9633B	M	° Vincennes Zoo, France	Anne-Laure Gourmand	Faeces
	G.c. antiquorum	ZAK1	M	* Zakouma National Park, Chad	Bertrand Chardonnet	Skin
		ZAK2	?	* Zakouma National Park, Chad	Bertrand Chardonnet	Skin
		Sarah / M9271	F	° Antwerp Zoo, Belgium	Francis Vercammen	Faeces
Reticulated	***G.c. reticulata***	Zénith / 4-3083	M	° Sigean African Reserve, France	Frédéric Tardy	Faeces
		Désirée	F	° Sigean African Reserve, France	Frédéric Tardy	Faeces
Nubian / Rotschild's	***G.c. rothschildi***	Eole / 1137	M	° La Palmyre Zoo, France	Thierry Petit	Faeces
		Robert / A4216	M	° Thoiry Zoological Park, France	Carine Alves	Faeces
Masai	***G.c. tippelskirchi***	Wari / 990681	F	° Basel Zoo, Switzerland	Friederike Von Holiwald	Faeces
		Saburi / 950677	M	° Basel Zoo, Switzerland	Friederike Von Holiwald	Faeces
Southern group	***G.c. angolensis***	Lisbon5A / 7382	M	° Lisbon Zoo, Portugal	Patricia Vilarinho	Faeces
		Lisbon6A / 6751	M	° Lisbon Zoo, Portugal	Patricia Vilarinho	Faeces
	G.c. giraffa	Tango / 97058	M	° Thoiry Zoological Park, France	Carine Alves	Faeces
		PHALA / GCG9	?	* Phalaborwa, Limpopo, South Africa	Hanneline Smit	Faeces
Okapi	***Okapia johnstoni***	Gunther / Z95145	M	° Vincennes Zoo, France	Geoffrey Fruleux	Faeces

for Thr- and Pro-tRNAs, and the 5′ part of the control region, also named D-loop in mammals. The sequences were obtained using several overlapping PCR amplifications. The exact matching between the overlapping portions of two different PCR fragments has been checked as a proof of authenticity of sequences. Most primers come from previous publications on the *Cyb* gene [9,10], but two new primers were specifically designed to amplify the D-loop region: 5′-CAT CGG ACA ACT AGC ATC TAT-3′ (direct, position 15222 in the sequence of *Muntiacus muntjak*, accession number NC_004563) and 5′-CCA GAT GTC TGA TAA AGT TCA-3′ (reverse, position 15882). Amplifications were done in 50 µl, using the following PCR standard conditions: buffer 10X with $MgCl_2$: 5 µl, dNTP: 5 µl (6.6 mM), Taq DNA polymerase (QBiogen, Illkirch, France): 0.3 µl (2.5 U), and primers: 2.5 µl at 10 µM. The standard PCR conditions used were: 94 °C for 4 min; 94 °C for 1 min, 50–58 °C for 1 min, 72 °C for 1 min (30 cycles). PCR products were sequenced by Genoscreen (Lille, France) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

2.3. Phylogenetic analyses

The DNA sequences were aligned by eye using SeAl v2.0a11 [11]. One ambiguous region, i.e., involving ambiguity for the position of the gaps, was excluded from the analyses to avoid erroneous hypotheses of primary homology: positions 15531–15603 in the sequence of *Muntiacus muntjak* (accession number NC_004563). The reduced alignment of mt sequences consists of 1689 nt. It is available upon request to AH.

Phylogenetic analyses were performed using Maximum Parsimony (MP), Maximum Likelihood (ML), Neighbour-Joining (NJ), and Bayesian methods. The MP analyses were conducted on PAUP 3.1.1 [12] with differential weighting of the character-state transformations using the product CI_{ex} S (CI_{ex}: consistency index excluding uninformative characters, S: slope of saturation) [9,13]. Bootstrap percentages (BP_{MP}) were computed after 1000 replicates using the closest stepwise addition option. ML and NJ analyses were carried out under PAUP 4.0b10 [14], and Bootstrap proportions (BP_{ML} and BP_{NJ}) were obtained after 1000 replicates. MrModeltest 2.2 [15] was used for choosing the model of DNA substitution that best fits the data. The selected likelihood model was the Hasegawa–Kishino–Yano model [16] with among-site substitution rate heterogeneity described by a gamma distribution (HKY + G). Bayesian posterior probabilities (PP) were calculated on MrBayes 3.1.2 [17] using five independent Markov chains run for 2 000 000 Metropolis-coupled MCMC generations, with tree sampling every 100 generations and a burn-in of 2000 trees.

3. Results

3.1. Mitochondrial DNA variation in Giraffa camelopardalis

A total of 12 mitochondrial haplotypes (mitotypes) was identified among the 23 samples. Sequences of these haplotypes have been deposited in the EMBL/GenBank/DDBJ database (accession numbers EF442263-EF442274). In Fig. 2, haplotypes are coded by geographical locality or zoological park with individual identification numbers. In general, giraffes of the same zoological park share the same haplotype (Basel, Sigean, and Vincennes). However, two mitotypes were found in the Lisbon zoo (Nos. 7382 and 6751), which differ by only one nucleotide (Fig. 2; transition C-T in the D-loop, position 1566 of the alignment).

Two haplotypes were found for the subspecies *antiquorum*: the first one diagnoses two wild giraffes of Zakouma (Chad, ZAK 1 and 2), and the second one characterizes the maternal lineage of the Antwerp zoo (specimens named Sarah and Valère; the latter is a hybrid produced from crossing a female *antiquorum* from the Antwerp zoo with a male *peralta* from the Vincennes zoo).

Three haplotypes were found for the subspecies *peralta*: the first one diagnoses all the wild giraffes of Niger (PER1-3, and DOSSO); the second one defines the three wild giraffes of northern Cameroon (BOUBA, WAZA1, and 2); the third one characterizes the giraffes of the Vincennes zoo (Uma and Rafiki).

The 12 haplotypes can be classified into three distinct lineages, i.e. northern giraffe, Angolan giraffe, and the southeastern group, composed of the subspecies *giraffa* and *tippelskirchi*, as their sequence divergences range from 3.1 to 4.4% for the total mitochondrial fragment (1765 nt), from 2.5 to 3.8% for *Cyb* (1140 nt), and from 3.9 to 6.9% for the 5′ region of the D-loop (464 nt). The northern group includes the populations previously included in the subspecies *peralta*, *antiquorum, rothschildi*, and *reticulata*.

3.2. Phylogenetic analyses

Out of 1689 total unambiguous characters kept for the phylogenetic analyses, 1239 were constant, 207 were variable but parsimony-uninformative and 243

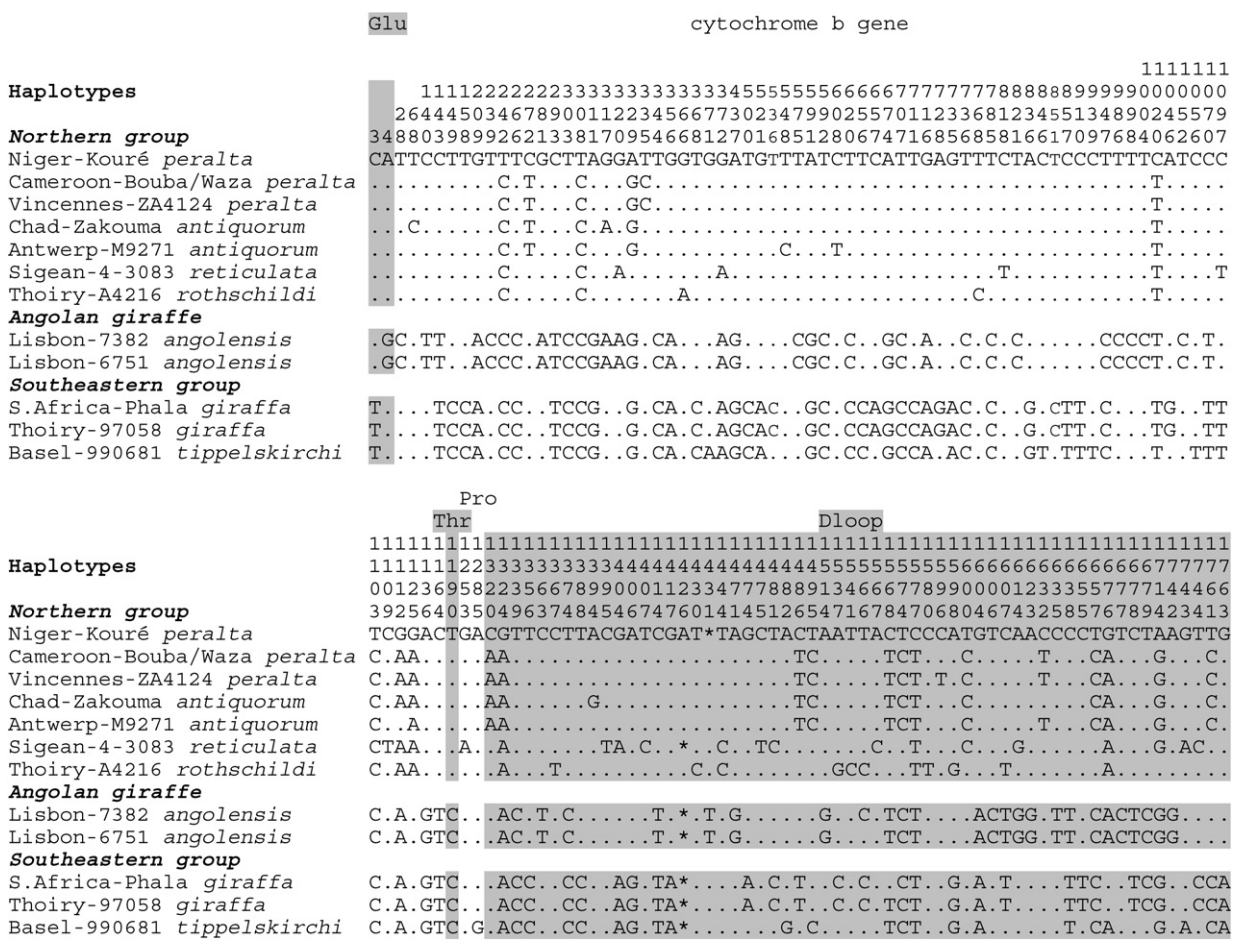

Fig. 2. List of the 134 variable sites identified between the 12 giraffe mitotypes. The total DNA alignment represents 1765 nucleotide sites and includes the 3′ end of the tRNA-Glu, the complete cytochrome *b* gene and tRNA genes for Thr and Pro, and the 5′ region of the D-loop. One hundred and thirty-four variable sites were identified between the 12 haplotypes of *Giraffa camelopardalis*. The region of the D-loop ranging from 1403 to 1486 was excluded from the phylogenetic analyses, given the ambiguity in the position of several gaps when outgroup species were included in the alignment.

were informative. When the three distant outgroup species were excluded from the analyses, only 119 characters were found variable between populations of *G. camelopardalis*, including 87 informative characters (see details in Fig. 2).

The four methods of tree reconstruction (MP, ML, NJ, and the Bayesian inference) show that *G. camelopardalis* is a monophyletic species (Fig. 3; $BP_{\mathrm{MP/ML/NJ}} = 100$, $PP = 1$). The three groups of giraffe haplotypes are supported by high values of robustness and several molecular signatures diagnose each group. The two haplotypes of Angolan giraffe are grouped together ($BP_{\mathrm{MP/ML/NJ}} = 100/89/100$; $PP = 0.98$), and share six exclusive synapomorphies, including four transitions A → G (pos. 4, 1514, 1623, and 1742), and two transitions T → C (pos. 1606 and 1677). The subspecies *giraffa* and *tippelskirchi* are grouped together ($BP_{\mathrm{MP/ML/NJ}} = 98/62/100$; $PP = 0.78$), and have in common three exclusive transitions (pos. 3: C → T, pos. 836: A → G, and pos. 1763: G → A). All giraffes of the northern group fall into a well-supported clade ($BP_{\mathrm{MP/ML/NJ}} = 99/88/100$; $PP = 0.98$) and share seven exclusive synapomorphies, including three transitions A → G (pos. 209, 356, and 716), three transitions C → T (pos. 592, 785, and 1190), and one transition T → C (pos. 1559). In this group, the subspecies *antiquorum* and *peralta* are not found to be monophyletic. The subspecies *antiquorum* is paraphyletic, as the giraffe of the Antwerp zoo is not directly related to the giraffe of Zakouma, but rather is allied to the giraffes *peralta* of northern Cameroon (Boubandjida and Waza National Parks) and to those held captive in the Vincennes zoo ($BP_{\mathrm{MP/ML/NJ}} = 66/61/78$; $PP = 0.87$). In addition, the subspecies *peralta* is polyphyletic, as the giraffe *per-*

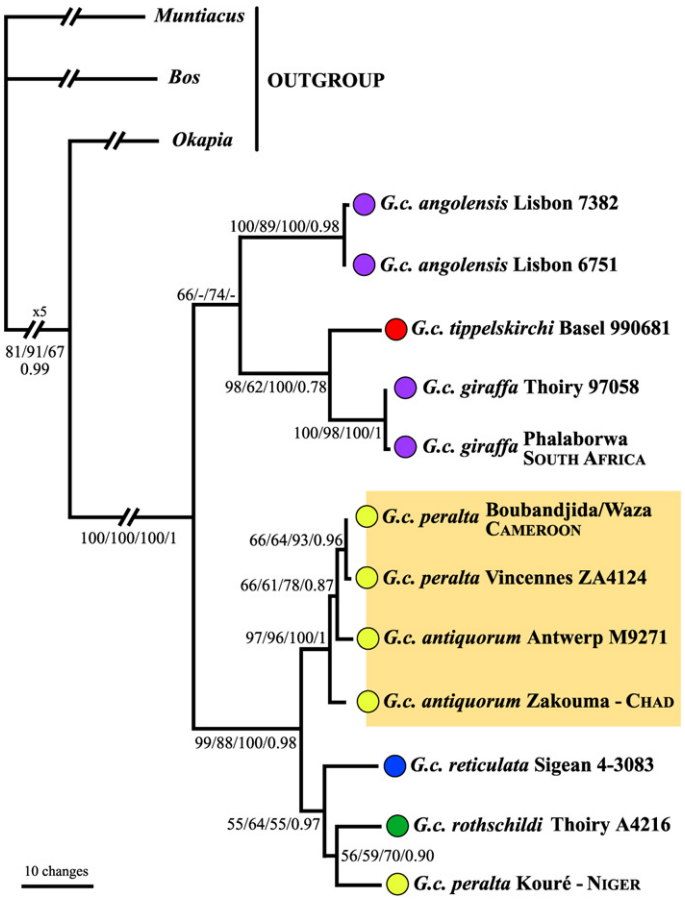

Fig. 3. Phylogenetic tree of the 12 giraffe mitotypes. The tree was constructed with the Maximum Parsimony method and values indicated on the branches are, from left to right, the bootstrap percentages (values less than 50% are not shown) obtained with either weighted Maximum Parsimony, Maximum Likelihood or Neighbour-Joining methods, and Bayesian posterior probabilities (values less than 0.5 are not shown). At the end of the terminal branches, the colour of the circle indicates to which group defined by the IUCN classification [5] the haplotype belongs (see in Fig. 1A for details). Our results suggest including the four haplotypes highlighted in orange in the subspecies *antiquorum* (For interpretation of the references to colour, the reader is referred to the web version of this article.).

alta from Niger is not grouped with other *peralta* from Cameroon and Vincennes, but rather is linked to the eastern giraffes of the subspecies *rothschildi* and *reticulata* ($BP_{MP/ML/NJ} = 55/64/55$; $PP = 0.97$). Within the northern group, only one clade appears strongly supported: the one joining the wild giraffes of Cameroon and Chad with the captive giraffes held in the Vincennes and Antwerp zoos ($BP_{MP/ML/NJ} = 97/96/100$; $PP = 1$). This clade is diagnosed by two exclusive synapomorphies, including one transition C → T (pos. 272) and one transversion C → A (pos. 1320).

The MP and NJ trees are identical to those reconstructed with ML and Bayesian methods, except for the position of *angolensis*. In the former trees, Angolan giraffes are grouped with other southern giraffes, i.e. the subspecies *giraffa* and *tippelskirchi* ($BP_{MP/NJ} = 66/74$), but in the latter trees, they are allied to northern giraffes, i.e. the clade including the subspecies *antiquorum*, *peralta*, *reticulata*, and *rothschildi* (data not shown, $PP = 0.55$, $BP_{ML} = 55$).

4. Discussion

4.1. Taxonomy of the western giraffes

The status of the northern giraffes has always been controversial. In Lydekker [18], the reticulated giraffe, from northeastern Kenya, Somalia and southern Ethiopia, was treated as a separate species, *G. reticulata*, whereas other northern populations were classified into five subspecies of *G. camelopardalis*: *antiquorum* (Kordofan, central Sudan), *cottoni* (northern Uganda), *peralta* (Nigeria in the neighbourhood of Lokoja, where the Niger and Benue rivers meet), *typica* (formerly Nu-

bia and Abyssinia, i.e. south of Egypt, northern and eastern regions of Sudan, Eritrea and north of Ethiopia) and *rothschildi* (western Kenya and eastern Uganda).

Most of these subspecies were adopted by Dagg and Foster [2], but the reticulated giraffe was defined as a subspecies of *G. camelopardalis* (*reticulata*), the subspecies *typica* was regarded as a synonym of *camelopardalis* and the subspecies *cottoni* was included in *rothschildi*. In addition, the former distribution of *peralta* was supposed to cover most countries of West and central Africa, including Senegal, southern Mauritania, northern Guinea, southern Mali, Burkina Faso, the North of Ghana, Togo and Benin, southern Niger, Nigeria, Cameroon, southern Chad, and the western part of the Central African Republic (Fig. 1B).

The view proposed by Kingdon [1,4] differed in three major points: (*i*) the subspecies *antiquorum* was discarded, as all populations inhabiting the Sahelian zone, from Senegal to Sudan, were included in the subspecies *peralta*; (*ii*) the woodland populations from Cameroon to Uganda were put in the subspecies *congoensis*; and (*iii*) possible hybrid populations were described in Cameroon between *congoensis* and *peralta*, and in Kenya between *congoensis* and *reticulata*. As a consequence, *rothschildi* was not regarded as a valid subspecies.

As pointed out by East [5], considerable uncertainty surrounds the validity and geographical limits of most described subspecies of giraffe. For instance, there is no major geographical barrier separating the distributions of supposed subspecies of northern giraffe, such as *peralta* and *antiquorum* in central Africa, *camelopardalis* and *rothschildi* in southern Sudan, or *reticulata* and *rothschildi* in central Kenya. As a consequence, East [5] recognized only three arbitrary groups of northern giraffe in the IUCN classification: the western giraffe, including *peralta*, *antiquorum* and *congoensis*, the Nubian/Rothschild's giraffe, including *camelopardalis* and *rothschildi*, and the reticulated giraffe (subspecies *reticulata*).

Our molecular data show that all previous classifications are invalid. Within the northern clade, the giraffes of the Vincennes zoo, which were referred to as *peralta*, are closely related to the *peralta* of Boubandjida and Waza National Parks (Cameroon), but they do not group with the *peralta* of Niger. However, they are included in a biogeographically-coherent clade, which contains giraffes living in three adjacent countries – Cameroon, Chad, and Sudan – as the giraffes of the Antwerp zoo were acquired from the Game Preservation Department of Sudan in 1948 and 1949 in the neighbourhood of Khartoum (B. Van Puijenbroeck, personal communication). Interestingly, this grouping agrees with their geographical origin, as the captive population of Vincennes grew from four wild animals originating from Cameroon (Zizi I, male), Chad (Lamy and Diamala II, males) and north of the Central African Republic (Negry, female). Since the mitochondrial genome is inherited by the maternal lineage, the mitotype of Vincennes characterizes one population of the Central African Republic. Consequently, our analyses of mitochondrial sequences suggest that two subspecies of giraffes can be distinguished in West and central Africa: the subspecies *peralta*, which consists of a very small population, representing less than 200 individuals, in the southwestern region of Niger, and the subspecies *antiquorum*, which comprises all populations found in northern Cameroon, southern Chad, north of the Central African Republic and southwestern and central Sudan.

4.2. Phylogeography of the giraffes

The different subspecies of giraffe exhibit moderate nucleotide divergence of the mitochondrial sequences. By comparing the 5′ end of the *Cyb* gene (417 nt), we found a maximum distance of 4.56% between northern and southern subspecies (*angolensis* and *peralta*) and 1.44% within the northern clade (*antiquorum* and *reticulata*). These values are higher than that calculated for various breeds of domestic cattle (0.48%, *Bos taurus taurus*, 75 sequences extracted from complete mitochondrial genomes, data not shown), which have diversified after their domestication some 11 000 years ago [19], and lower than that found between southern and northern subspecies of hartebeest (5.76% between *Alcelaphus buselaphus swaynei* and *A. b. caama*), which are supposed to have diverged some 500 000 years ago [20]. Although estimates of divergence time based on the molecular clock are questionable, because the nucleotide substitution rates may vary extensively among mammals, these data suggest that the diversification of extant subspecies of giraffes took place during the Late Pleistocene. This result agrees with the fossil record, as the most ancient remains described for the species *G. camelopardalis* were dated at around 1 Myr [21].

The climate of the Pleistocene was characterized by cold, arid glacial periods oscillating with warmer, wetter interglacial periods, with associated expansion and contraction of savannah habitats [22]. Recurring isolations of populations into one or more favourable refugia in West, East and South Africa during wetter periods with range expansion events during drier periods have been postulated to explain the phylogeographical patterns in other ruminant species occupying savannah habitats,

such as the greater kudu (*Tragelaphus strepsiceros*), hartebeest (*Alcelaphus buselaphus*), impala (*Aepyceros melampus*), roan antelope (*Hippotragus equinus*), topi (*Damaliscus lunatus*), and wildebeest (*Connochaetes taurinus*) [20,23–25]. Our phylogenetic analyses show that northern giraffes are divergent from two southern groups corresponding to the southwestern subspecies *angolensis* and to the southeastern clade uniting *giraffa* and *tippelskirchi*. The important nucleotide divergence between these three groups suggests that previously existing ecological or topographic barriers effectively prevented gene flow over extensive periods of time, leading to reproductive isolation of these lineages. As the giraffes are found predominantly in open or broken savannah habitats, we suggest that the existence of dense tropical forests, and also of vast deserts, may have acted as strong ecological barriers. Rivers, lakes and mountain ranges may have also served as effective barriers to giraffe dispersal. For this reason, the Great Rift Valley and lakes of East Africa may explain the important differentiation between northern and southern populations of giraffes. In southern Africa, there is a significant division between the subspecies *angolensis* and eastern populations. Since a similar phylogeographical pattern was previously obtained for bovids [23,24], we suggest that the Mega Kalahari sand sea [26] may have acted as a strong ecological barrier during the Late Pleistocene.

The phylogeny of northern giraffes shows that the Nigerian giraffe (subspecies *peralta*) is more closely related to the giraffes of East Africa (subspecies *rothschildi* and *reticulata*) than to those of central Africa (subspecies *antiquorum*). This result suggests that the current distributions of giraffes in Western and central Africa result from two independent biogeographical events and that a physical barrier prevents gene flow between *peralta* and *antiquorum*, given that both share the same latitudinal distribution in the Sahelian zone. The first biogeographical event may have led to the separation of giraffes of Central and East Africa, corresponding respectively to the subspecies *antiquorum*, and the common ancestor of the subspecies *reticulata*, *rothschildi*, and *peralta*. The Nile River and the Great Lakes of East Africa may have stopped gene flows between these two groups during most parts of the Pleistocene and Holocene epochs. A second, more recent biogeographical event was the dispersal of the ancestor of *peralta* from East to West Africa. Due to the earlier presence of *antiquorum* in central Africa, it may be inferred that the ancestor of *peralta* dispersed at first from East to North Africa and thereafter to West Africa. This hypothesis is confirmed by the many prehistoric rock paintings and engravings indicating that the giraffe was found all over the region now covered by the Sahara desert during the Holocene [27]. Around 6000 years ago, the development of hyperarid conditions resulted in the formation and extension of the Sahara desert [28,29], which may have forced the giraffes of West Africa to migrate in the southwards and to occupy their current Sahelian distribution. Several authors have proposed the existence of a Holocene giant lake in the central part of North Africa. Known as Lake Mega-Chad, it included the present-day Lake Chad, but extended over the Tibesti and Ennedi mountains in the North and East of Chad, respectively [30,31]. Since Lake Mega-Chad was at its maximum level between 7700 and 5500 years BP, and was still well developed between 3700 and 3000 years BP, we can assume that it acted as a natural physical barrier preventing communication between *peralta* and *antiquorum* populations. More recently, three biogeographical barriers may have limited the distribution of the Nigerian giraffe in West Africa: in the north, the southern limit of the Sahara desert, in the south, the Niger and Benue Rivers, together with the Upper Guinea rainforests extending from Guinea into Sierra Leone and eastward through Liberia, Ivory Coast, and Ghana into western Togo, and in the East, the forests and mountains (Mandara and Alantika) on the border between Nigeria and Cameroon [32].

4.3. Consequences for conservation of the giraffe in western Africa

Our genetic study on the mtDNA sequences is an important first step that can have a direct and important impact on conservation management. We show, firstly, that the giraffes of western and central Africa belong to two different subspecies, *peralta* and *antiquorum*, and secondly, that the giraffes in the Vincennes zoo, which were referred to as *peralta*, in fact belong to the subspecies *antiquorum*. This means that the subspecies *peralta* is today only represented in Niger, with a small population of around 160–180 giraffes. As no giraffe *peralta* exists in European zoos, no re-introduction programme could be conducted in the future to deal with the conservation of this endangered population. We recommend therefore that urgent and major measures be taken to protect the last giraffes of West Africa.

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