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Contribution of small rodent communities (Muridae) to the history of West Central African savannas (case of Gabonese savannas): diversity, biogeographic affinities, phylogeography and paleoenvironmental analyses

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ABSTRACT

Savannas of West Central Africa stand around or inside the large rainforest block. They result from paleo-climatic fluctuations, but the details of their history remain poorly known. The aim of the present study is to explain the present distribution of savannas in West Central Africa, considering the two classic anthropic or paleoclimatic hypotheses about their origin. The study was focused on Gabonese savannas and based on small terrestrial mammal patterns. The phylogeography of small species of strictly savanicolous rodents was compared using nested clade analysis of haplotype networks based on cytochrome *b* mitochondrial DNA, coupled with other approaches. The data were also compared with literature data on vegetation changes in tropical West Africa over the last 150,000 years to test scenarios on the origin and history of Gabonese savannas. The results showed several fragmentation events of small rodent populations and revealed redundant biogeographic relationships. These events were dated, and the results about the history of rodent species were congruent with palaeobiological data about the successive phases of savanna expansion and fragmentation: the results support the hypothesis of a paleoclimatic origin of the Western Congolian forest-savanna mosaic. These rodent population fragmentation events occurred more than 50,000 years ago.
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Keywords: Biogeographic analysis, Genetic diversity, Small rodents, Cytochrome *b*, Savanna landscape, Middle Pleistocene.

INTRODUCTION

West Central Africa (Gabon, the Republic of Congo, Cameroon, the Central African Republic and Equatorial Guinea) is mainly covered by rainforest, but peripheral savannas and islands of savanna are also present (Noupa and Nkongmeneck, 2008; Figure 1). Most studies on the small rodent communities (Rodentia, Muridae) of West Central Africa have focused on forested areas, particularly in Gabon (Goodman et al., 2001; Nicolas and Colyn, 2003). Studies on savanna mammals have focused on large mammals (King and Chamberlan, 2013; Nakashima, 2015; Barnett et al., 2018) and ignored small rodent communities.

Situated in the middle of the Guinean-Congolese region, the Gabonese landscape is

characterised by an extensive forest cover, but also harbours many examples of relict savanna and coastal savanna forest. These areas are located in the Guineo-Congolese forest massif (in the case of the Lopé area) or on the periphery of the main rainforest block (to the east and the south) and occupy about 15% of the country (Peyrot et al., 2003; Maloba Makanga, 2011). The West Congolese forest-savanna mosaic is part of the Congolese rainforest. The savannas located on the Batéké plateaux and in the Nyanga (south-west Gabon) and Niari (south-west Gabon and Congo) basins have a strong affinity with the flora and fauna of the Zambezian savanna (Peyrot, 2008; King and Chamberlan, 2013). At the beginning of the 20th century, several species or sub-species of mammals otherwise strictly recorded in the Zambezian savannas were found in these Western Congolian savannas, e.g. *Redunca arundinum occidentalis Sylvicapra grimmia splendidula, Pelomys campanae,* and *Cercopithecus pygerythrus (aethiops) cynosuros.*

The question of the origin of these marginal ecosystems is also regional because a large part of these environments extends to the Republic of Congo and the Democratic Republic of Congo (DRC), including the left bank of the Congo River. The presence of these islands of savanna and peripheral savannas in Gabon and the Republic of Congo is rather surprising because the present climate favours forest habitats. The origin of these savannas has given rise to considerable discussion. Two main hypotheses have been proposed to explain their presence: a human origin (Elenga et al., 1994; Cornelissen, 2002; Bayon et al., 2012) or a paleoclimatic origin (Kouka, 2002; Peyrot et al., 2003; Ngomanda et al., 2005; Oslisly et al., 2013).

The hypothesis of a paleoclimatic origin has attracted considerable support. The savannas of Gabon, like all those of West Central Africa (Congo Basin) are natural savannas of paleoclimatic and edaphic origin (Maley, 2001; Peyrot et al., 2003; Oslisly et al., 2013). Data on vegetation changes in tropical West Africa over the last 150,000 years have been summarised by Dupont et al. (2000), and more detailed data about the last 70,000 years are available for Central Africa (Maley, 2001; Nieto-Quintano et al., 2018). They show that this landscape has often been much more open, and characterised by the presence of large savannas. These data highlight several successive phases of forest and savanna expansion and fragmentation, and the latest episode of savanna expansion is dating back to around 3,000-2,500 years before present (BP) (Maley, 2001; Walters et al., 2012). The rainforest expanded widely between 130,000-115,000, 107,000-95,000, 85,000-75,000 and 40,000-30,000 years BP, and from 12,000 years BP to the present day (except a short episode of catastrophic forest destruction *ca*. 2,500 years BP). The forest was strongly reduced between 145,000-130,000, 70,000-40,000 and 30,000-12,000 years BP when open, grass-rich vegetation prevailed (Maley, 2010).

Gabonese savannas are distributed in four distinct zones (Richard and Leonard 1993; Schwartz et al, 1995). They are found in stretches along the coastal border (Giresse et al., 2009) to the south-west (Coastal Basin), in larger blocks to the south (Ngounié/N'yanga) and south-east of the country (Batéké Plateaux), and in a series of small islands included in the central forest block (Lopé/Okanda). These savannas differ in their vegetation, soils, animal species, climate, and degree of geographic isolation.

Despite these differences, Gabon harbours a diversity of savanna landscapes suited for a comparative study of rodent communities. The diversity of small rodents in Gabonese savannas has been extensively documented (Mboumba et al., 2020; 2021). The present study is aimed at assessing the species distribution of the murid community in these four very different savannas in order to assess their possible relationships with the different savanna types and the biogeographic pattern of species distribution.

The history of the geographic distribution of small rodent communities is expected to be closely tied to that of the

Gabonese savanna landscape (Keita et al., 2021), considering i) the two classic hypotheses of a human or a paleoclimatic origin, and ii) the successive phases of relative forest or savanna expansion or fragmentation that occurred in this region. The history of the dynamics of the savannas of Gabon or West Central Africa has often been presented through observations of plant macrofossils, and archaeological, pedological, paleoenvironmental and human influences. Very little recent and fossil fauna data supports this hypothesis. Van Neer (1990) highlighted the existence of fossil mammalian fauna (7,000 years BP) and the current characteristics of savanna habitats. Therefore, strictly savanicolous animal species could be a complementary source of information to test hypothetical scenarios on the origin and history of savannas based on paleobiological and archaeological data (applied on fossil pollen).

The abundance, ease of capture and limited dispersal capacities of small mammals make them potentially reliable biogeographic markers for reconstructing the history of the regional Gabonese savanna landscape, provided that they are strongly linked to particular types of habitats and their phylogenic and phylogeographic histories and statuses are well documented. Mboumba et al. (2011) highlighted a singular vicariance between neighbouring populations and fragmentation events of small rodent species of the Gabonese savannas. These historical events have never been dated. Consequently, the main objectives of the present study are to i) analyse the composition and structure of small rodent communities from forest-embedded and peripheral savannas; ii) compare the phylogeography of the two most abundant species of small rodent communities (*Mus* and *Lemniscomys*); iii) identify areas of fragmentation of populations; and iv) in the event of fragmentation, identify the periods (dates) and determine whether they correspond to the periods of the great history of the landscape of West Central Africa, and try and reconstruct a finer history of Gabonese

savannas, based on molecular data so as to shed additional light on the origin of savannas.

MATERIALS AND METHODS
Study area

The sites were selected (Figure 1) to represent the following four different savanna types:

- the savannas of the Coastal Basin, situated in the south-west of the country, form a continuous and often narrow strip along the ocean coast, from Mayumba to Setté-Cama in the north. The climate is tropical, and annual rainfall is 1,850 mm on average. The mean annual temperature is 25-26°C. These savannas cover recent sedimentary soils and are generally sandy. They are generally herbaceous, shrubs are absent and they have a low plant species diversity mostly consisting of the following grasses: *Andropogon gabonensis*, *Anadelphia arrecta*, *Pobeguinea arrecta*, *Pleiadelphia gossweileri*, *Rynchelytrum* sp., *Nauclea latifolia*, *Bridelia ferruginea* and *Crossopteryx febrifuga*. Sampling was carried out in June 2004 in the locality of Mayumba (hereafter MYB: 03°27'S, 10°40'E) in the buffer zone of Mayumba National Park;

- the savannas of Ngounié/N'yanga in southern Gabon (in the Niari basin) form two corridors separated by forest. They are mostly grassy and also harbour a few wide-ranging shrub species. Shrubs are pioneer species highly resistant to fire (the dominant ones are *Hymenocardia acida, Anonna arenaria, Bridelia ferruginea*, and *Sarcocephalus esculentus*). The climate is of the equatorial transition type. Annual rainfall fluctuates from 1,600 mm to 2,000 mm (Dibakou et al., 2018). Temperature is more or less uniform throughout the year. The soils are shale-limestone (schisto-shale-calcareous). Sampling was conducted in April 2004 in the locality of Moukalaba (MKB: 02°16' S, 157 10°30'E) in the buffer zone of Moukalaba-Doudou National Park;

- the savannas of Lopé/Okanda, in Central Gabon, are embedded within the larger

rainforest block. The regional climate is a hot and humid equatorial type. Mean annual rainfall is 1,500 mm. Temperature varies little over the year, within ranges of 20-23°C in the dry season and 26-33°C in the rainy season. The geological substrate primarily consists of archean granitoids (Peyrot et al., 2003). The soils are ferrallitic type with a clay-sandy texture. The landscape is made up of a mosaic of forest and shrubby savanna or savanna interspersed with trees, hence highly varied landscapes. *Hyparrhenia diplandra*, *Anedelphia arrecta*, *Eleochari acutangula* and *Fuirena umbella* are found in the sampled zones. These savannas occasionally harbour shrubs of four species: *Crosopteryx febrifuga*, *Nauclea latifolia*, *Psidium guineensis* and *Bridellia ferruginea* (Mboumba et al., 2021). Three localities were sampled in this region: Mingenzi (MGZ: 00°05'S, 11°36'E), on the right bank of Ogooué river in the buffer zone of Lopé National Park (LNP); a second savanna inside LNP (00°04' S, 11°34'E), on the left bank of the Ogooué close to MGZ; and Kazamabika (KZM; 00°08'1S, 11°42'E), 20 km south-east of LNP, also on the left bank of the Ogooué, in the buffer zone of LNP. Sampling was conducted in July 2003 and May 2004;

- the savannas of the Batéké Plateaux, in south-eastern Gabon, constitute a mosaic of forests and savannas in the Franceville Basin. The Batéké Plateaux lie in an area of strong rainfall compared to much of south-western Gabon. Mean annual rainfall at Batéké Plateaux is 1,800-2,000 mm minimum and 2,000-2,250 mm maximum. This area has a transitional tropical climate (Ondo Ovono et al., 2014). The grassy flora is poorer than in Ngounié/N'yanga, but shrub species are the same, dominated by *Hymenocardia acida* (a fire-resistant shrub species), *Sarcocephalus latifolius*, *Bridelia ferruginea*, *Anona arenaria* and *A. senegalensis* (another fire-resistant shrub species). The area is essentially defined by its geology (Precambrian or Francevillian sedimentary), and poorer psammitic ferrallitic soils dominate (Mboumba et al., 2021). The soils are highly permeable. Sampling was performed in the locality of Mbaya, at two sites separated by the Ogooué river: on the right bank (MBD: 01°32'S, 13°29'E) and the left bank (MBG: 01°37' S, 13°20'E) of the river (figure 1), in July 2003 and May 2004. Additional sites were sampled in West Central Africa to better understand the relationships between Gabonese savannas and all the savannas of West Central Africa (Figure 1).

Rodent sampling and species identification

The sampling protocol is detailed in Mboumba et al. (2020). Studies on the structure of small mammal communities are dependent on adequate sampling of the whole community, which is strongly influenced by the capture technique. The line transect method was used. To minimise possible bias caused by different trapping techniques, three different trap types were used: pitfalls (using 10-L plastic buckets that are efficient for catching shrews), Sherman live traps (7.5 x 9 x 23 cm), and snap traps (10 x 15 cm; Mboumba et al., 2021). To maximise the probability to attract as diverse a suite of species as possible, two bait types were used: palm (*Elaeis guineensis*) nuts and manioc root, both of which are very attractive to most murid species (Mboumba et al., 2021).

All specimens were euthanised by cervical dislocation for further species identification, sexed, weighed and measured (total length, tail length and ear length). A muscle tissue sample was taken and preserved in 95% ethanol for complementary genetic study (Mboumba et al., 2011), and bodies were fixed in formalin 10%.

Data and statistical analyses

The rodent communities of each site were characterised by their species richness (S), the relative abundance of each species (P_i) and their species diversity. Species diversity at each site was calculated using the Shannon-Weaver index H'. Then, the two species that dominated the different populations of the small rodent communities of the Gabonese savannas were selected. A molecular analysis of these species was carried out, using

supplemental data from other localities in West Central Africa.

Molecular analyses

The central African rodents *Mus* and *Lemniscomys* are widely distributed across West Central Africa (and outside) and are found in a broad range of savanna habitats, making them well-suited to landscape-level studies of genomic variation. The phylogeographical study of these species was based on the analysis of partial mitochondrial DNA sequences of the cytochrome *b* (Cyt*b*) gene.

Specimens examined and mitochondrial DNA sequencing

One hundred and eighteen (118) individuals from two of the most abundant and most widely distributed species in the sampled savannas were sequenced (Figure 1). Eight hundred and thirty-eight (838) and 846 base pairs (bp) of the cytochrome *b* sequence were obtained from 52 and 66 *Lemniscomys striatus* and *Mus minutoides* individuals, respectively (Table 1). Using maximum-parsimony, maximum-likelihood, nested clade and genetic structure analysis, intraspecific relationships were inferred and the above-mentioned hypothesis was tested for the historical patterns of gene flow within these taxa. The mitochondrial DNA extraction, amplification and sequencing techniques are described in Mboumba et al. (2011). Sequence alignment for cytochrome *b* (Cytb) was non-problematic (no gap); 838-bp (*L. striatus*) and 846-bp (*M. minutoides*) portions of the gene were retained for the analysis. Three taxa (*Rattus norvegicus*, *L. rosalia* and *L. zebra*) were used as outgroups for rooting the *Lemniscomys* tree. Three more distant taxa (*Apodemus mystacinus*, *Apodemus sylvaticus* and *R. norvegicus*) were used as outgroups for rooting the *M. minutoides* tree.

Reconstruction of the evolutionary history of a lineage

L. striatus is characterised by a local structured pattern similar to that of *M. minutoides* (Mboumba et al., 2011). The evolutionary history of a line can be understood by analysing nested groups or by nested clade analysis (NCA; Posada et al., 2000), which uses three sources of information – geographic distribution (spatial data), phylogenetic structure, and allele frequency, incorporating principles from the coalescence theory.

The aim was to try and to obtain a molecular phylogeny of each species before embarking on phylogeographic analyses.

Phylogeographic analyses

Phylogeography (the combined analysis of gene genealogical data and geographic distribution) has become a powerful tool for inferring historical biogeographic events. In the present study, the phylogeography of the savanna species *M. minutoides* (66 individuals) and *L. striatus* (52 individuals) from 19 localities distributed across the study site was addressed. Using maximum-parsimony, maximum-likelihood, nested clade and genetic structure analyses, intraspecific relationships were inferred and hypotheses for historical patterns of gene flow within these two taxa were tested.

Time of divergence

Using the clades identified in the phylogenetic analysis, a Bayesian approach was applied to calculate the time to the most recent common ancestor (TMRCA) of all *L. striatus* and *M. minutoides* populations from the study sites. TMRCA can be used as a proxy for ancestral population age. Clades were dated using the BEAST v1.4.6 program (Mboumba et al., 2011). For this last analysis, two calibration points derived from paleontological data were used to estimate the time of divergence based on cytochrome *b* sequences, namely the *Mus*/*Rattus* lineage split estimated to have occurred 11-12.3 million years ago (Mya) (Benton and Donoghue, 2007), and the divergence between the 'big' *Apodemus mystacinus* and all the 'small' *Sylvaemus* (*A. flavicollis*, *A. sylvaticus*, *A. alpicola, A. uralensis*) estimated to have occurred 7 Mya (Michaux et al., 2005).
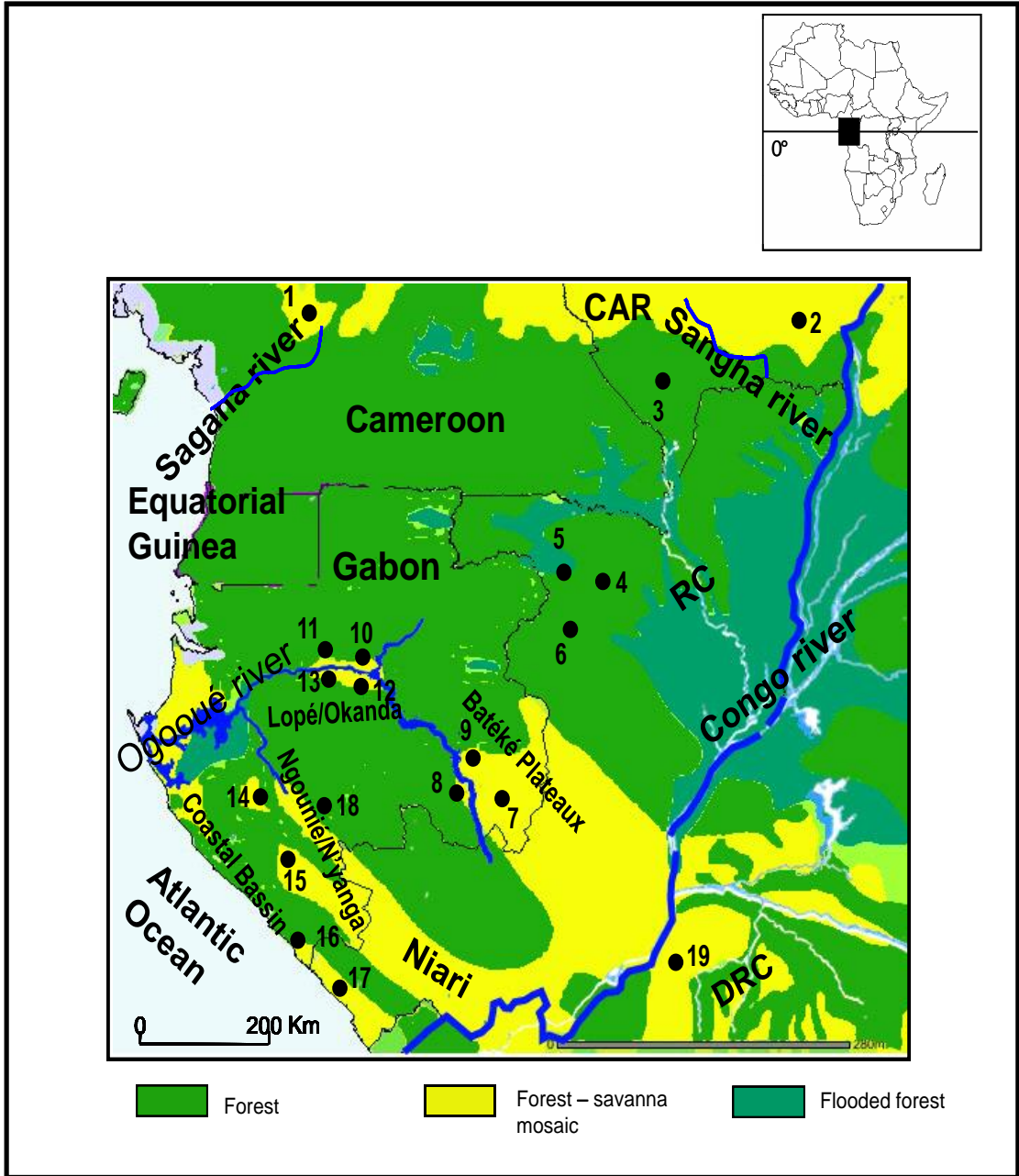
Figure 1. *Mus minutoides* and *Lemniscomys striatus* sampling sites (modified from Mboumba et al., 2011). **Cameroon:** 1, Nditam (NDI). **Central African Republic (CAR):** 2, Ngotto (NGO); 3, Salo (SAL). **Republic of Congo (RC):** 4, Illego site (SEI); 5, Mboko (MBK); 6, Mbomo (MBM); **Gabon:** Batéké Plateaux (south-eastern peripheral savannas) = 7, Franceville (FRA); 8, Mbaya, left bank of Ogooué river (MBG); 9, Mbaya, right bank of Ogooué river (MBD); Lopé/Okanda (forest-embedded savannas) = 10, Mingenzi (MGZ); 11, Mokekou (MOK); 12, Kazamabika (KZM); 13, Lopé (LOP); Ngounié/N'yanga (south-western peripheral savannas) = 14, Kili (KIL); 15, Moukalaba (MBK); Coastal Basin savannas = 16, Mayumba (MYB); 17 Tchissanga (TCH); 18, Petite savane (PET). Democratic Republic of Congo (DRC): 19, Kisangani (KIN).

Table 1. List of specimens with locations and haplotype codes. GenBank accession numbers of the *Mus minutoides* and *Lemniscomys striatus* specimens used in the molecular and phylogeographic analyses.

Species	Country	Locality	Locality codes	Number of specimens used	Haplotype codes	GenBank number
	Cameroon	Nditam	NDI	1	Ndi1	DQ789937
	Congo	Mbomo (Odzala)	MBM	3	MBM1-4	DQ789934 to DQ789936
		Mboko	MBK	2	MBK1-2	DQ789932 to DQ789933
		Illégo study site	SEI	3	SEI1-3	DQ789929 to DQ789931
		Franceville	FRA	3	FRA1-3	DQ789906 to DQ789908
		Lopé	LOP	6	LOP1-6	DQ789944 to DQ789949
		Mbaya, right bank of Ogooué river	MBD	6	MBD 1-6	DQ789909 to DQ789914
Mus minutoides		Mbaya, left bank of Ogooué river	MBG	4	MBG1-4	DQ789940 to DQ789943
	Gabon	Kazamabika, left bank of Ogooué river	KZM	5	KZM1	DQ789950 to DQ789954
		Mayumba	MYB	6	MYB1-6	DQ789959 to DQ789964
		Mokékou	MOK	7	MOK1-7	DQ789922 to DQ789928
		Mingenzi, right bank of Ogooué river	MGZ	7	MGZ1-7	DQ789915 to DQ789921
		Kili	KIL	4	KIL1-4	DQ789955 to DQ789958
		Moukalaba	MKB	7	MKB1-7	DQ789965 to DQ789971
	CAR	Ngotto	NGO	2	SAL1-2	DQ789938 to DQ789939
	CAR	Ngotto	NGO	1	NGO1	DQ537285
		Salo	SAL	2	SAL1-2	DQ537267 to DQ537268
	Congo	Illégo study site	SEI	6	SEI1-6	DQ537257 to DQ537260
		Mbomo (Odzala)	MBM	2	MBM1-2	DQ537255 to DQ537256
		Tchissanga	TCH	1	TCH1	AF141210
	DRC	Kinsagani	KIN	2	KIN1-2	DQ537310 to DQ537311
		Abeilles forest (Petite Savane)	PET	3	PET1-3	DQ537264 to DQ537266
		Franceville (left bank of Ogooue river)	FRAg	2	FRAg1-2	DQ537243 to DQ537244
		Franceville (right bank of Ogooué River)	FRAd	3	FRAd1-3	DQ537245 to DQ537247
		Kazamabika, left bank of Ogooué river	KZM	4	KZM1-4	DQ537228 to DQ537231
Lemniscomys striatus		Malounga	MAL	1	MAL1	DQ537254
	Gabon	Mingenzi	MGZ	6	MGZ1-6	DQ537232 to DQ537237
		Mbaya (left bank of Ogooue River)	MBG	2	MBG1-2	DQ537241 to DQ537242
		Mbaya (right bank of Ogooué River)	MBD	6	MBD 1-6	DQ537248 to DQ537253
		Mokékou	MOK	3	MOK1-3	DQ537238 to DQ537240
		Moukalaba	MKB	8	MKB1-8	DQ537269 to DQ537276

RESULTS
Species richness and relative abundance

No new endemic species was found. Gabonese savannas harbour six species of Muridae overall (two to five species in the different savanna types). All these species had a wide geographic distribution outside Gabon. Species richness was relatively high in the Ngougnié/N'yanga savanna (south-western peripheral savannas: five species), followed by Batéké Plateaux (south-eastern peripheral savannas) and Lopé/Okanda savannas (forest-

embedded savannas) (four species each), and was very low in the Coastal Basin savannas.

Species composition of the small rodent communities of the four savanna types

The dominant species in Gabonese savannas was *M. minutoides,* followed by *L. striatus*, *Lophuromys sikapusi* and *Pelomys campanae*. This ranking was the same across all sampled localities.

Species composition and diversity indexes according to the savanna type

The small rodent community structure differed in the various habitats, and followed four distribution types: (i) *M. minutoides* was common to all four savanna types; (ii) two species (*L. striatus* and *L. sikapusi*) occurred in three savanna types; (iii) *P. campanae* had austral affinities from Zambesian savannas and occurred in all three southern savanna types; (iv) two locally rare species widely distributed in West Central Africa were only found at one locality: *D. cf. mystacalis* was found in Ngougnié/N'yanga savannas, and *Mastomys* sp. in Lopé/Okanda. Overall, diversity indexes were low in all four savanna types, with the highest value at Ngounié/N'yanga (H' =1.2), followed by Batéké Plateaux, and the lowest values at Lopé/Okanda and at the Coastal Basin site.

Phylogeographic analysis and genetic diversity

The coalescence theory predicts that haplotypes are more likely to be connected to haplotypes from the same locality than to haplotypes occurring in distant populations. NCA revealed fragmentation events in the *L. striatus* and *M. minutoides* populations (Figures 2 and 3). Fifteen different haplotypes were identified within the *M. minutoides* sample out of 66 individuals from 10 Gabonese localities (Figure 2A). The whole network of haplotypes included three groups, namely i) a group of haplotypes of populations composed of individuals from different peripheral savannas of the south and southwest (clade 1-5); ii) a group of

haplotypes of populations from peripheral savannas of the south-east (Batéké Plateaux, clade 2-3); iii) a group of haplotypes of the populations of central Gabon (Lopé/Okanda; clade 1-2). Locally, the populations from the Lopé/Okanda area (five derived haplotypes) and the Batéké plateaux (four derived haplotypes) were genetically more diverse than the populations of the southwestern peripheral savanna (one derived haplotype).

Chi-squared analysis identified three nested haplotype groups significantly associated with geography (clades 2-2, 2-3 and 3-2; Figure 2A). Contiguous range expansion was inferred within clade 2-3 (Batéké Plateaux). Within clade 3-2, allopatric fragmentation was inferred between clade 2-2 (restricted to KIL, KZM, LOP, MBK, MGZ, MKB, MOK, MYB, NDI, NGO and SEI) and clade 2-3 (restricted to FRA, MBD and MBG). However, restricted gene flow with isolation by distance was found within clade 1-5.

Twenty-one haplotypes were found among *L. striatus* individuals (Figure 2B). Sixteen haplotypes were identified out of 38 individuals from eight Gabonese localities. They belonged to two major groups: a group of haplotypes of populations from peripheral savannas of the south-east (Batéké Plateaux + MOK; clade 2-1) and a group of haplotypes of populations composed of individuals from different peripheral savannas of the south, southwest + the Lopé/Okanda sector (clade 2-3). The haplotypes of the south-west + Lopé complex were very diversified (10 derived haplotypes: Figure 2B). At a local scale, haplotype diversity of *L. striatus* was higher in the peripheral savannas of the southeast (nine derived haplotypes), followed by those of the savannas of Lopé/Okanda (seven derived haplotypes) and finally the peripheral savannas of the southwest (three derived haplotypes).

Chi-squared analysis identified two major groups of nested haplotypes significantly associated with geography (clades 2-1 and 2-3), and a fragmentation event was observed (clades 3-1 and 3-2).

Time of divergence

Based on the *M. musculus/R. norvegicus* calibration (estimated to have occurred approximately 12 Mya), the fragmentation events of the haplotype clades corresponding to coherent geographic areas were dated (Figure 3). *M. minutoides* from Lopé/Okanda region was estimated to have diverged from all the other peripheral southwestern and southern savanna populations (savannas of the Coastal Basin and Ngounié/N'yanga) about 82,000 years ago. Fragmentation between the Batéké Plateaux populations and populations from these regions was estimated to have occurred 88,000 years BP. In this same region, a population fragmentation event between the localities was estimated to have occurred 92,000 years BP (Figure 3A). The structure of the network of haplotypes provided a glimpse of a succession of events: the Batéké Plateaux (southeast) population first isolated itself from the populations of all the other savannas, and then from the Lopé/Okanda sector. The dating results confirmed a divergence of the southeast (Batéké plateaux) populations before the divergence of the Lopé/Okanda savanna populations from those of the southwestern peripheral savanna populations (88,000 years *versus* 82,000 years BP) (Figure 3A).

L. striatus dating showed that the Batéké plateaux populations diverged from the other Gabonese savanna populations (south-western peripheral savannas and the central Lopé/Okanda savanna) about 100,000 years ago (Figure 3B).

The phylogeographic patterns of *M. minutoides* based on the *A. mystacinus/A. sylvaticus* dichotomy (estimated to have occurred approximately 7 Mya; Aguilar and Michaux, 1996) was also dated to compare the succession of population fragmentation events with that of the *Mus/Rattus* dichotomy. The results showed that the Lopé/Okanda populations diverged from those of the southern and southwestern peripheral savannas about 50,000 years BP. In contrast, the divergence of the Batéké plateaux populations from those of the complex of peripheral savannas of the south and southwest + Lopé/Okanda was dated back to 55,000 years BP.
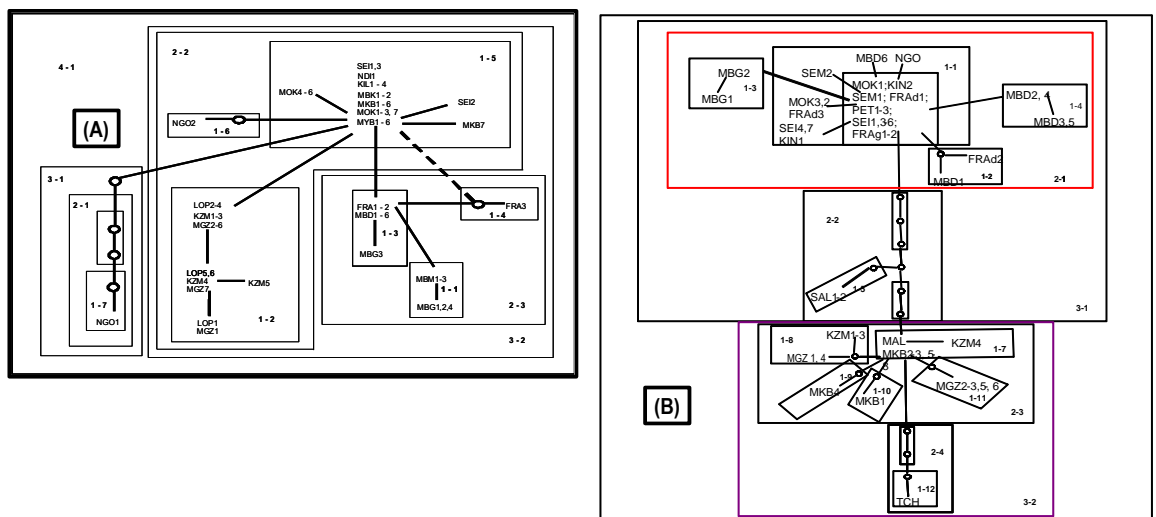

Figure 2. *Mus minutoides* (A) and *Lemniscomys striatus* (B) haplotype network - Cladogram expressing the genetic distance between individuals based on Cyt*b*. Letters, locations from which the haplotypes originate; numbers, numbers of individuals (see Table1). Bold-type figures, numbers of clades and sub-clades.
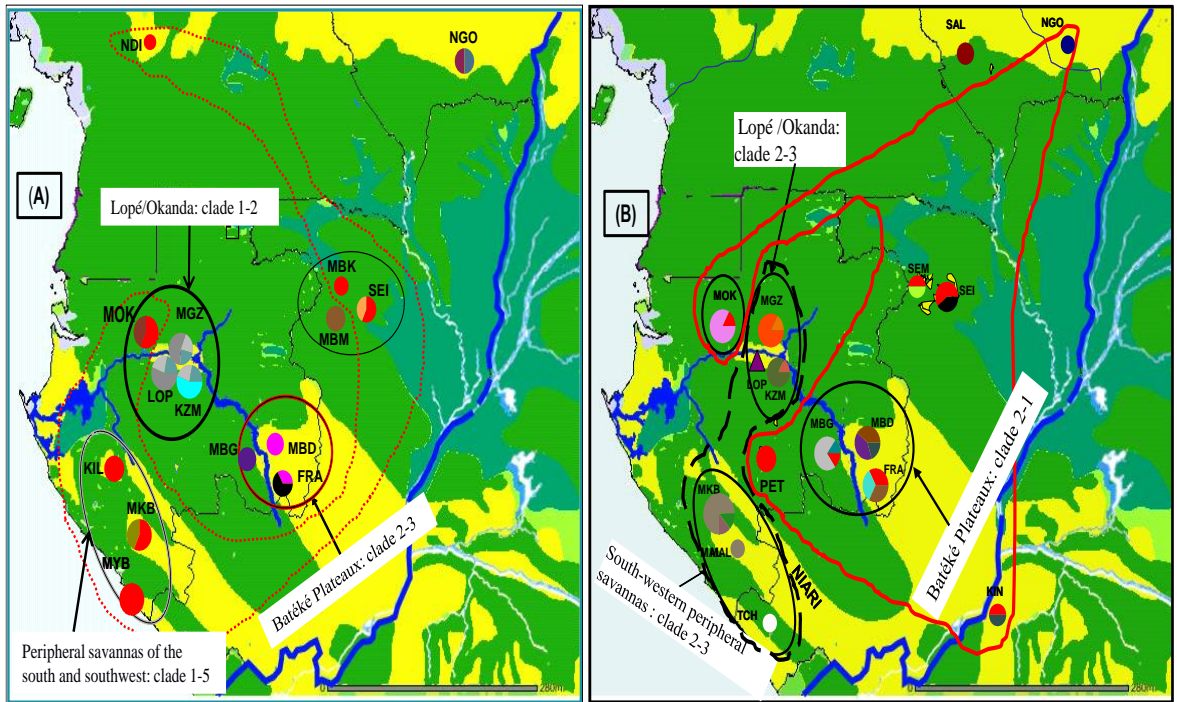
Figure 3. Map showing the geographic distribution of *M. minutoides* (A) and *L. striatus* (B) haplotype clades. Each population is represented by a plain circle whose diameter is proportional to the number of sampled individuals; each haplotype is figured by a differently coloured sector, whose size is proportional to the haplotype frequency among the sampled individuals.

DISCUSSION

Data about the structure of small rodent savanna communities in Gabon illustrate the low species richness of Gabonese savannas. The highest diversity was recorded in peripheral savanna areas (south-western and south-eastern peripheral savannas). *M. munitoides* and *L. striatus* were the two most represented species, also found in all the savannas of West Central Africa. This result revealed an old connection between the Gabonese savannas and the other savannas of West Central Africa. The data also revealed a species with austral affinities known from Zambesian savannas: *Pelomys campanae* was present only in the savannas of southern (Ngounié/N'yanga) and southeastern (Batéké Plateaux) Gabon. This result may be due to i) an ancient contact between the southern Gabon savannas and the more austral ones, or ii) recent colonisation via an ancient savanna

corridor located between the southern savannas of Gabon and the austral ones. This last assumption would imply that this species crossed the Congo River. This last hypothesis should be taken with caution because rivers can be major obstacles to the distribution of mammalian fauna. This first analysis was supplemented by an analysis of the molecular data of two main species of small rodent communities of the Gabonese savannas and West Central Africa.

Genetic diversity

The Lopé/Okanda sector displayed similar haplotypic diversity to the other two large savanna sectors. The strong genetic diversity of the Lopé/Okanda sector was expressed by barely different haplotypes (one to two mutations between populations); three haplotypes out of four were common on both sides of the Ogooué, suggesting gene flow

between the savannas of each riverside. Locally, the savanna populations from the Lopé/Okanda area (five derived haplotypes) and Batéké plateaux (four derived haplotypes) were genetically more diversified than the populations of the southwestern peripheral savanna. This result suggested the presence of several forest patches that divided savannas and isolated populations of small savanna rodents in these two regions and prevented contact between populations. This result is accordance with Maley (2010) and Maley et al. (2018). On a local scale, the number of derived haplotypes in these same regions increased with *L. striatus* (nine derived haplotypes for Batéké plateaux, seven for the Lopé/Okanda sector, and three for the southwestern peripheral savannas). This result confirmed a pattern already observed in *M. minutoides* populations. It also confirmed that the peripheral savannas of the southwest were very little affected by forest transgression.

Phylogeographic and paleoenvironmental analyses

The taxa used to calibrate the molecular clock diverged much earlier than the taxon under study. Therefore, the substitution rate may have been underestimated and divergence times overestimated because of a certain amount of saturation of the substitutions. In agreement with this hypothesis, divergence time estimates based on the split between *Mus* and *Rattus* were constantly higher than those based on the split between *A. mystacinus* and *A. sylvaticus*. Therefore, the values should be interpreted cautiously and only viewed as preliminary hypotheses of the timing of important events in the history of the species.

The results showed fragmentation events among the populations of these two species (*L. striatus and M. minutoides*), leading to isolation of the populations of the Lopé/Okanda savannas (in the center of Gabon) from those of peripheral savannas. This result indicated the presence of a biogeographic barrier or forest expansion events in this region. In addition, no new endemic species was found despite the isolation of these savannas (Lopé/Okanda). This indicates that the geographic isolation of these savannas did not last long enough for the speciation of new taxa to occur.

Two fragmentation events were observed for *M. minutoides* populations: one between the Lopé/Okanda localities (LOP-KZM-MGZ: clade 1-2) and the peripheral localities (KIL, MKB, MOK, MYB; clade 1-5), and one between the Batéké Plateaux localities (clade 2-3) plus the complex of western and southern peripheral localities (MOK-MKB-MBK-MYB-SEI-NDI-KIL; clade 1-5) and Lopé/Okanda savannas (clade 1-2); this result points to several events of forest expansion that separated central Gabon savannas from the southern peripheral savanna. For *L. striatus* populations, data also showed a fragmentation event between the complex of western and southern peripheral localities (MAL-MKB and TCH) + Lopé/Okanda savannas (KZM and MGZ; clade 3-2) and Batéké Plateaux (clade 3-1). This result suggested persistent biogeographic barriers between central Gabon and the southern peripheral savanna complex. However, it is estimated that fragmentation occurred 80,000-90,000 and 100,000 years ago for *M. minutoides* and *L. striatus*, respectively. These results are in accordance with literature data according to which the rainforest was much more widespread than it is now 130,000-115,000, 107,000-95,000, 85,000-75,000 and 40,000-30,000 years BP (Dupont et al., 2000; Maley, 2001; Nieto-Quintano et al., 2018). This may have contributed to the fragmentation of the savannas and in turn of *M. minutoides* and *L. striatus* populations. During the last 150,000 years, several phases of forest expansion and fragmentation occurred in West Central Africa, and these changes affected the genetic structure of species. These new results further support the scenario of historical and ongoing fragmentation of the local savanna landscape that started in the upper Pleistocene, despite repeated savanna expansion episodes as documented by the landscape history.

In Batéké Plateaux, contiguous range expansion of *M. minutoides* populations was observed between the localities of this region and dated back to 92,000 years BP (*Mus/ Rattus*). At these periods the rainforest had expanded across West Central Africa (Dupont et al., 2000; Maley, 2001; 2010; Nieto-Quintano et al., 2018). This phylogeographic scheme appears to be at odds with literature results showing concomitant forest expansion. An explanation for this event might come from the end of the climate crisis in West Central Africa during which the reconstitution of primary forest was not synchronous (or the savannas never all disappeared, even during the most humid episodes of the last 40,000 years). This hypothesis is in agreement with Maley (2001). The calibration bias may also lead to suspect overestimated dates (Ho et al., 2005). On the other hand, contrary to the dating based on *Mus/Rattus*, the dating based on the *A. mystacinus/A. sylvaticus* dichotomy was 58,000 years BP for this same event. This latter result is in agreement with literature results showing that the rainforest was strongly reduced in West Central Africa at these periods (Dupont et al., 2000; Maley, 2001; 2010), and possibly favoured species dispersal. However, calibration based on the more distant groups *Mus* and *Rattus* provided a 1.6-fold older estimate. This corresponds exactly to the criticism of Ho et al. (2005) that calibration based on ancient fossils will tend to overestimate the age of divergence. Therefore, reservations can still be expressed about a possible overestimation. The latest results of *M. minutoides* population dating based on the *A. sylvaticus/A. mystacinus* dichotomy revealed two dates differing from the date revealed by the *Mus/Rattus* dichotomy, i.e. divergence of the Lopé/Okanda populations from those of the southern and western southern peripheral savannas 50,000 years BP; then, divergence between the Batéké Plateaux populations and those of the complex formed by the southern and south-western peripheral savannas + Lopé/Okanda occurred 70,000 to 40,000 years BP. However, according to literature data (Dupont et al., 2000; Maley, 2010), savannas were widely distributed in West Central Africa between 70,000 and 40,000 years BP, so it is unlikely that a fragmentation event of the populations of strictly savanicolous species (*M. minutoides* and *L. striatus*) occurred during this period. Two explanations are possible for this: i) despite savanna expansion, the forest never disappeared, forest islands persisted and prevented savannas being in contact at a continental or even a local scale, and ii) dating was overestimated. However, regardless of the marker (*Mus/Rattus* or *A. mystacinus/A. sylvaticus*) used to date *M. minutoides* population fragmentation events, the results showed an identical scenario: the populations of the Batéké Plateaux savannas diverged from the populations of the southern and south-western peripheral savannas first, followed by rodent populations from Lopé/Okanda. Therefore, these events were not synchronous.

Conclusion

The forest-embedded savannas of Lopé/Okanda harbour a low specific diversity of small rodents (Muridae) but a high genetic diversity. In terms of origin, all the results of the present study support the hypothesis of an ancient origin of the West Central African savannas. The phylogeographic history of those species is congruent with current knowledge about the successive phases of savanna expansion and fragmentation. It would be interesting to undertake a comparative study of several co-distributed taxa other than small terrestrial rodents to determine if their phylogenetic history is similar. Combining results from several taxa should provide a better documented biogeographic history of this region. However, the savannas of the study area can be considered as natural environments that host a spontaneous fauna: the savannas of Gabon are natural savannas of paleoclimatic and edaphic origin, like all of the savannas of Central Africa (Congo Basin).

COMPETING INTERESTS

The authors declare that there is no competing interest.

AUTHORS' CONTRIBUTIONS

The authors have accepted responsibility for the entire content of this submitted manuscript and approved submission; individual contributions: 90% for JFM and 10% for VG.

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