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Mitochondrial and Nuclear Genes-Based Phylogeography of *Arvicanthis niloticus* (Murinae) and Sub-Saharan Open Habitats Pleistocene History

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Abstract

A phylogeographic study was conducted on the Nile grass rat, *Arvicanthis niloticus*, a rodent species that is tightly associated with open grasslands from the Sudano-Sahelian regions. Using one mitochondrial (cytochrome b) and one nuclear (intron 7 of Beta Fibrinogen) gene, robust patterns were retrieved that clearly show that (i) the species originated in East Africa concomitantly with expanding grasslands some 2 Ma, and (ii) four parapatric and genetically well-defined lineages differentiated essentially from East to West following Pleistocene bioclimatic cycles. This strongly points towards allopatric genetic divergence within savannah refuges during humid episodes, then dispersal during arid ones; secondary contact zones would have then stabilized around geographic barriers, namely, Niger River and Lake Chad basins. Our results pertinently add to those obtained for several other African rodent as well as non-rodent species that inhabit forests, humid zones, savannahs and deserts, all studies that now allow one to depict a more comprehensive picture of the Pleistocene history of the continent south of the Sahara. In particular, although their precise location remains to be determined, at least three Pleistocene refuges are identified within the West and Central African savannah biome.

Introduction

Current climatic changes and their consequences on the evolution of biodiversity are a hotly debated topic with sometimes highly divergent predictive scenarios [1–6]. In most groups, long-term surveys are still lacking to provide robust predictions under various potential scenarios. In such a context, though at a very different scale, the reconstruction of past eco-climatic modifications may provide helpful pieces of information. In particular, when interpreted through available paleo-environmental frameworks, phylogeographic studies of habitat-specialist species can join the cortege of data that is necessary to draw the complete picture [7,8]. Even more instructive is the comparison of phylogeographic patterns obtained in several species sharing the same biogeographic regions [9–11].

In Africa, a majority of phylogeographic investigations have been conducted on large mammals (see references below; review in [10]). Yet, the latter are now usually confined to protected areas where recent demographic and genetic effects may mask signatures of more ancient evolutionary events. In contrast, rodents appear as excellent alternative phylogeographic markers owing to their usually strong affinities to particular ecological niches and wide geographic distribution, their short generation time, their weak dispersal abilities as well as their small size that may render them highly sensible to geographic barriers such as mountains or large rivers, thus limiting admixture between allopatrically differentiated lineages. Altogether, these characteristics are expected to greatly facilitate detection of phylogeographic patterns. Moreover, most of rodent species being abundant and non-protected (when not pest organisms), they are relatively easy to sample, thus facilitating the gathering of datasets that are appropriate for phylogeographic/population studies. This may explain why all African species investigated so far led to strong phylogeographic signals, as well as remarkable congruence between sometimes highly divergent taxa. Indeed, data are now available for rodent species inhabiting arid zones (*Jaculus jaculus*: [12]), steppes, shrub and tree savannahs (*Lemniscomys striatus*: [13]; *Praomys daltoni*: [14]; *Mastomys erythrocephalus*: [15,16]; *Mastomys natalensis*: [17]), rocky habitats (*Acomys chabaudi*: [18]), humid areas (*Mastomys huberti*: [19]), forest-savannah mosaics (*Mus (Nanomys) minutoides*: [20] and forests (*Praomys rostratus* and *P. tullbergi*: [21]; *P. misonner*: [22,23]).

As part of this process, we here focused on the Nile grass rat, *Arvicanthis niloticus* (*Muridae*, *Murinae*). This species ranges all along the Southern limit of the Sahara desert, throughout the Sahelian and Sudanian regions, from the Atlantic to the Indian Oceans [24,25]. As such, *A. niloticus* biogeographic evolution is expected to provide interesting insights on the effects of past eco-climatic changes on sub-Saharan steppes and savannahs. The genus *Arvicanthis* contains seven currently recognized species [24], all found south of the Sahara desert, from the Atlantic coast in Senegal to Ethiopia, and down south to Zambia, with the
exceptions of populations along the Nile Valley in Egypt, south west of the Arabian Peninsula, and a mention from the Hoggar mountains, south east Algeria [24,25]. Many of them represent sibling species whose boundaries and phylogenetic relationships have essentially been resolved thanks to cytogenetic and molecular studies [26–33]. Nevertheless, systematic investigations are still required, particularly for several East African taxa (see [32], and below) as well as other ambiguous taxonomic units (e.g. ANI-2 and ANI-4; see [34]).

The Nile grass rat *Arvicanthis niloticus* is a well-defined species characterized by a 2N = 62/number of autosomeal arms (NFa) = 62–64 karyotype ([26,28–30]. This species exhibits the widest distribution within the genus [24]. It is rather generalist and inhabits steppes, savannahs as well as humid zones and human-modified biotopes (e.g. villages, gardens, rice fields, sometimes within cities) of the Sahelian and Sudanian bioclimatic zones where it is considered a major agricultural pest [25]. *Arvicanthis niloticus* has also been shown to carry a number of pathogens, such as *Leishmania major* spp. [38], *Schistosoma* spp. [39], *Leptospira* spp. [37], *Rickettsia* spp. [38], *Shistosoma* spp. [39] or *Toxoplasmagondii* [40]. Yet, our understanding of evolutionary patterns in the Nile grass rat still relies on very scarce data, and no wide scale survey has been performed on this species to date. Previous cytogenetic studies have suggested the possible existence of two lineages, a West African one (ANI-1a, sensu [30]) and another Central and East African one (ANI-1b) whose karyotypes would differ by a pericentric inversion on pair 30 (N Fa = 62 and 64, respectively; [30]). Recently, Abdel Rahman et al. [33] proposed some preliminary hypotheses about *A. niloticus* phylogeography and demogenetics using only mitochondrial DNA (mtDNA) sequence data. Unfortunately, their study was based on only 26 specimens, 23 of which were sampled in Sudan, making their results likely biased and uninformative at a wider scale.

In order to fill this gap, we performed a comprehensive phylogeographic survey of *A. niloticus* based on a sample that covers all the species range. In contrast with previous phylogeographic studies dealing with West and Central African rodents (see above), we used both a mitochondrial (cytochrome b) and a non-translated part of a nuclear (Beta Fibrinogen intron 7) coding genes to infer the evolutionary history of our species of interest. We also implemented more thorough time calibration analyses, by using independent set of constraints based either on fossil data or on secondary calibrations. Finally, our results are discussed in regards to those previously obtained for other rodent as well as non-rodent species in this part of Africa.

**Materials and Methods**

**Sampling Effort**

In total, 105 individuals of *A. niloticus* and two *A. ansorgei* individuals were trapped during various field surveys conducted in seven countries (Burkina-Faso, Cameroon, Chad, Mali, Niger, Mauritania and Senegal; Table 1). Organs or hind feet digits were conserved in ethanol until use in the laboratory. In addition, fibroblast cell pellets from one *A. niloticus* specimen from Egypt and two *A. cf. niloticus* individuals from Kenya were kindly provided by V. Volobouev (Museum National d’Histoire Naturelle, Paris, France). Four ethanol-preserved samples of *A. niloticus* from Sudan (courtesy of E. Abdel Rahman, Natural Science Museum, Durban, South Africa) and one *A. ansorgei* from Burkina-Faso (courtesy of M. Deniaux) were also available. All these specimens (115 in total) were sequenced for the mitochondrial cytochrome b gene (cytb), while some representatives were selected among them for the intron 7 of the nuclear gene Beta Fibrinogen (Fib7) sequencing (see details below). In addition, 54 cytb sequences were downloaded from GenBank, among which 34 *A. niloticus* representatives (one from Egypt, one from Senegal, two from Niger, seven from Cameroon and 23 from Sudan), nine representatives of other *Arvicanthis* species as well as 11 representatives of other genera (*Aethomys, Desmonomys, Galunda, Mylomys, Otomys, Pelomys*, and *Rhabdomys*). For the fib7 dataset we also used a sequence from a specimen of *Mastomys erythrocephalus* that was trapped in Mali. Finally, one *Lemniscomys* cytb sequence was kindly provided by V. Nicolas (Museum National d’Histoire Naturelle, Paris, France). The rationale here (see also the *Phylogenetic and dating analyses section for details*) was: (i) to have a dense sampling of *Arvicanthis* species in order to properly enforce the corresponding fossil constraint; (ii) include species used in previous studies within *Arvicanthis* and *Murinae* [41,42] in order to perform secondary calibrations. Associated voucher specimens are deposited in the collections of the Centre de Biologie pour la Gestion des Populations (CBGP, France), Muséum National d’Histoire Naturelle (MNHN, France) and Durban Natural Science Museum (DM); they can be recovered using numbers provided in Table 1.

**Ethics Statement**

Each trapping campaign was validated by national and local authorities. At the French level, all sampling procedures were conducted by biologists from the IRD holding a certificate to carry out experiments on live animals (‘certificat d’autorisation à expérimenter sur animaux vivants’; agreement number C34-106, valid until December, 16th 2016). The CBGP joint research unit is also holding an agreement to conduct experiments on live animals (‘établissement agréé pour expérimenter sur animaux vivants’; agreement number C34-169-1, valid until July, 25th 2017). Within each country where sampling was performed, research was systematically made possible thanks to extant conventions between the IRD and local governments (see the regional sections as well as IRD Ethical Guidelines on the IRD website: www.ird.fr). Additional authorizations were not required because *Arvicanthis* species are considered as pest species (especially *A. niloticus*) and have no protected status (see IUCN and CITES lists). At the local level, traps were systematically set only after the agreement of the village head and the field owner was explicitly obtained. Moreover, in cultivated fields, traps were always placed on the edge of the exploited area, so that no damage could be cause to crops. Nile rats were caught alive in wire-mesh and Sherman traps. All animals were euthanized by cervical dislocation. Animals were treated in a humane manner, and in accordance with guidelines of the American Society of Mammalogists [43]. No ethical country-specific agreement could be obtained since countries where sampling occurred and the IRD have no ethics committee that oversees animal experimentation.

**DNA Extraction and Sequencing**

Total genomic DNA was extracted using the Puregene DNA Purification Kit (Genta Systems). The complete cytb mitochondrial gene was then amplified using primers H15915 and L14123 following procedures detailed in Lecompte et al., [44]. The resulting PCR products were purified and then sequenced in both directions on a MegaBACE 1000 (Amersham Biosciences). Sequences were carefully checked by eye, and aligned using the ClustalW Multiple Alignment option implemented in BioEdit v.7.0.4.1. In total, our cytb dataset includes 169 sequences encompassing 146 *A. niloticus*, two *A. cf. niloticus*, 11 other *Arvicanthis* spp. and 10 representatives of other rodent genera (Table 1). The complete non translated intron 7 of the Fibrinogen nuclear gene was amplified using primers BFIBR1 and BFIBR2, following
| Individuals and species | Origin | Lat. | Long. | cyt b | Fib7 | Labels |
|------------------------|--------|------|-------|-------|------|--------|
| *Arvicanthis niloticus* |        |      |       |       |      |        |
| GenBank: AF004568.1*    | Ethiopia, Koka | 08' 26 N | 39' 02 E | x   | Eth 1 |
| CBGP: M5659, M5678      | Burkina-Faso, Niassan | 13' 07 N | 03' 26 W | x   | x   | BF 1,2 |
| GenBank: HM635824       | Cameroon, Gamnaga | 10' 57 N | 14' 03 E | x   | Cam 1 |
| GenBank: HM635825       | Cameroon, Kongola | 10' 37 N | 14' 25 E | x   | Cam 5 |
| GenBank: HM635826       | Cameroon, Kongola | 10' 37 N | 14' 25 E | x   | x   | Cam 4 |
| GenBank: HM635827; CBGP: C344, C345, C352, C353 | Cameroon, Maga | 10' 50 N | 14' 57 E | x   | Cam 2, 6–9 |
| CBGP: C358, C374; GenBank: HM635835, HM635836 | Cameroon, Maga | 10' 50 N | 14' 57 E | x   | x   | Cam 10–13 |
| MNHN: VV1998-060       | Chad, Farcha | 12' 06 N | 15' 03 E | x   | x   | Cha 5 |
| MNHN: VV1998-061; VV1999-213 | Chad, Farcha | 12' 06 N | 15' 03 E | x   | Cha 7,8 |
| MNHN: 2000-060         | Chad, Goz Djerat | 10' 59 N | 19' 55 E | x   | x   | Cha 4 |
| MNHN: 2000-061         | Chad, Goz Djerat | 10' 59 N | 19' 55 E | x   | Cha 6 |
| CBGP: M4134            | Chad, Zakouma NP | 10' 44 N | 19' 40 E | x   | x   | Cha 1 |
| CBGP: M4159, N2085     | Chad, Zakouma NP | 10' 44 N | 19' 40 E | x   | Cha 2,3 |
| MNHN: VV1995-073       | Egypt, breeding colony | ?   | ?   | x   | x   | Egy 1 |
| GenBank: AF004569      | Egypt, breeding colony | ?   | ?   | x   | Egy 2 |
| CBGP: M5240            | Mali, Abeibara | 19’ 01 N | 01’ 45 E | x   | x   | Mal 6 |
| CBGP: KM0139           | Mali, Ansongo | 15’ 40 N | 00’ 30 E | x   | Mal 29 |
| CBGP: M5647            | Mali, Bintagoungou | 16’ 44 N | 03’ 44 W | x   | x   | Mal 9 |
| CBGP: M4060            | Mali, Boulou | 15’ 11 N | 09’ 31 W | x   | x   | Mal 1 |
| CBGP: M4189            | Mali, Dialo | 14’ 28 N | 11’ 30 W | x   | x   | Mal 3 |
| CBGP: M5645            | Mali, Dianké | 15’ 45 N | 04’ 39 W | x   | x   | Mal 25 |
| CBGP: M4260            | Mali, Dirimbé | 15’ 01 N | 02’ 54 W | x   | x   | Mal 15 |
| MNHN: VV1999-054      | Mali, Edjerir | 18’ 12 N | 01’ 24 E | x   | Mal 13 |
| MNHN: VV1999-046      | Mali, Edjerir | 18’ 12 N | 01’ 24 E | x   | x   | Mal 10 |
| CBGP: M5385            | Mali, Emmalhere | 14’ 28 N | 04’ 05 W | x   | x   | Mal 23 |
| CBGP: M4605            | Mali, Farabougou | 14’ 53 N | 06’ 08 W | x   | x   | Mal 17 |
| CBGP: M4675            | Mali, Farabougou | 14’ 53 N | 06’ 08 W | x   | Mal 19 |
| CBGP: M4680            | Mali, Gono | 15’ 03 N | 02’ 47 W | x   | x   | Mal 20 |
| CBGP: M4771            | Mali, Gono | 15’ 03 N | 02’ 47 W | x   | Mal 21 |
| CBGP: M5649            | Mali, Goubolobo | 15’ 54 N | 03’ 59 W | x   | x   | Mal 26 |
| CBGP: M4065            | Mali, Makana | 15’ 08 N | 09’ 26 W | x   | x   | Mal 2 |
| MNHN: VV1999-076      | Mali, Ménaka | 15’ 55 N | 02’ 25 E | x   | x   | Mal 14 |
| CBGP: M5367            | Mali, Niono | 14’ 17 N | 05’ 59 W | x   | x   | Mal 22 |
| CBGP: KM01081         | Mali, Ouattagouna | 15’ 12 N | 00’ 42 E | x   | x   | Mal 28 |
| CBGP: M5639            | Mali, San | 13’ 22 N | 04’ 56 W | x   | x   | Mal 24 |
| CBGP: M4209            | Mali, Sare Mama | 14’ 53 N | 04’ 02 W | x   | x   | Mal 4 |
| CBGP: M4934            | Mali, Sare Mama | 14’ 53 N | 04’ 02 W | x   | Mal 5 |
| CBGP: M5682            | Mali, Tanda | 15’ 46 N | 04’ 38 W | x   | x   | Mal 27 |
| MNHN: VV1999-049; VV1999-070 | Mali, Tararabat | 19’ 24 N | 01’ 14 E | x   | Mal 11,12 |
| CBGP: M5265            | Mali, Tidérémé | 17’ 01 N | 02’ 07 E | x   | x   | Mal 7 |
| CBGP: M5287            | Mali, Tidérémé | 17’ 01 N | 02’ 07 E | x   | Mal 8 |
| CBGP: M4589            | Mali, Tira | 15’ 41 N | 04’ 44 W | x   | x   | Mal 16 |
| CBGP: M4619            | Mali, Tira | 15’ 41 N | 04’ 44 W | x   | Mal 18 |
| MNHN: VV1995-017      | Mauritania, Chott Boul | 16’ 37 N | 16’ 25 W | x   | x   | Mau 1 |
| MNHN: VV1995-041      | Mauritania, Chott Boul | 16’ 37 N | 16’ 25 W | x   | Mau 2 |
| CBGP: N3214           | Niger, Agadez | 16’ 58 N | 07’ 59 E | x   | Nig 4 |
| CBGP: N3222           | Niger, Agadez | 16’ 58 N | 07’ 59 E | x   | Nig 5 |
| Individuals and species | Origin | Lat. | Long. | cyt b | Fib7 | Labels |
|------------------------|--------|------|-------|-------|------|--------|
| CBGP: N3143            | Niger, Bosso | 13°41N | 13°18E | x | x | Nig 21 |
| CBGP: N4251            | Niger, Boumba | 12°25N | 02°50E | x | x | Nig 31 |
| CBGP: N4211            | Niger, Boumba | 12°25N | 02°50E | x | x | Nig 32 |
| CBGP: N3266            | Niger, Chëtimari | 13°10N | 12°28E | x | x | Nig 10 |
| CBGP: N4078, N4092     | Niger, Chëtimari | 13°10N | 12°28E | x | x | Nig 12,13 |
| CBGP: N4094, N4152     | Niger, Chëtimari | 13°10N | 12°28E | x | x | Nig 24,25 |
| CBGP: N4096            | Niger, Djirataoua | 13°24N | 07°08E | x | x | Nig 16 |
| CBGP: N4134            | Niger, Djirataoua | 13°24N | 07°08E | x | x | Nig 17 |
| CBGP: N4146            | Niger, Gaya | 11°53N | 03°27E | x | x | Nig 19 |
| CBGP: N4147            | Niger, Gaya | 11°53N | 03°27E | x | x | Nig 20 |
| CBGP: N3267            | Niger, Gouré | 14°03N | 10°13E | x | x | Nig 7 |
| CBGP: N4135            | Niger, Guidimouni | 19°24N | 09°30E | x | x | Nig 18 |
| CBGP: N3106, N3107     | Niger, Guilayni | 13°26N | 02°42E | x | x | Nig 1,2 |
| CBGP: N4271            | Niger, Kary Kopto | 12°33N | 02°38E | x | x | Nig 33 |
| CBGP: N4273            | Niger, Kary Kopto | 12°33N | 02°38E | x | x | Nig 34 |
| CBGP: N4093            | Niger, Kojimairi | 13°24N | 11°05E | x | x | Nig 14 |
| CBGP: N4098, N4109, N4133, N4154, N4159 | Niger, Kojimairi | 13°24N | 11°05E | x | x | Nig 15, 27–30 |
| GenBank: AF004571, AF004570 | Niger, Kollo | 13°21N | 02°17E | x | x | Nig 23,24 |
| CBGP: N3124            | Niger, N’Guigmi | 14°15N | 13°06E | x | x | Nig 3 |
| CBGP: N3144            | Niger, Niamey | 13°31N | 02°05E | x | x | Nig 22 |
| CBGP: N4001            | Niger, Tabelot | 17°36N | 08°56E | x | x | Nig 9 |
| CBGP: N3268            | Niger, Tanout | 14°57N | 08°53E | x | x | Nig 8 |
| CBGP: M5295            | Niger, Tila | 15°09N | 02°04E | x | x | Nig 11 |
| CBGP: N3219            | Niger, Tondibia | 13°34N | 02°01E | x | x | Nig 6 |
| CBGP: KB2807, KB2816   | Senegal, Darou-Wolof | 14°00N | 14°47W | x | x | Sen 23,24 |
| CBGP: JMD441, JMD447   | Senegal, Gouniang | 14°50N | 12°26W | x | x | Sen 1,2 |
| CBGP: KB932, KB933     | Senegal, Kaolack | 14°10N | 16°07W | x | x | Sen 17,18 |
| CBGP: KB1243           | Senegal, Lampar Peuhl | 16°05N | 16°20W | x | x | Sen 5 |
| CBGP: KB1244           | Senegal, Lampar Peuhl | 16°05N | 16°20W | x | x | Sen 9 |
| CBGP: KB924, KB925     | Senegal, Lindiane | 14°10N | 16°09W | x | x | Sen 15,16 |
| CBGP: KB3125, KB3126   | Senegal, Mibess | 15°25N | 16°42W | x | x | Sen 25,28 |
| CBGP: KB2407           | Senegal, Ndya | 14°34N | 12°45W | x | x | Sen 11 |
| CBGP: KB2408           | Senegal, Ndya | 14°34N | 12°45W | x | x | Sen 12 |
| CBGP: KB1191           | Senegal, Niaga | 14°49N | 17°16W | x | x | Sen 10 |
| CBGP: KB3143           | Senegal, Pekh Tall | 15°27N | 16°24W | x | x | Sen 26 |
| CBGP: KB3150           | Senegal, Pekh Tall | 15°27N | 16°24W | x | x | Sen 27 |
| GenBank: AF004572     | Senegal, Richard Toll | 16°28N | 15°45W | x | x | Sen 29 |
| CBGP: KB2765, KB2766   | Senegal, Saré-Gayo | 13°51N | 13°58W | x | x | Sen 21,22 |
| CBGP: KB1437           | Senegal, Savoigne | 16°09N | 16°18W | x | x | Sen 6 |
| CBGP: JMD645, JMD646   | Senegal, Sinthiane Doudé | 15°25N | 12°57W | x | x | Sen 3,4 |
| CBGP: KB2755           | Senegal, Sinthiou-Maleme | 13°49N | 13°55W | x | x | Sen 19 |
| CBGP: KB2758           | Senegal, Sinthiou-Maleme | 13°49N | 13°55W | x | x | Sen 20 |
| CBGP: KB2441           | Senegal, Sinthiou-Doudé | 14°11N | 12°45W | x | x | Sen 13 |
| CBGP: KB2442           | Senegal, Sinthiou-Doudé | 14°11N | 12°45W | x | x | Sen 14 |
| CBGP: KB1335, KB1336   | Senegal, Wouro-Aib | 16°28N | 15°37W | x | x | Sen 7,8 |
| GenBank: EF128062, EF128063, EF128064 | Sudan, Dongola | 19°00N | 30°29E | x | x | Sud 5–7 |
| GenBank: EF128067, EF128068, EF128069 | Sudan, El Sabagola | 17°34N | 33°26E | x | x | Sud 10–11 |
| GenBank: EF128070, EF128071, EF128072; DM: 9106 | Sudan, El Sabagola | 17°34N | 33°26E | x | x | Sud 12–15 |
| DM: 8993               | Sudan, El Sabagola | 17°34N | 33°26E | x | x | Sud 4 |
Seddon et al. [46]. These PCR products were purified and then sequenced in both directions by Macrogen (Seoul, Korea). In total, our Fib7 dataset groups 67 sequences that represent 60 A. niloticus, two A. cf. niloticus from Kenya, four representatives of other Arvicanthis species as well as one representative of genus Mastomys, to be used as an outgroup (Table 1). As stated previously, all individuals sequenced for Fib7 were sequenced for cytb (except for the A. niloticus specimen labelled ‘Mal 29′, and the representative of genus Mastomys), thus allowing us to build a combined dataset of 1,871 nucleotides (nt) encompassing 171 individuals. Importantly, all 10 countries that are represented for A. niloticus or A. cf. niloticus individuals in our cytb dataset are also represented in our Fib7 dataset. Similarly, 57 out of the 72 localities sampled in the A. niloticus or A. cf. niloticus cytb dataset were also sampled for Fib7 (see Table 1), thus providing a very similar and fully overlapping geographic coverage for both genes. All the sequences generated

### Table 1. Cont.

| Individuals and species | Origin | Lat. | Long. | cyt b | Fib7 | Labels |
|-------------------------|--------|------|-------|-------|------|--------|
| DM: 9103, 9108          | Sudan, El Suki | 13′19″ | 33′54″ | x     | x    | Sud 1,2 |
| GenBank: EF128083, EF128084 | Sudan, El Suki | 13′19″ | 33′54″ | x | 26,27 |
| GenBank: EF128073, EF128074, EF128075 | Sudan, Khartoum | 15′40″ | 32′35″ | x | Sud 16–18 |
| GenBank: EF128076, EF128077 | Sudan, Khartoum | 15′40″ | 32′35″ | x | Sud 19,20 |
| GenBank: EF128078, EF128079, EF128080 | Sudan, Medani | 14′23″ | 33′29″ | x | Sud 21–23 |
| GenBank: EF128081, EF128082 | Sudan, Medani | 14′23″ | 33′29″ | x | Sud 24,25 |
| GenBank: EF128065, EF128066 | Sudan, Shandi | 16′42″ | 33′29″ | x | Sud 8,9 |
| Arvicanthis cf. niloticus** | Kenya, Masai Mara NP | 01′495 | 35′20E | x | x | 1,2 |
| Arvicanthis abyssinicus | GenBank: AF004567.1*** | Ethiopia, Menagesha | 09′03N | 38′34E | x | 1 |
| Arvicanthis neumanni | GenBank: AF004566.1*** | Ethiopia, Sululta | 09′11N | 38′46E | x | 2 |
| Arvicanthis abyssinicus | GenBank: AF004574.**** | Tanzania, Berega | 06′11S | 37′09E | x | 1 |
| Arvicanthis ansorgei | GenBank: AF004573.**** | Tanzania, Berega | 06′11S | 37′09E | x | 2 |
| Arvicanthis rufinus | CBGP: Leish NEG 024M51 | Burkina-Faso, Pissy | 12′20N | 01′35W | x | x | 3 |
| Arvicanthis rufinus | CBGP: M6068 | Burkina-Faso, Toumbani | 11′00N | 00′58E | x | x | 2 |
| Arvicanthis rufinus | CBGP: M5619 | Mali, Sénènêa | 10′50N | 05′40W | x | x | 1 |
| Arvicanthis sp. (ANI-2) | GenBank: HM635839 | Cameroon, Gamnaga | 10′57N | 14′03E | x | x | ANI-2 1 |
| Arvicanthis sp. (ANI-2) | GenBank: AF004584 | CAR, Koumbala | 09′14N | 20′42E | x | ANI-2 2 |
| Arvicanthis sp. (ANI-2) | GenBank: AF004582 | Benin, Lokossa | 06′38N | 01′43E | x | 1 |
| Arvicanthis sp. (ANI-2) | GenBank: AF004583 | Benin, Tanougou | 10′49N | 01′26E | x | 2 |
| non-Arvicanthis species | Aethomys chrysophilus (GenBank: AJ604526.1) | Tanzania | x |
| Desmomyos harringtoni (GenBank: AF141206) | Ethiopia | x |
| Golunda ellioti (GenBank: AM408338.1) | India | x |
| Lemniscomys striatus (MNHN: ZM-2008-020) | Benin | x |
| Mastomys erythrocleucens (CBGP: M4136) | Chad | x |
| Melomys dybowski (GenBank: AF141212.1) | Ivory Coast | x |
| Pellomys fallax (GenBank: DQ022382.1) | Tanzania | x |
| Otomys irroratus (GenBank: EU874434) | South Africa | x |
| Otomys sungae (GenBank: JF795993) | ? | x |
| Otomys tropicalis (GenBank: JF795995) | ? | x |
| Rhabdomys pumilio (GenBank: AF533116) | South Africa | x |

*"CBGP", “DM” and “MNHN” stand for Centre de Biologie pour la Gestion des Populations (Montpellier, France), Durban Natural Science Museum (Durban, South Africa) and Museum National d’Histoire Naturelle (Paris, France), respectively. Individuals’ labels corresponding to Figures 1 to 3 are also provided.

**referred to as A. dembeensis in [29];

***most probably referable to A. somalicus (cf. text for details);

****referred to as A. cf. abyssinicus in [29];

****referred to as A. cf. somalicus in [29].

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in this study were deposited in GenBank (accession numbers KF478244 to KF478310 for the Fib7 and KF478311 to KF478426 for the cytb).

**Phylogenetic and Dating Analyses**

Bayesian inference (BI) was used to co-estimate phylogenetic relationships and divergence times using the BEAST v1.7.5 package [47]. BEAST uses Bayesian Markov Chain Monte Carlo (MCMC) procedures to approximate phylogenies and simultaneously infer nodes ages. To infer the time-calibrated phylogeny, we used the Bayesian relaxed clock (BRC) approach [48] implemented in BEAST. This method accounts for rate variation across lineages and assumes that substitution rates are uncorrelated across the tree (there is thus no a priori correlation between a lineage rate and that of its ancestor). To maximize the amount of available information, analyses were performed on the combined dataset, using specific sets of parameters for each gene [49]. Best-fit models of evolution for each gene were selected with jModelTest [50] using the Bayesian information criterion (BIC).

For the molecular dating analyses, each gene was associated with a specific uncorrelated lognormal relaxed clock (ULRC) model. A coalescent model tree prior with a constant population size was also preferentially used to account for the fact that our trees mostly describe intra-specific relationships [51]. BEAST. xml files were also modified to implement the path-sampling (PS) procedure [52], which allows a better approximation of the marginal likelihood of runs [53]. In a complementary way, we carried out maximum likelihood (ML) analyses with the software RAxML v.7.0.8 [54] using default settings and 100 random-addition replicates. For the corresponding partitioned analyses, the same best-fit models of evolution (as in BI) were used. Clade support for ML was assessed with non-parametric bootstrap values (BV; 1,000 replicates were used).

Three distinct sets of calibration were used in this study. The first two sets rely on the oldest known fossil occurrence for the genus *Arvicanthis* [55]. This fossil (individual BPSP#76) is a relatively complete specimen found in Kenya, in the Lukuino formation, which was radiometrically dated at 5.9-5.7 million years ago [Ma] [55]. We used two distinct parametric distributions (exponential and lognormal) to set a minimum age of 5.7 Ma for the genus *Arvicanthis* (see [36], for more rationale on these settings). The corresponding 5.7 Ma minimum age constraint was enforced as follows: exponentialPrior mean = 5.0, offset = 5.444 (for the exponential distribution), and lognormalPrior mean = 1.0, stddev = 1.0, offset = 5.175 (for the lognormal distribution). For the third set of constraints we used the time-calibrated phylogeny of Lecompte et al. [42] to set a series of secondary calibrations for the nodes that are shared between our respective datasets. Though the use of secondary calibration is not advocated [56,57], we conducted these analyses for comparison purpose. The fact that our outgroup choice was based on the result of the study of Lecompte et al. [42] allowed us to identify six shared nodes that were used for secondary calibrations. To do so, we used normal parametric distributions to set minimum and maximum ages for the corresponding nodes. These constraints were enforced as follows: (i) most recent common ancestor (MRCA) of *Arvicanthis* and *Aethomys*, normalPrior mean = 6.9 stddev = 1.0; (ii) MRCA of *Arvicanthis* and *Desomys*, normalPrior mean = 8.4 stddev = 1.0; (iii) MRCA of *Arvicanthis* and *Lebomys*, normalPrior mean = 4.8 stddev = 1.0; (iv) MRCA of *Arvicanthis* and *Othonys*, normalPrior mean = 11.0 stddev = 1.0; (v) MRCA of *Desomys* and *Rhabdomys*, normalPrior mean = 5.1 stddev = 1.0; (vi) MRCA of *Myomys* and *Pelomys*, normalPrior mean = 3.83 stddev = 1.0.

For each calibration set (‘exponential distribution’; ‘lognormal distribution’; ‘secondary calibrations’), two distinct runs were carried out, each one with 50 million generations, default priors and trees sampled every 5000th generation. After applying a conservative burn-in of 12.5 million generations for each run, convergence of runs was further assessed by examining the effective sample size (ESS) of parameters with Tracer available at [www.tree.bio.ed.ac.uk/software/tracer/](http://www.tree.bio.ed.ac.uk/software/tracer/). The resulting log and tree files were then combined using LogCombiner. We then directly estimated both node support (posterior probabilities; PP) and node age (median age estimates and 95% higher posterior densities; 95% HPD) using TreeAnnotator. Only nodes with posterior probabilities (PP) ≥0.95 were considered strongly supported [58]. Bayes factor (BF) [59] were then estimated using the log files of the three distinct calibration sets, using scripts detailed in Baele et al. [53]. The resulting time-calibrated trees (one per calibration set) were further modified under Mesquite v2.75 [60] by pruning the most basal outgroup taxa. For comparison purpose, additional runs (same settings for the MCMC) were also conducted for the cytb dataset (169 taxa; 1,113 nt) and the Fib7 dataset (67 taxa; 758 nt).

Additional phylogenetic-based analyses were also conducted to precise the geographic origin of the various *Arvicanthis niloticus* populations. To do so, we relied on the tree resulting from the analyses of the combined dataset. This tree was further pruned under Mesquite in order to include one specimen for each of the major lineages of *A. niloticus*, and one specimen for each other *Arvicanthis* species. Character optimizations were then performed to infer the ancestral areas of distribution. Three major areas were categorized for this analysis: (i) ‘West Africa’, which includes Benin, Burkina-Faso, Ghana, Mali, Niger, Senegal, Togo; (ii) ‘Central Africa’, which includes Cameroon, Central African Republic, Chad; and (iii) ‘North-East and East Africa’, which here includes Egypt, Ethiopia, Kenya, Somalia, Sudan, Tanzania. We then performed a single state parsimony optimization with Mesquite in order to identify the geographic origin of the MRCA of each *A. niloticus* phylogroups.

**Genetic Differentiation, Genetic Diversity and Network Analyses**

Analyses at the intraspecific level were performed on datasets that encompass only *A. niloticus* individuals (146 individuals for the cytb dataset and 60 individuals for the Fib7 one). The genetic differentiation between specimens belonging to the major phylogroups inferred by the phylogenetic analyses of the combined dataset was assessed for each gene using DnaSP v.5.1.0 [61]. However, the fact that DnaSP does not take into account missing data was problematic for the Fib7 dataset since it led to the exclusion of 113 nucleotide positions. Therefore, we analyzed a subset of the original dataset from which two sequences with a lot of missing data were removed [62]. In order to assess the level of genetic differentiation among the four phylogroups, we used three distinct statistics (FST, KST, and Snn). The FST (fixation index) is a statistic that compares the level of diversity of randomly chosen alleles in a given population with those found in the entire geographical sample; the KST is a statistic that takes into account the number of nucleotide differences between different haplotypes but does not give much weighting to large numbers of differences [63]; the nearest-neighbour statistic (Snn) measures how often the nearest neighbours within a matrix of sequences originate from the same population [64]. Because these three indices are known to be more or less sensitive to specific dataset features (such as low level of genetic diversity or low sample size), we used them in combination to ensure a more robust detection of genetic
differentiation [65]. For each statistics, a permutation test of 1,000 replicates was performed to assess the significance of the subdivision parameters. DnaSP was then used to infer the following parameters of genetic diversity: number of segregating sites (S), number of haplotypes (θ), haplotypic (Hθ) and nucleotide (π) diversities. We also performed neutrality tests for each gene using Tajima’s D [66] and Fu’s F statistics [67]. For the latter, values close to zero are expected for historically stable populations, whereas negative values would be indicative of recent population expansion.

Finally, networks analyses were conducted on the reduced cyt b and fib7 datasets using the reduced median joining method [68], which has the ability to deal with missing data as well as to infer ancestral haplotypes. This method also performs well against, or outperforms other network approaches [69]. It was implemented using the software Network v.4.6.1.1 (http://www.fluxus-engineering.com), with ε set to 0 in order to minimize alternative median networks.

Results

Phylogenetic Analyses

The BIC returned a general time reversible (GTR) model+Γ as best-fit model for each gene. For all analyses (combined dataset, or Fib7 and cyt b gene alone), convergence was reached as indicated by the high ESS values (>200) recovered for all runs.

Analyses of the combined dataset yield a similar topology whatever the method used (BI or ML; Figure 1). Overall the corresponding trees are well supported, and most interspecific nodes are supported by PP≥0.95 under BI or bootstrap values ≥70% under ML (Figure 1). Within the genus Arvicanthis, two main plurispecific clades emerge: specimens of A. ansorgi, A. cf. rufinus and ANI-2 cytotype (sensu [30]) form a well-supported monophyletic group (PP of 1.0/BV of 93%) whose sister assemblage contains (PP of 1.0/BV of 99%) representatives identified as A. neumanni, A. abyssiniclus, A. cf. niloticus (two specimens from Kenya) and A. niloticus sensu stricto (i.e. all A. niloticus but the two latter Kenyan specimens) here below referred to as A. niloticus. Within the former group, A. ansorgi forms a first robust monophyletic lineage (PP of 1.0/BV of 100%), while the second one contains A. cf. rufinus and ANI-2 individuals (PP of 1.0/BV of 98%). Within the second main Arvicanthis assemblage, three lineages were clearly retrieved: lineage A (PP of 0.88/BV ≤50%) is basal and made of two A. cf. niloticus and two A. neumanni specimens from Kenya and Tanzania, respectively; then, lineage B (PP of 1.0/BV of 100%) is represented by the two A. abyssiniclus specimens from Ethiopia; lineage C (PP of 0.71/BV of 84%) groups all A. niloticus from Ethiopia, Sudan, Egypt throughout all other westward countries specimens (Figure 1).

Central to the present study, lineage C splits into four phylogroups (C-1 to C-4), which also correspond to remarkably well-defined and exclusive geographic areas: (i) phylogroup C-1 (PP of 1.0/BV of 99%) contains all A. niloticus from Ethiopia, Sudan and Egypt; (ii) phylogroup C-2 (PP of 1.0/BV of 100%) groups all A. niloticus from Chad and Northern Cameroon, as well as one specimen from the Western bank of Lake Chad (Bosso) in Niger; (iii) A. niloticus individuals from West Mali to Senegal and Mauritania all gather in phylogroup C-3 (PP of 0.48/BV of 57%); (iv) specimens from phylogroup C-4 (PP of 0.48/BV of 60%) are all Nile grass rats originating from Niger, Burkina-Faso, North and East of Mali. In other words, in addition to a perfect match between genetic and geographic structure, a clear differentiation from East (phylogroup C-1 as sister to all remaining group) to West (phylogroups C-3 and C-4 as the most derived ones) is suggested. The latter pattern is also supported by the result of the character optimization of ancestral areas (see Figure 1), which indicates that the MRCA of all A. niloticus phylogroups is of East African origin. On a side note, it is also worthy to notice that individuals from Adrar des Ifoghas massif (specimens ‘Mali.10’, ‘Mali.11’, ‘Mali.12’ and ‘Mali.13’) appear genetically very close to each other, while those from Air massif (specimens ‘Nig.4’, ‘Nig.5’ and ‘Nig.9’) do not convincingly do so.

All the aforementioned lineages and phylogroups are recovered by the separate analysis of the cyt b gene (see Figure 2), with moderate (PP of 0.38 for lineage C) to high supports (PP of 0.98 for lineage A, and PP of 1.0 for lineage B and phylogroups C-1, C-2, C-3 and C-4). By contrast, a less resolved and supported topology is inferred by the analysis of the Fib7 gene alone (see Figure 3), which only identifies two well-supported (PP of 1.0) clades within A. niloticus: the first one mixes together individuals that are found in previously mentioned phylogroups C-1 and C-2, while the second one gathers representatives from phylogroups C-3 and C-4. The two Kenyan representatives of A. cf. niloticus were found basal to all other A. niloticus.

Divergence Time Estimates

Regarding dating analyses, the ‘secondary calibrations’ set yields younger age estimates (Table 2). The latter is problematic since the age of the oldest known Arvicathanthus fossil (5.7–5.9 Ma) significantly predates the median age resulting from the ‘secondary calibrations’ calibration set (4.71 Ma). The two other calibration sets recover very similar median ages, which never differ from more than 1 Ma when considering Arvicanthini lineages. Because the ‘exponential distribution’ calibration set is significantly favoured by the corresponding BF comparison (Ln BF = −10831.60 +11332.01 = 450.41), we chose to preferentially present the corresponding age estimates in Figure 1.

With respect to the age of Arvicanthini, a mid-Miocene origin was inferred with ages ranging from 11.29 Ma (95% HPD: 6.7–19.3; ‘lognormal distribution’ calibration) to 12.13 Ma (95% HPD: 7.04–10.924; ‘exponential distribution’ calibration). A Late Miocene origin was recovered for the genus Arvicathanthus with ages ranging from 6.52 Ma (95% HPD: 5.27–10.24; ‘lognormal distribution’ calibration) to 6.6 Ma (95% HPD: 5.44–11.41; ‘exponential distribution’ calibration). The lineage encompassing all A. niloticus specimens (lineage C) appeared in the Late Pleistocene approximately 2.72–2.92 Ma. Several major splits then occurred in the Pleistocene (1.85–1.92 Ma, 1.16–1.43 Ma), leading to A. niloticus phylogroups C-1 to C-4.

Genetic Differentiation, Genetic Diversity and Network Analyses

For the cyt b gene, all statistics (FST, KST*, and Snn) recovered a significant level of genetic differentiation among the four major phylogroups (Table 3). By contrast, the level of genetic structure was lower for the Fib7 gene since no significant genetic structure was evidenced between C-1 and C-2, or between C-3 and C-4. For the latter, the statistics were not even computable because of the absence of genetic variation (when excluding gaps and positions with missing data) between the 50 sampled specimens of the two groups.

In contrast with the cyt b gene (S = 182, h = 79, Hd = 0.985), genetic diversity was extremely low for the Fib7 gene (S = 9, h = 4, Hd = 0.233) (Table 4). For both genes, the highest levels of genetic diversity were found in the phylogroup C-1. Interestingly, this result was recovered despite the fact that the number of sampled individuals for phylogroup C-1 is lower than those of phylogroups C-3 and C-4 (and even C-2 when considering the Fib7 dataset).
The results of the neutrality tests for the two genes showed that *A. niloticus* populations were stable over time. We only find evidence for expansion of populations' size for the phylogroup C-1 (both Tajima's $D$ and Fu's $F$ statistics) and in the phylogroup C-3 (Fu's $F$ statistics).

Network analyses recovered a relatively congruent pattern for both genes (Figure 2 and Figure 3), in which the haplotypes from East Africa (phylogroup C-1) are connected with haplotypes from Central Africa (phylogroup C-2), which are further connected with haplotypes from West Africa (phylogroups C-3 and C-4), thus indicating a clear East to West pattern of colonization. Furthermore, the cytB dataset suggests that the genetic pool that is now present in the eastern part of West Africa (phylogroup C-4) originated from the haplotypic population that currently lies in the western part of West Africa (phylogroup C-3).

Figure 1. Time-calibrated tree resulting from the partitioned Bayesian analysis of the combined dataset. Age estimates correspond to the results of the BRC analysis using the fossil constraint with an exponential distribution. Labels on nodes correspond to the nodes listed in Table 2. Posterior probabilities (left) and bootstrap values (right) are also indicated for major nodes (values below 0.50 are not figured). On the bottom left, a map is included to figure the localities of almost all sampled *Arvicanthis niloticus* specimens (with the exception of the specimen from Ethiopia and the two specimens from Egypt). Additional information on mountain and hydrogeographic formations of interest is also provided. A red line is also used to figure the distribution limits of the two distinct *A. niloticus* cytotypes (ANI-1a and ANI-1b; see text for details). On the right side, the general origin of *Arvicanthis niloticus* specimens is represented using circles filled with colours corresponding to those used on the map. In addition, vertical sidebars highlight the three major lineages (A, B and C) and the four major clades within the lineage C (C-1, C-2, C-3 and C-4). On the bottom left again, the simplified phylogenetic topology of *Arvicanthis* studied here is represented with corresponding geographic origin of extant lineages as well as of MRCA as inferred by the ancestral area optimization under Mesquite (see text for details). doi:10.1371/journal.pone.0077815.g001
Discussion

All *Arvicanthis* individuals cluster in a monophyletic clade, whose sister group is *Lemniscomys striatus*, in agreement with Lecompte et al. [42]. Within *Arvicanthis*, three distinct clades are retrieved. A first one, that would be the sister group of all other *Arvicanthis* specimens studied here, comprises two subgroups of specimens: one includes individuals of *A. ansorgei* and the other contains individuals of *A. cf. rufinus* as well as those referred to as ANI-2. Such an association was already retrieved and discussed by Ducroz.
et al. [29], Volobouev et al. [70], and Dobigny et al. [34], and it will thus not be commented further here.

A second clade comprises two specimens of *A. neumanni* and two *A. cf. niloticus* specimens from Kenya. The two specimens labelled *A. neumanni* (from Tanzania) are those studied under the name *A. somalicus* by Ducroz et al. [29] who found them to be characterized by a $2N = 54/NFa = 62$ karyotype. Ducroz [27] further proposed that they correspond to *A. neumanni*, as acknowledged by Castiglia et al. [32] to distinguish them from the true *A. somalicus* that they considered as having a karyotype with $2N = 62$ and $NFa = 62–63$. As in Ducroz et al. [29], these two *A. neumanni* specimens have a sister-group relationship with the *A. niloticus*/*A. abyssinicus* clade. Yet, they here share this position with two *A. cf. niloticus* from Kenya, that were also karyotyped by Ducroz [27] as $2N = 62/NFa = 62$. The clear distinctness of these two *A. cf. niloticus* from the remaining *A. niloticus* specimens on molecular grounds strongly suggests that another name should be given to them. The latter may well be *A. somalicus* since their karyotypes seem to match quite well the one proposed by Baskevich & Lavrenchenko [71] for this species (for further comments, see [32]).

The third main clade is characterized by the two individuals of *A. abyssinicus* that lie in a basal position relative to all other *Arvicanthis niloticus*. This result, also found using a much smaller sample of *A. niloticus* specimens by Ducroz et al. [29], tends to consolidate the sister status of *A. abyssinicus* and *A. niloticus*. The two species are sympatric, but apparently not syntopic, in Ethiopia where the former is considered an endemic of the High Plateaus, whereas the latter would range at lower altitudes, west of the Rift Valley [24]. The remaining part of this clade corresponds to *Arvicanthis niloticus*, whose phylogeography is discussed in detail below, thanks to a large and wide-ranging sample.

Figure 3. Haplotype network and phylogenetic tree resulting from the analysis of the Fib7 dataset. The haplotype network reconstruction takes into account missing data and gaps, so that the inferred number of haplotypes is higher than the one presented in Table 4. Red values on nodes indicate the inferred number of mutations steps between haplotypes or ancestral haplotypes (symbolized by a red node). The absence of value means that the number of step is equal to 1. The phylogenetic tree corresponds to the results of a Bayesian inference analysis (see text for details); posterior probabilities (PP) are indicated for major nodes (values below 0.50 are not figured). On the right side, the general origin of *Arvicanthis niloticus* specimens is figured using circles filled with different colours that directly refer to the map presented in Figure 1. doi:10.1371/journal.pone.0077815.g003
Western parts of the species range, respectively, with no geographic overlap ([30]; see Fig. 1). Nevertheless, the two chromosomal lineages were also found paraphyletic on DNA grounds, and the taxonomic significance of this chromosomal

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Table 4. Parameters describing genetic diversity and genetic differentiation.

| Gene | phylogroup (n) | S  | h   | H_{D} | π   | D   | F   |
|------|----------------|----|-----|-------|-----|-----|-----|
| Cytb | (All) (146)    | 182| 79  | 0.985 | 0.0465 | 0.078 | n.s. | 8.008 (ns) |
| C-1  | 30            | 83 | 24  | 0.983 | 0.0151 | 1.897 | -11.536 |
| C-2  | 22            | 16 | 9   | 0.870 | 0.0055 | 0.318 | -0.695 (ns) |
| C-3  | 47            | 32 | 24  | 0.946 | 0.0043 | 1.713 | -14.579 |
| C-4  | 47            | 57 | 22  | 0.946 | 0.0107 | -0.863 | -2.781 (ns) |
| Fib7 | (All) (58)    | 9  | 4   | 0.253 | 0.0026 | -0.162 | 3.310 (ns) |
| C-1  | 2             | 1  | 1.000 | 0.0014 | n/a  | n/a  |
| C-2  | 6             | 1  | 2.000 | 0.0008 | 1.445 | 0.795 (ns) |
| C-3  | 24            | 0  | 1   | 0.000 | 0.0000 | n/a  | n/a  |
| C-4  | 26            | 0  | 1   | 0.000 | 0.0000 | n/a  | n/a  |

The following abbreviations were used: number of sequences per phylogroup (n); number of polymorphic sites (S); number of haplotypes (h); haplotypic diversity (H_{D}); nucleotide diversity (π); Tajima’s D statistic (D); Fu’s F statistic (F).

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mutation has already been questioned [30]. Since then, reasonably large series of animals were karyotyped on the Eastern part of A. niloticus' range (Sudan: 33; Northern Cameroon: 34) and, accordingly, they were found with the ANI-1a karyotype. Moreover, the geographic distributions of ANI-1a and ANI-1b (see Fig. 7 in [30]) resemble that of the genetic clades retrieved here, with C-3 range (this study) widely overlapping ANI-1b's one [30]. However, this geographic match is imperfect since C-3 does not extend beyond the Bani River, while ANI-1b reaches the Adrar des Ifoghas massif (Northern Mali) and South-Western Niger (i.e., Andelamboukane). More precisely, several localities were included in both the cytogenetic and the molecular studies (e.g., Menaka, Ouatagouna and Tararabat, all in North-Eastern Mali) and provided animals with the ANI-1b karyotype but belonging to the C-4 (and not C-3) mtDNA clade. In addition, the two cytotypes were found sympatric in Sare Mama (Central Mali; Granjon, unpubl. data), and one heterozygous individual (2N = 62/NA = 63) was trapped in Gaya (Southern Niger; Dobigny, unpubl. data). Taking into account the strict geographic exclusion of the C-3/C-4 mtDNA clades observed here, such an imperfect geographic match between chromosomal and molecular patterns may be due to asymmetric introgression between the nuclear (here the karyotype) and mitochondrial genomes following secondary contact of clades C-3 and C-4. Unfortunately, the resolution power offered by the Fib7 gene is too weak here to test for this hypothesis. Karyotypic vs. mtDNA profiles mismatch could also be due to incomplete lineage sorting of a long-standing secondary contact of clades C-3 and C-4. Furthermore, the eastern part of West Africa was recolonized by the clade from Central Africa (phylogroup C-2) in contact with phylogroup C-1. Thanks to the results of the network analyses, we can also infer a more precise pattern for A. niloticus phylogroups. Overall, there is a clear East to West differentiation of populations, with Egyptian and Sudanese individuals (phylogroup C-1) being connected to Chadian or Northern Cameroonian (phylogroup C-2) populations. Unfortunately, a wide sampling gap exists between Sudanese (phylogroup C-1) and Chadian (phylogroups C-2) samples which precludes any precise localization of the contact zone between these two lineages. Surprisingly, the clade from Central Africa (phylogroup C-2) is genetically more related to a lineage that is not in direct contact (phylogroup C-3), thus suggesting that the ancestors of these lineages were likely isolated during a past episode. After this episode, the eastern part of West Africa was recolonized by another population (phylogroup C-4), which is in contact with phylogroup C-3 in Central Mali (more precisely along the Eastern side of the Niger River valley) and in contact with phylogroup C-2 in the Lake Chad surroundings.

As already suggested for many other rodent as well as non-rodent African organisms (see below, and Table S1), such a pattern, which is referable to phylogeographic category I of Avise [76], strongly suggests that extant genetic structure in A. niloticus results from the divergence of allopatric populations, either through ecological local adaptation, through vicariance (i.e. on each side of persisting geographic barriers) or through isolation in refuges and subsequent dispersal until secondary contact. West African steppes and savannas represent a rather homogenous habitat from Senegal to the Red Sea. This is why the East-West differentiation of A. niloticus lineages can hardly be accounted for by ecology-driven local adaptive processes. Furthermore, the numerous individuals that were trapped in very close localities but that yet belong to different genetic clades (e.g., in Central Mali and around Lake Chad) poorly support the local adaptation hypothesis. In the same manner, although putative geographic barriers that currently delimit A. niloticus intraspecific clades (Niger River, Lake Chad basin) were identified, vicariance appears as an inaccurate explanation here since no admixture at all was detected in our dataset. Indeed, this would imply that propagules crossed each of those barriers only ‘once’ and then remained reproductively isolated for hundreds thousand years, something that sounds highly improbable.
On the contrary, the refuge theory [77] fits well to the phylogeographic pattern observed in the Nile grass rats: lineages’ splits all occurred during the Pleistocene which is characterised by deep climatic variations that induced extensive modifications of the open grasslands habitat range in Sub-Saharan Africa. Indeed, some 5 million years ago, the African climate started to get generally cooler and drier, along with successive arid-pluvial cycles, more or less following glacial-interglacial ones at higher latitudes [78–81]. Whatever sudden [78,79] or progressive [90], these recurrent aridification events have led to cyclic contractions of the moisted habitats (i.e., riverine and tropical forests, swampy areas), which were reduced to refugial patches in several instances during the Plio-Pleistocene surrounded by savannah-forest mosaic landscapes [82]. In parallel, arid phases induced wide-scale expansion of more xeric and open habitats. These climatic oscillations translated into deep modifications of all major biomes distribution ([79–81,83–85]; among many others). In particular, C4 grasses, which are considered as valuable biomarkers of open grassland habitats, emerged during the Upper Miocene but became a major component of African biomes only from the Plio-Pleistocene [86]. Recurrent phases of grassland expansion following Pleistocene climatic cycles were accompanied by major turnovers of mammalian faunas towards more numerous and more abundant open habitat-adapted species [85]. Conversely, phases of savannah fragmentation have triggered intra- and interspecific diversification [10], and references below). These variations in grassland habitats of the Sahelian region during the Pleistocene have probably had major consequences on *A. niloticus* evolution since this species is highly specialized in this habitat type: its diet is mainly herbivorous, and it also forages along runways through matted grass around its nests which are mainly made of intermingled blades of grass themselves [25,87].

The refuge theory is also strongly supported by an increasing number of studies dealing with other Sub-Saharan rodent species (see below as well as Table S1), and where geographic distributions perfectly or almost perfectly match with genetic assemblages. All these studies similarly point towards isolation and genetic differentiation within refuges during unfavourable periods, with subsequent dispersion phases during favourable ones. Secondary contacts then tend to stabilize around strict (or even partial) geographic barriers that stop (or slow down) dispersal and gene flow—the so-called suture zones *sensu* Hewitt [88]. Of course, the nature of refuges, favourable/unfavourable periods and/or barriers depend on species-specific characteristics.

For instance, the Sahelo-Saharan spiny mouse *Acomys chateaui* was most probably restricted to rocky areas that were surrounded by sand deserts during arid episodes, but may have dispersed through steppe-like environments during less arid periods [18]. The humid habitat-adapted *Mastomys huberti* underwent isolation around water-body relics during arid phases, while it colonized hydrographic basins of Senegal, Mali and Guinea during humid ones [19]. As far as forest-dwelling rodent species are concerned, they are expected to be trapped in forest relic patches during arid episodes but to extend during forest expansions associated with humid periods (e.g., *Pranomys talbarg* and *Pranomys rustratus*: [21]; *Pranomys misonnei*: [22,23]). Similarly, populations of open tree savannah species would diverge allopatrically during moist episodes, when fragmented throughout extended savannah-forest mosaic landscapes (e.g., *Pranomys daltoni*: [14]). On the contrary, species from the drier scrub savannah and steppes are thought to be isolated when surrounded by forests during the moisted phases, and then to disperse widely with expanding open grasslands (*Mastomys erythroleucus*: [15]; *Lemniscomys striatus*: [13]). *A. niloticus* is to be considered as belonging to the latter category.

Geographic barriers to dispersal were also identified in most of African rodent phylogeographic investigations. Once again, many similarities appear between species-specific case studies. The tectonic complex of the Rift Valley was identified as a contact zone between parapatric phylogroups in *L. striatus* [13] and *Mastomys natalensis* [17]. Similarly, the relationships between some hydrographic features and genetic structure have been documented in several Sub-Saharan rodent species (e.g., *Taterillus* spp.: [89]; *M. erythroleucus*: [15,16]; *L. striatus*: [13]; *P. talbarg*: [21]; *P. misonnei*: [22,23]; *P. daltoni*: [14]). In particular, it is striking to see how the same rivers and/or lakes have been pointed out as putative barriers in several taxa. In regards to the present study, this is notably the case for the Niger River valley that tends to separate clades C-3 and C-4 of *A. niloticus* as well as several parapatric clades within (*L. striatus*: [13]; *M. erythroleucus*: [15,16]; *P. daltoni*: [14]) and between (*Taterillus* spp.: [89]) other unrelated rodent species. In the same manner, Lake Chad and its surroundings coincide with the contact zone between *A. niloticus* clades C-2 and C-3, while this area also signs genetic hiatus in *Mastomys erythroleucus* [15,16], *Gerbillus nigeriae* [90] and *Taterillus* spp. [89]. Besides, it has already been suggested that the latter region constitutes a ‘phylogeographic crossroad’, i.e. a centre of diversification where faunas may have diversified following the recurrent Plio-Pleistocene cycles of transgression/regression of the Palaeolake Chad [34,91].

So, in essence, ancestral *A. niloticus* populations would have diverged allopatrically during humid periods within at least four different Pleistocene refuges of open habitat. They would have dispersed during more arid ones until secondary contacts around geographic barriers such as the Niger River and the Lake Chad basins. The precise timing of isolation within Pleistocene refuges and of dispersal outside these refuges is not feasible here since molecular inferences of dates for such events are associated with confidence intervals that are usually of the same magnitude—when not larger—than Pleistocene climatic cycles themselves, thus precluding any robust conclusion. Also, the putative existence of a third barrier eastwards will require further sampling in Eastern Chad and Sudan in order to identify the precise geographic range of clades C-1 and C-2.

Finally, although we did not attempt to investigate formally sub-structure within each clade, we clearly retrieve a well-supported group of individuals that all originate from Adrar des Ifoğhas in Mali (4 specimens from 2 different localities). No such signal could be obtained for individuals from Aïr in Niger (3 specimens from 2 different localities), thus supporting a previous hypothesis [92,93] that the Malian Adrar may represent a more isolated Sahelian refuge within the Sahara desert than the Aïr.

Interestingly, the picture drawn from studies on rodents often resembles those obtained on other mammals that we are aware of: refuges and allopatry during successive climatic cycles in the past were advocated to explain the differentiation of genetic lineages in cercopithecine monkeys (e.g., [94,95]), baboons [96], hyenas [97], wild dog [98], common warthog [99], giraffe [100], buffalo [101] and many large antelopes like topi, hartebeest [102,103], impala, kudu [104], waterbuck [105], kob [106], African bushbuck [107], common eland [108] and roan [109]. Similar conclusions were also reached in plants (e.g., shea tree: [110]; giant lobelia: [111]; coffee tree: [112]); insects (e.g., maize stalk borer: [113]), reptiles (e.g., puff adder: [114]); Southern rock agama: [115]) and birds (e.g., starred robin: [116]; ostrich: [117]). Zoogeographic barriers to dispersal have also been pointed out in several species, including the Rífi Valley (e.g., lion: [118]; giraffe: [110]; wildebeest: [102]; waterbuck: [105]; bushbuck: [107]; maize stalk borer: [113]; giant lobelia: [111] and great rivers (e.g., [95]).
As a whole, this reaches what was inferred for other continents, especially the well-studied Europe and North America [119–121]. Sub-Saharan Africa was once claimed to be understudied [119], but data have accumulated during the last decade (reviewed in [10], and references hereabove). Among studies that have concerned ungulates, a clear sampling bias towards Eastern and Southern Africa is obvious. West African megafauna’s phylogeography remains very poorly documented and this part of the continent is usually dramatically absent from most datasets: Lorenzen and colleagues’ recent review [10] clearly illustrates such a gap of knowledge, with several highly differentiated ungulate lineages west of the Rift Valley (see their Figure 3) that, unfortunately, are documented through extremely reduced number of localities (but see [101], for a slightly higher number of sampling sites in buffaloes). This emphasizes the importance of studies conducted on rodents that, in the contrary, have mainly focused on West and Central African deserts, grasslands and forests. In particular, the present study adds to the three other available ones that are typical of open grasslands in West and Central Africa, namely Mastomys natalensis [17], M. erythroleucus [15] and Lennioscomys striatus [19]. Together, these latter works allow us to draw an integrative picture of West and Central African open habitats history during the Pleistocene, something that could not be reached with ungulates models. In particular, one refuge was hypothesised in West Africa (i.e., between Senegal and the Rift Valley) on the basis of large mammals data [10], while at least three and potentially four major ones can be speculated from patterns obtained in these four rodent species: one most probably westward of the Niger River, one somewhere between the Niger River and the Lake Chad, and one or two between the Lake Chad and the Nile River. Nevertheless, the precise locations of these Pleistocene refuges for open habitat species are still to be precisely assessed.

Supporting Information

Table S1 Summary of previous phylogeographic studies conducted on Sub-Saharan African rodents. The species, their preferred habitat, the molecular marker used, the sample size, the methods for date inference, the major evolutionary events as well as putative corresponding geographic aspects are indicated. References can be found in the main text. (DOCX)

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Author Contributions

Conceived and designed the experiments: GD CT PG. Performed the experiments: GD CT PG. Analyzed the data: GD GJK. Contributed reagents/materials/analysis tools: GD CT PG KR JMD LG. Wrote the paper: GD CT JMD LG GJK.

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