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Mitochondrial phylogeny reveals differential modes of chromosomal evolution in the genus *Tatera* (Rodentia: Gerbillinae) in Africa

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Abstract

The African gerbils of the genus *Tatera* are widespread and abundant throughout sub-Saharan Africa. There is still today a certain controversy concerning the taxonomy of these rodents and very few attempts have been made to assess their systematic relationships. The present paper introduces findings based on the partial sequences of cytochrome *b* (495 bp) and the 16S rRNA (469 bp) mitochondrial genes of six (*T. robusta*, *T. nigricauda*, *T. vicina*, *T. leucogaster*, *T. valida*, and *T. kempi*) species together with two additional taxa. We also report the karyotypes of *T. vicina* and *T. leucogaster*. We propose that *T. vicina* should be considered as a valid species and show the monophyly of the *robusta* species group, with the exclusion of *T. leucogaster*. Our results show there is a different chromosomal evolutionary pattern within the two major lineages, which is recognizable through molecular phylogenetics. One is characterized by karyotype stability and the other by a considerable number of chromosomal rearrangements. The lineage divergence coincides with the formation of the East African Rift. The processes that led to the origin of the East African species seem to be related to the subsequent climatic changes, which caused cyclic contraction and expansion of the savannah biomes. Furthermore, geological activities that characterized East Africa during Plio-Pleistocene may also have contributed to lineage divergence.

Keywords: African Rodents; *Tatera*; Chromosomal speciation; Karyotype evolution; Phylogeny; Cytochrome *b*; 16S; mtDNA

1. Introduction

The gerbils of the genus *Tatera* Lataste 1882 (Rodentia, Gerbillinae) are widespread in the sandy plains, grasslands and savannahs of sub-Saharan Africa, Near East and the Indian subcontinent. They are also common in cultivated areas, where they nowadays cause considerable agricultural damage (see Staplerat, 2003). Given their abundance and the fact they are frequently prominent in rodent pest control programmes in agriculture (see Singleton et al., 1999), there have been a number of morphological (Bates, 1985; Davis, 1949, 1965, 1966, 1975) and karyological (Benazzou et al., 1984; Codjia et al., 1984; Colangelo et al., 2001; Fadda et al., 2001; Matthey, 1969; Matthey and Petter, 1970; Qumsiyeh, 1986; Qumsiyeh et al., 1987; Tranier, 1974; Viegas-Pequignot et al., 1982) studies directly regarding their taxonomy and systematics. However,

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they all failed to provide a clear overall picture of species distinction and relationships. Therefore, it is generally accepted that a revision of the genus is very much required (Bates, 1988; Musser and Carleton, 1993).

In their last rodent taxonomic review Musser and Carleton (1993) listed 12 species, 11 of which occur in Africa (*T. afra*, *T. brantsii*, *T. boehmi*, *T. guineae* Thomas 1910, *T. inclusa*, *T. vicina*, *T. kempi*, *T. leucogaster*, *T. nigricauda*, *T. phillipsi* (De Winton, 1898) and *T. robusta*) and only one in Asia (*T. indica*). Furthermore, Pavlinov (1999) maintained that morphological characters demonstrate that only the species from Asia can be considered as *Tatera sensu stricto* and consequently he placed all the African species in the one genus *Gerbilliscus* Thomas, 1897. However, given the fact a complete revision of the genus has yet to be carried out, we will use the name *Tatera* for the African species as well.

The African species were clustered by Davis (1975) into two groups on the basis of their morphological traits, with the exception of *T. boehmi* (Noack, 1887): the *afra* group, including *T. afra* (Gray, 1830), *T. valida* (Bocage, 1890), *T. brantsii* (Smith, 1836), *T. inclusa* (Thomas and Wroughton, 1908); and the *robusta* group, including *T. leucogaster* (Peters, 1852), *T. nigricauda* (Peters, 1878), *T. robusta* (Cretzschmar, 1826). No test, however, has been performed to allocate them to monophyletic units. Cytogenetics has cast some light (see above on this matter), but an appropriate comparison of karyotypes through banding has been done for only a part of the species (Qumsiyeh, 1986; Qumsiyeh et al., 1987).

The present paper investigates the phylogenetic relationships between samples from part of the range of the genus, i.e., Ethiopia, Kenya, Tanzania, Benin, Burkina Faso, and the Democratic Republic of Congo. We test the correspondence of the *robusta* and *afra* groups to monophyletic units and we attempt to clarify the taxonomy and systematics of identified taxa through molecular genetics (16S rRNA and cytochrome *b* mitochondrial genes) and cytogenetics (standard chromosome description and G-banding). This form of integrated approach is particularly appropriate in attempt to identify the possible occurrence of sibling species (Denys et al., 2003). The study was performed in the context of the "bar-coding taxon identification" (Blaxter, 2004), where, specimens are defined by their unique sequences and compared with known taxa and only then are they assigned to a known or new species. Finally, we hypothesize a possible scenario of mitochondrial lineage dispersion and appearance and fixation of chromosomal changes in the eastern African savannahs and their possible relationship to geological and climatic modifications that took place in the area during the formation of the Great Rift Valley.

2. Materials and methods

2.1. Source of material and species attribution

A total number of seventy-three samples from 34 localities in Tanzania, Kenya, Ethiopia, Democratic Republic of Congo (DRC), Benin, and Burkina Faso were analysed (Table 1, Fig. 1). Due to the current taxonomic uncertainty, samples defined by their unique sequences were attributed to a species only after comparison with type specimens and after morphometric analyses (both multivariate and geometric; Rohlf and Marcus, 1993) on a large data set (unpublished data).

By following these criteria, all samples were attributed to one of the five species occurring in East Africa. These are: *T. robusta*, *T. nigricauda*, *T. vicina* (Peters, 1878), *T. leucogaster,* and *T. valida* (Table 1). *T. vicina* is actually a synonym of *T. robusta*, but our morphometric comparison indicated that the main part of the Tanzanian and the Kenyan samples should be considered *T. vicina*. The samples from Benin and Burkina Faso were previously identified by Colangelo et al. (2001) as *T. kempi* on the basis of morphological and chromosomal analyses (they all show $2n=48$; NFa ranges from 62 to 64). Species attribution for the specimens KE102 (Rongai, Kenya) and KIK1703, KIK1704 (Kikwit, Democratic Republic of Congo) (Table 1) was impossible as they could not be properly compared with any adequate series from those localities. Therefore, these specimens were considered in the analysis as MOTUs (molecular taxonomic units; Blaxter, 2004) and labelled as *Tatera* sp. followed by their codes.

Samples were directly live trapped or came from the tissue data bank of the Museo di Anatomia Comparata (Università di Roma "La Sapienza") and the Royal Belgian Institute of Natural Sciences (Brussels). The study of the karyotypes and of the sequences of the cytochrome *b* and 16S rRNA genes was been performed on a different number of individuals. These are reported in detail in Table 1.

2.2. Chromosome preparation

Live trapped specimens were transported to the Sokoine University of Agriculture, Morogoro (Tanzania), Addis Ababa University (Ethiopia), and Kenyatta University (Kenya), where chromosome metaphases were obtained from the bone marrow following Hsu and Patton (1969). Cell suspensions in fixative were then transported to the Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza," where slides were prepared. Metaphases were stained by the Giemsa standard method (pH 7). G-bands were enhanced with trypsin following the protocol of Seabright (1971). Pictures of metaphases were collected using the digital camera Photometrics Sensys 1600 and the Iplab software (Scanalytics, version 2.420).

Table 1
Species, country, sampling locality names, geographic coordinates, museum codes, diploid and autosomal fundamental numbers, and EMBL accession numbers

	Species	Country	Localities	Latitude and longitude	Museum code	2*n*	NFa	EMBL accession	
								Cytochrome	16S rRNA
1	*T. robusta*	Ethiopia	Zeway	07°55′N–38°43′E	ET107[a,b,c], ET119[a,b], ET127[a,b,c]	36	68	AJ875234A, J875235, AJ875336	AJ878516, AJ878517
2	*T. nigricauda*	Kenya	Nguruman	01°50′S–36°06′E	NK2010, NK2034[c]			AJ875237, AJ875238	AJ878518
3	*T. nigricauda*	Tanzania	Jipe	03°41′S–37°42′E	T50453[b], T50456, T50475[c], T50476	36	68	AJ875239, AJ875240, AJ875241, AJ875242	AJ878519
4	*T. nigricauda*	Tanzania	Lwami	03°41′S–37°32′E	T50494[b], T50496[c], T50230	36	68	AJ875243, AJ875244, AJ875245	AJ878520
5	*T. nigricauda*	Tanzania	Same	04°04′S–37°43′E	14416			AJ875246	
6	*T. nigricauda*	Tanzania	Mkomazi	04°37′S–38°04′E	T50216[b], T50217[b,c]	36	68	AJ875247, AJ875248	AJ878521
7	*T. vicina*	Kenya	Nairobi	01°15′S–36°47′E	KE135[a,b], KE138[a,b,c]	36	68	AJ875249, AJ875250	AJ878522
8	*T. vicina*	Tanzania	Tpri	03°16′S–36°41′E	14361, 14383			AJ875251, AJ875252	
9	*T. vicina*	Tanzania	Kisiwani	04°07′S–37°57′E	T50342			AJ875253	
10	*T. vicina*	Tanzania	Mombo	04°54′S–38°13′E	T50214[b], T50215[b], T50226, T50227[b], T50229[b]	36	68	AJ875254, AJ875255, AJ875256, AJ875257, AJ875258	
11	*T. vicina*	Tanzania	Ngasumet	04°29′S–37°10′E	T50180, T50189, T50190[b]	36	68	AJ875259, AJ875260, AJ875261	
12	*T. vicina*	Tanzania	Ndaleta	05°12′S–36°30′E	T50144[b], 50153[b], T50158 [b]	36	68	AJ875262, AJ875263, AJ875264	
13	*T. vicina*	Tanzania	Kanyelele	02°38′S–33°08′E	1739, 1828, 2371			AJ875265, AJ875266, AJ875267	
14	*T. vicina*	Tanzania	Tabora	05°01′S–32°49′E	T50620			AJ875268	
15	*T. vicina*	Tanzania	Inala	05°25′S–32°49′E	T50579[b,c], T50581, T50582	36	68	AJ875269, AJ875270, AJ875271	AJ878523
16	*T. vicina*	Tanzania	Itigi	05°41′S–34°28′E	T50339[b,c]	36	68	AJ875272	AJ878524
17	*T. vicina*	Tanzania	Mvumi	06°20′S–35°50′E	T50302, T50320			AJ875273, AJ875274	
18	*T. vicina*	Tanzania	Matongolo	05°46′S–36°28′E	T50000, T50031[c], T50062[b]	36	68	AJ875275, AJ875276 AJ875277	AJ878525
19	*T. vicina*	Tanzania	Berega	06°14′S–37°10′E	14271, 14276			AJ875278, AJ875279	
20	*T. vicina*	Tanzania	Dakawa	06°26′S–37°34′E	5667, 5709			AJ875280, AJ875281	
21	*T. vicina*	Tanzania	Morogoro	06°50′S–37°39′E	2892, 2941, 2870			AJ875282, AJ875283, AJ875284	
22	*T. vicina*	Tanzania	Mlali	06°57′S–37°33′E	2033			AJ875285	
23	*T. vicina*	Tanzania	Msimba	07°26′S–36°57′E	14623			AJ875286	
24	*T. vicina*	Tanzania	Mbarali	08°15′S–33°55′E	3036			AJ875287	
25	*T. vicina*	Tanzania	Jipe	03°41′S–37°42′E	T50443, 50472, T50473[b], T50474[c]	36	68	AJ875288, AJ875289, AJ875290, AJ875291	AJ878526
26	*T. leucogaster*	Tanzania	Dakawa	06°25′S–37°31′E	T50545[b], T50546[b,c]	40	66	AJ875292, AJ875293	AJ878527
27	*T. leucogaster*	Tanzania	Chunya	08°31′S–33°24′E	SWT98[c], SWT99			AJ875294, AJ875295	AJ878528
28	*T. leucogaster*	Tanzania	Muhuwesi	10°54′S–37°29′E	5451[c]			AJ875296	AJ878529
29	*T. leucogaster*	Tanzania	Mnara	10°07′S–39°24′E	4573, 4574			AJ875297, AJ875298	
30	*T. valida*	Tanzania	Ilanga	07°50′S–31°34′E	13387[c]			AJ875299	AJ878530
31	*T. kempi*	Benin	Setto	07°30′N–02°04′E	B93 [a,b,c]	48	64	AJ875300	AJ878531
32	*T. kempi*	Benin	Atchérigbé	07°34′N–02°02′E	B68[a,b,c]	48	62	AJ875301	AJ878511
33	*T. kempi*	Benin	Tanogou	10°49′N–01°25′E	B81[a,b]	48	63	AJ875302	
34	*T. kempi*	Burkina Faso	Goden	12°23′N–02°17′W	BKF7[a,b]	48	64	AJ875303	
35	*Tatera* sp.	Kenya	Rongai	01°10′S–35°36′E	KE102[a,b,c]	36	68	AJ8753034	AJ878512
36	*Tatera* sp.	Dem. Rep. of Congo	Kikwit	05°03′S–18°51′E	KIK1704[c], KIK1703[c]			AJ875305, AJ875306	AJ878513, AJ878514

Numbers in column one correspond to the localities in the map represented in Fig. 1. For all samples a 495 base pair fragment of the cytochrome *b* was obtained.

[a] Collection of the Museo di Anatomia Comparata, Università di Roma "La Sapienza," Italy; otherwise, collection of the Royal Museum for Central Africa, Tervuren, Belgium.

[b] Samples for which karyotypes were prepared or are known.

[c] Samples for which the 16S rRNA gene was sequenced.

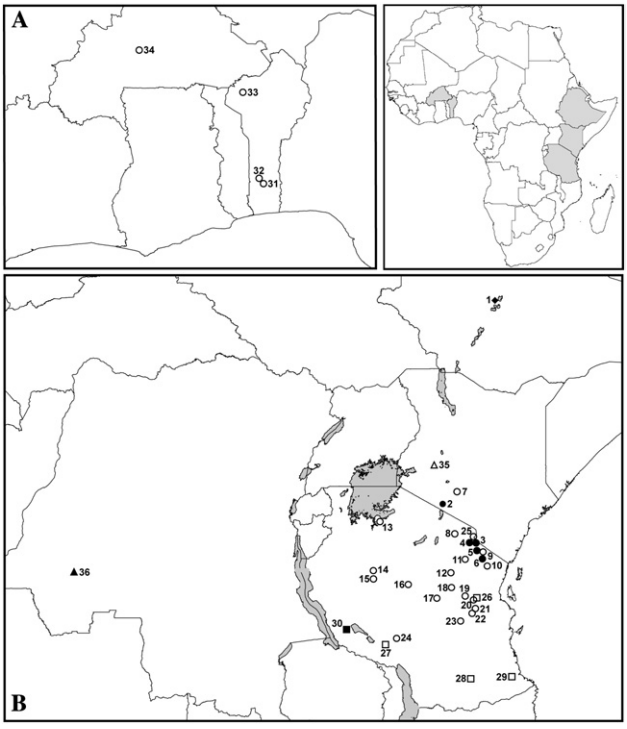

Fig. 1. Map with the location of the samples. (A) West Africa: *T. kempi*, open circle. (B) East Africa: *T. robusta*, filled diamond; *T. vicina*, open circles; *T. nigricauda*, filled circle; *T. leucogaster*, open square; *T. valida*, filled square; *Tatera* sp. (KE102), open triangle; *Tatera* sp. (KIK1704, KIK1703), filled triangle. For locality identification numbers see Table 1.

2.3. DNA extraction, amplification, and sequencing

DNA was obtained from liver, kidney, heart, muscle, and tail, which were preserved in 80% ethanol. DNA extraction was performed using the DNeasy tissue kit (Qiagen). The mitochondrial DNA sequences were isolated and amplified by polymerase chain reaction (PCR).

A 495 base pair fragment of the cytochrome *b* was obtained from all the samples. To test the reliability of the deepest phylogenetic relationships obtained with the cytochrome *b* sequences, a representative subset of 20 samples (Table 1) was used to sequence the more slowly evolving mitochondrial 16S rRNA gene.

The cytochrome *b* gene fragment was amplified combining the primers L14723 (5′-ACC AAT GAC ATG AAA AAT CAT CGT T-3′) and H15553 (5′-TAG GCA AAT AGG AAA TAT CAT-3′) (Irwin et al., 1991). The 16S rRNA gene fragment was amplified using the primers 16S rRNAar (5′-CGC CTG TTT AAC AAA AAC AT-3′) (Palumbi et al., 1991) and 16S rRNA-Hm (5′-AGA TCA CGT AGG ACT TTA AT-3′) (Quérouil et al., 2001).

The amplifications were performed in a MJ Research PTC-150HB MiniCycler as follows—(1) cytochrome *b*: a first cycle of an initial denaturation at 94 °C for 1 min, then 35 cycles with denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min. These steps were followed by a 10 min extension at 72 °C; (2) 16S rRNA: a first cycle of an initial denaturation at 94 °C for 3 min, then 30 cycles with denaturation at 94 °C for 1 min, annealing at 49 °C for 1 min, and extension at 72 °C for 1 h 30 min. These steps were followed by a 4 min extension at 72 °C. The reactions were carried out in 50 μL reaction volume including 200 ng of each primer, 5 μL of 10× Tris buffer, 3.5 μL $MgCl_2$, 0.2 mM dNTP, 2 U *Taq* polymerase (Promega), and 50–500 ng of template DNA. Double stranded PCR products were purified using an Amersham GFX/PCR purification kit and prepared for automated sequencing using the same primers utilized for amplification.

The 16S rRNA sequence alignment was obtained with the Clustal X program (Thompson et al., 1997) using default settings and subsequently corrected by the naked eye.

2.4. Genetic diversity and phylogenetics

The MEGA 2.1 program (Kumar et al., 2001) was used to measure the levels of genetic diversity and the transition/transversion ratio. Interspecific sequence divergence was estimated by pairwise comparisons using the Kimura 2-parameters model (K2P) (Kimura, 1980). Saturation analyses for the cytochrome *b* and 16S rRNA sequences were performed by plotting the *P* distances against the absolute number of substitutions for both transitions (Ts) and transversions (Tv).

The phylogenetic relationships were reconstructed using the PAUP 4.0b10 program (Swofford, 1998). The cytochrome *b*, 16S rRNA and the combined datasets were analysed using the maximum parsimony (MP), neighbour-joining (NJ), and maximum likelihood (ML) methods.

MP was performed using a heuristic search with tree-bisection-reconnection (TBR). An MP analysis down weighting transitions in 3rd base position (Tv:Ts = 10:1) was also performed for the cytochrome *b* dataset to correct for possible saturation. Support for each clade was assessed by 500 bootstrap replications.

NJ was performed using the distances computed from the GTR + G + I model; the robustness of reconstruction was assessed through 500 bootstrap replications.

The ML tree was constructed using the GTR + G + I model. The robustness was evaluated through 100 bootstrap replicates for the 16S rRNA dataset only. This was not done for the complete cytochrome *b* and the combined datasets due to excessive computing time.

The appropriate substitution model for NJ and ML analyses was chosen using the Modeltest 3.04 program (Posada and Crandall, 1998).

As outgroups, we used two other Gerbillinae, i.e., *Taterillus* sp. from the Central African Republic (sequenced by us; EMBL Accession Nos. for cytochrome *b*:

AJ875307 and for the 16S rRNA: AJ878515) and *Gerbillus nigeriae* (EMBL Accession Nos. are AF141226, for the cytochrome *b* and AF141257, for the 16S rRNA).

Both the cytochrome *b* and 16S rRNA genes were tested for homogeneity in substitution rates along the different lineages through the likelihood ratio test using PAUP 4.0b10, and by the relative rate test using the PHYLTEST 2.0 program (Kumar, 1996). A linearized tree was constructed using the Mega 2.1 program.

3. Results

3.1. Cytogenetics

Two different karyotypes were found. The most common one is characterized by $2n = 36$ and by an autosomal fundamental number (NFa) = 68 (Fig. 2A, only G-banded karyotype shown; chromosome numeration follows Qumsiyeh, 1986). It is shared by the specimens attributed to *T. vicina*, *T. nigricauda*, *T. robusta*, and the one from Rongai (KE120) (Table 1). All autosomes are biarmed and decreasing in size. The X chromosome is a large metacentric, and the Y chromosome is a small acrocentric.

This chromosomal formula has been reported in several other localities: Central African Republic (Matthey and Petter, 1970; although incorrectly attributed to *T. kempi*), Kenya (Qumsiyeh et al., 1987; the two samples, however, differ in two chromosome pairs and were attributed to *T. robusta* and *Tatera* sp., respectively), Tanzania (Fadda et al., 2001; *T. robusta*), Niger (Dobigny et al., 2004; one sample attributed to *Tatera* sp.), and Chad (Granjon and Dobigny, 2003; *T. robusta*).

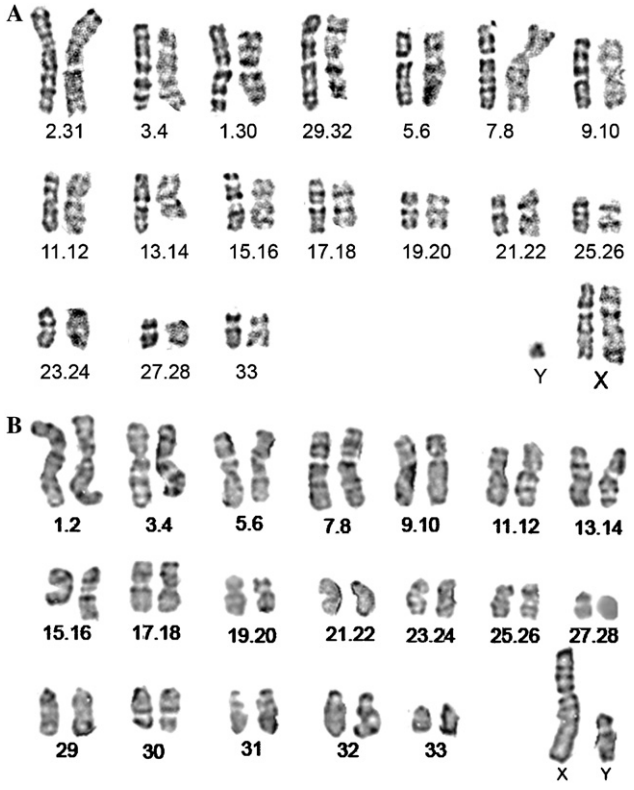

Fig. 2. G-banded karyotypes. (A) Comparison between *T. vicina* (left) and *T. nigricauda* (right), $2n = 36$, NFa = 68. (B) *T. leucogaster* (♂) $2n = 40$, NFa = 66. Chromosome numeration follows Qumsiyeh (1986).

The karyotype of *T. vicina* (Fig. 2A) shows extensive homology in G-banding with that of *T. nigricauda*, except for some minor differences in pairs 13.14 (a deletion/addition in arm 14) and 17.18 (a deletion/addition in arm 18). However, it is likely that the low resolution provided by the G-banding technique made it impossible us to identify other possible chromosomal rearrangements.

The second karyotype (Fig. 2B, G-banded karyotype shown; chromosome numeration follows Qumsiyeh, 1986) is characterized by $2n = 40$ and NFa = 66 and was found in only two specimens from Dakawa, Tanzania (Table 1, Fig. 1). It is composed of 14 pairs of biarmed chromosomes decreasing in size and by five pairs of medium size acrocentrics. The X chromosome is a large metacentric and the Y is a medium-sized submetacentric. The chromosomal morphology and G-banding pattern are comparable to those reported by Qumsiyeh (1986) for the karyotype of *T. leucogaster* from South Africa, except for a small deletion in acrocentric 33. This would confirm our attribution of these two specimens to *T. leucogaster* (see previous section). Qumsiyeh et al. (1987) also found a single specimen from Kenya with a karyotype of $2n = 40$ which they attributed to *T. nigricauda*. This differed from the South African *T. leucogaster* due to a pericentric inversion and a translocation. However, the taxonomic status of this Kenyan sample is cause for some debate.

It would seem that the $2n = 40$ and $2n = 36$ karyotypes of our samples from East Africa show the same chromosomal homologies found by Qumsiyeh et al. (1987), and differ due to three Robertsonian rearrangements with monobrachial homology, one pericentric inversion, and the X.

The limited chromosomal variation and the low diploid numbers found in *T. vicina*, *T. nigricauda*, and *T. robusta* contrast with the high karyotype variability and high diploid numbers recorded in the western ($2n = 46$, 48, 50, and 52) and southern ($2n = 40$ and 44) African species (Benazzou et al., 1984; Codjia et al., 1984; Colangelo et al., 2001; Matthey, 1969; Matthey and Petter, 1970; Qumsiyeh, 1986; Qumsiyeh et al., 1987; Tranier, 1974). For this reason, all further mtDNA analyses included samples from West Africa. These samples were of *T. kempi* from Benin and Burkina Faso. In a previous work (Colangelo et al., 2001) we showed their diploid number and an intraspecific polymorphism, with NFa ranging from 62 to 64, due to a pericentric inversion in a small autosomal pair.

3.2. Sequence composition and phylogenetic relationships

Out of the 495 base pairs obtained for the cytochrome *b*, 206 are variable (41.61%) and 178 informative (35.96%); 39 parsimony informative sites were found at the first codon position, 11 at the second and 128 at the third codon position. The scatter plots of the *P* distances against the absolute number of substitutions showed that saturation for transitions in the third codon position occurs above 15% sequence divergence (data not shown). After alignment, the 16S rRNA fragment was found to be 469 base pairs long, with 121 (25.80%) variable sites, 93 of which (19.79%) are parsimoniously informative. No saturation was observed either in transitions or transversions.

All NJ, MP, and ML trees based on the cytochrome *b* sequences show the same topology (Fig. 3) except for the branching order within the *T. vicina* clade. Weighted and unweighted MP trees show the same topology but differ in the bootstrap values at some nodes (Fig. 3).

Two main clades can easily be recognized: the first (named A) includes only samples from East Africa, while

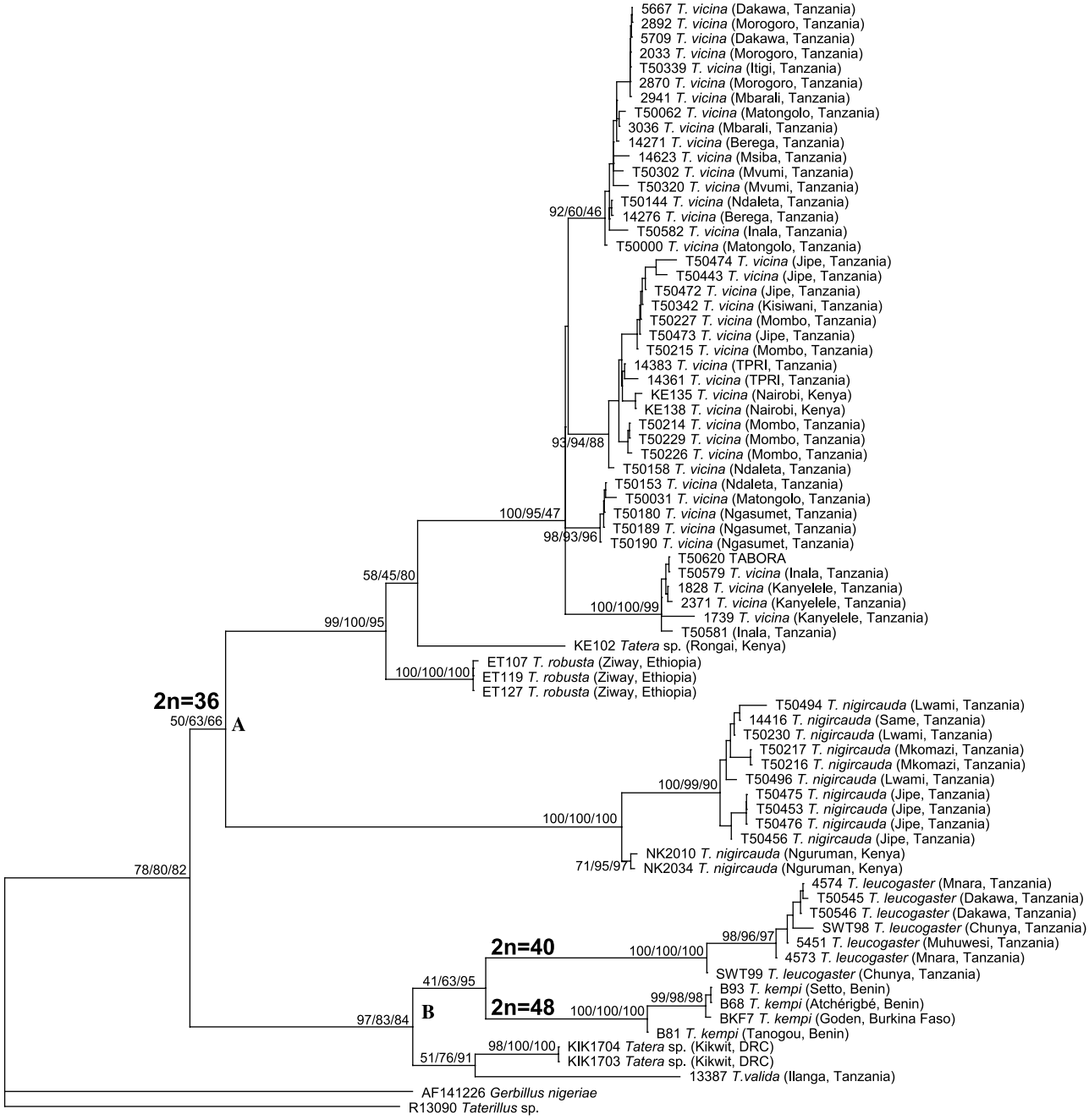

Fig. 3. Cytochrome *b* NJ tree (GTR + G + I model). Numbers are the bootstrap values (500 replications) obtained with NJ, unweighted MP, and weighted MP, respectively. Diploid numbers (2*n*) are reported at each branch when known. Letters A and B indicate the two main clades.

the second (named B) includes samples from West, Central (KIK1703, KIK1704) and East Africa.

All the specimens of clade A are characterized by the same chromosomal formula ($2n = 36$, NFa = 68) and were attributed to *T. vicina*, *T. robusta*, and *T. nigricauda*. Bootstrap values do not support this clade unambiguously, although they do support monophyly for each species (>95%). The specimen from Rongai (KE120) does not clearly belong to any of these three species and is phylogenetically intermediate between *T. vicina* and *T. robusta*.

Tatera nigricauda is the most basal species in clade A; the branch length within this clade implies an early divergence of *T. nigricauda* from the other East African species. Two subclades are recognizable within *T. nigricauda*: one includes the two specimens from Nguruman in Kenya and the other all the remaining specimens from Tanzania. This would suggest the occurrence of population fragmentation due to some restriction in dispersal between northern Tanzania and southern Kenya or to lineage sorting.

Tatera robusta and *T. vicina* are siblings with a recent divergence as suggested by their branch lengths. Within the *T. vicina* lineage, four groups can be recognized with an apparent parapatric distribution (Fig. 1). This would suggest the existence of a population structure, which is similar to what found in *T. nigricauda*.

Reliable values of bootstrap (>80%) support clade B. Two monophyletic subclades occur within, one represented by *T. valida* and the two specimens from Kikwit (DRC), and the other by *T. leucogaster* ($2n = 40$) and *T. kempi* ($2n = 48$; Colangelo et al., 2001) (Fig. 3). However, the dichotomy between these two subclades is questionable, as a good bootstrap is given by weighted MP only. Nonetheless, monophyly of each taxon is highly supported. Within *T. kempi* and *T. leucogaster* two specimens (B81 and SWT99, respectively) show a consistent divergence from their conspecifics, which is possibly due to lineage sorting and divergent mitochondrial lineage retaining.

All phylogenetic analyses (NJ, MP, and ML) on the 16S rRNA sequences produced trees with very similar topologies (Fig. 4, only MP tree show), but with some differences at the deepest node level.

The monophyletic hypothesis and topology for clade A is strongly supported (100% of bootstrap replications).

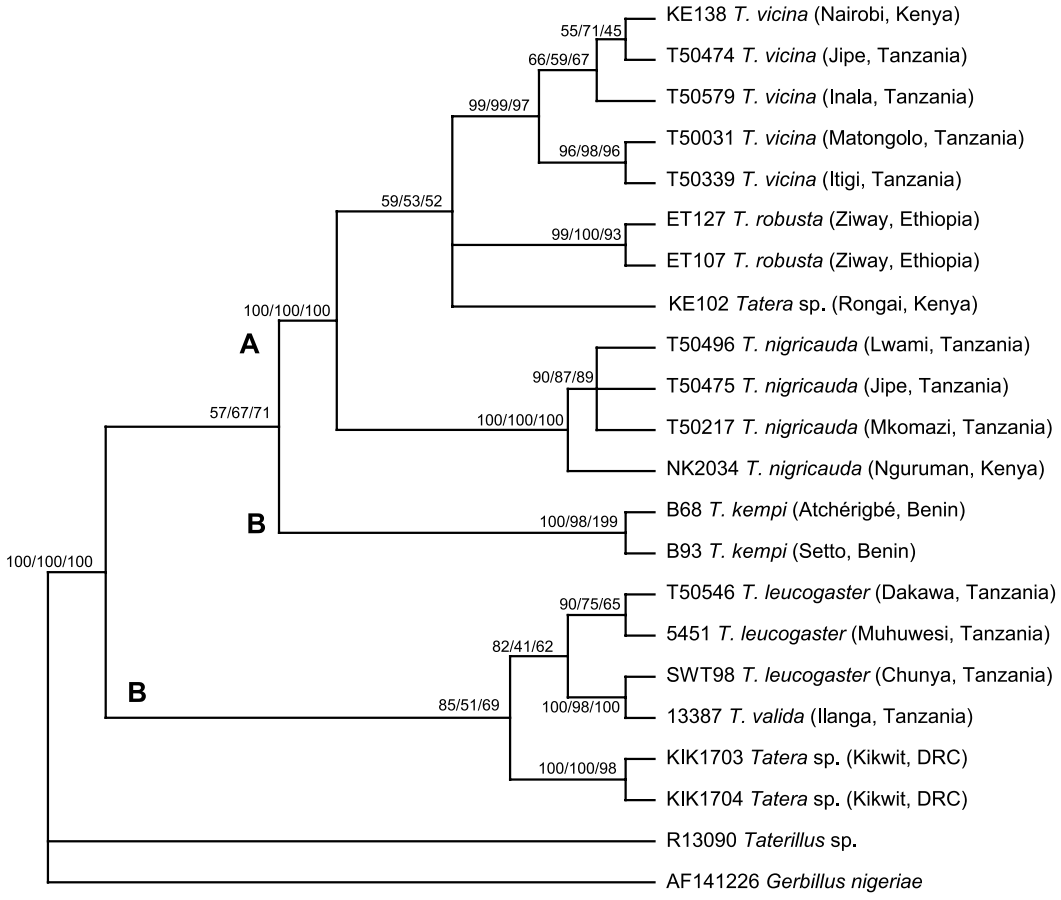

Fig. 4. 16S rRNA maximum parsimony 50% majority-rule consensus tree for a restricted dataset of 20 samples. Tree length = 217, consistency index = 0.71, rescaled consistency index = 0.62. Numbers are the bootstrap values (500 replications) obtained with MP, NJ, and ML, respectively. Letters indicate clades as defined by cytochrome *b* analysis (Fig. 3).

The position of the specimen from Rongai (KE102), however, varies according to the method employed, symptomatic of the 16S rRNA gene limited resolution for more recent cladogenetic events.

The cytochrome *b* monophyly for clade B (Fig. 3) is contradicted by the analysis of the 16S rRNA (Fig. 4): the group including *T. leucogaster*, *T. valida*, and *T. kempi* is polyphyletic because the species do not share a most recent common ancestor, and the *T. leucogaster–T. valida* group is paraphyletic. It should be stressed, however, that low bootstrap values do not support a polyphyletic hypothesis for clade B and a paraphyletic one for *T. leucogaster–T. valida.*

The combined analysis of the cytochrome *b* and 16S rRNA datasets produced a tree (not shown) with a topology that was close to the one obtained using only the cytochrome *b*. However, the branching order of clade B in the combined tree is not supported by reliable bootstrap values.

In conclusion, monophyly is supported for clade A only by both the cytochrome *b* and 16S rRNA analyses, but it is rejected for clade B by the 16S. It is not possible to definitively resolve the phylogenetic relationships within clade B and the analyses do not help to clarify the taxonomic status of the samples from Rongai (KE102) and from Kikwit (KIK1703, KIK1704). The 16S rRNA does not seem to be as appropriate as a molecular marker as the cytochrome *b* in the assessment of the systematic relationships within *Tatera*. Likelihood ratio tests (see below) show the occurrence of heterogeneity in substitution rates among 16S rRNA sequences, and therefore the use of its sequences should be considered with caution.

3.3. Genetic divergence

The mean genetic divergence (K2P) is 13.8% for the cytochrome *b* and 8.4% for the 16S rRNA. The examination of the transition/transversion ratio (Table 2) shows the common pattern found in other Rodent genera (e.g., *Arvicanthis*, Ducroz et al., 1998). As would be expected, phylogenetically close species, i.e., *T. robusta–T. vicina* and *T. kempi–T. leucogaster* have a higher ratio than species that are more distant, e.g., *T. nigricauda–T. kempi* (Table 2). These values increase to 1:27 in within-species comparisons. This also aligns with other findings in other Eutherian orders (Primates, Rodents, and Whales) reported by Yang and Yolder (1999).

The cytochrome *b* genetic divergence between species (Table 2) ranges from 9.7 to 23.1%, and falls in the upper part of the range of values found between species of other rodent genera (Bradley and Baker, 2001). The high divergence (21.6%) between *T. vicina* and *T. nigricauda* (Table 2) is considerable; these two species have been found in sympatry in one locality at the northern limits of the Maasai Steppe (Jipe, Table 1 and Fig. 1) and share the same chromosomal formula. Genetic divergence between *T. vicina* and *T. robusta* is 10.6% (Table 2). The specimens from Rongai KE102 show a lower genetic divergence with *T. robusta* (9.7%) than with *T. vicina* (11.4%).

The intraspecific genetic divergences within the *T. vicina* (from 3.2 to 5.4%) and *T. nigricauda* (5.1%) subclades are quite high, supporting an hypothesis of population fragmentation.

The levels of genetic divergence among *T. leucogaster*, *T. kempi*, and *T. valida* are comparable and range from 14.1 to 15.7% (Table 2). The specimens from Kikwit (KIK1703, KIK1704) show less genetic divergence from *T. valida* (10.2%) than from *T. leucogaster* (15.6%) and *T. kempi* (15.4%).

The between species divergence based on 16S rRNA ranges from 2.1 to 12.4% (Table 2). Similarly to the cytochrome *b*, the values found among *T. vicina*, *T. robusta*, and KE102 are low, while those of *T. nigricauda* are the highest within the *robusta* group (Table 2). Likewise, the 16S genetic divergence between *T. leucogaster* and *T. valida* is the lowest found within the genus (2.1%) and the high genetic affinity between the specimens from Kikwit with *T. valida* (3.9%) and *T. leucogaster* (3.8%) is confirmed.

3.4. Times of divergence

One of our aims was to relate the appearance of chromosomal rearrangements to mitochondrial lineage dispersion and to the past environmental changes which lead to the creation of the East African savannahs. Therefore, we estimated the temporal origin of the genus, that of the split of the *robusta* group (*T. nigricauda*,

Table 2
Genetic divergence (Kimura two-parameters) and the transition/transversion ratio for the cytochrome *b*

	T. vicina	*T. robusta*	*T. nigricauda*	*T. leucogaster*	*T. valida*	*T. kempi*
T. vicina		0.057	0.073	0.117	0.122	0.103
T. robusta	0.106/6.037		0.082	0.119	0.123	0.098
T. nigricauda	0.216/2.562	0.195/2.995		0.120	0.124	0.118
T. leucogaster	0.231/2.486	0.219/3.028	0.215/1.445		0.021	0.067
T. valida	0.226/2.438	0.202/2.486	0.204/1.582	0.145/3.089		0.073
T. kempi	0.227/2.382	0.201/2.666	0.203/1.481	0.157/6.486	0.141/2.756	

Below the diagonal: cytochrome *b* distances and the transition/transversion ratio; above the diagonal: 16S rRNA distances.

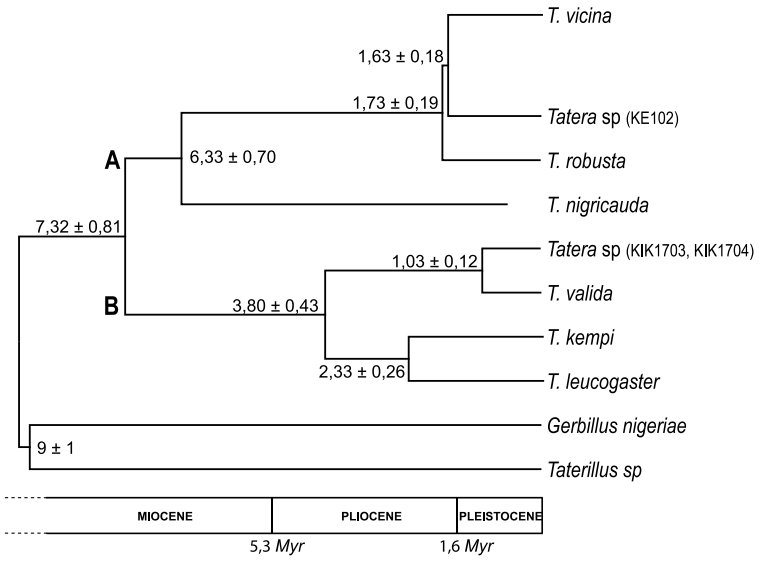

Fig. 5. Linearized tree showing divergence times (values are in Myr) at each dichotomy estimated from the uncorrected distances. Only transversional substitutions were used (1st + 2nd + 3rd codon positions). Letters A and B identify the two major clades.

T. robusta, and *T. vicina*) from the other species and its successive branching within the East African lineage.

Tong (1989), on the basis of fossil records, placed the dichotomy between the two tribes of Gerbillinae, i.e., Taterillini (including *Taterillus*, *Tatera*, and *Gerbillurus*) and Gerbillini (including *Gerbillus* and the other genera) in a period ranging between 8 and 10 Myr. We, therefore, used these dates to calibrate the split between *Gerbillus* and *Taterillus*. The likelihood ratio test did not reject constancy in the molecular clock for the cytochrome *b* dataset ($p > 0.01$), although the hypothesis is rejected for the 16S rRNA ($p < 0.01$). Furthermore, the relative rate tests using the *P* distances based on transversions revealed heterogeneity in substitution rates for the 16S rRNA (data not shown) but constancy for the cytochrome *b*. Consequently, the cytochrome *b* dataset only was used for the estimation of the times of divergence.

A linearized tree (Fig. 5) was constructed from a NJ tree obtained using the *P* distances based on transversions only (for all three codon positions) as these accumulate linearly as shown by saturation plots and, therefore, provide a more reliable estimate.

The substitution rate in transversions ranges between 0.36 and 0.45% per Myr. The molecular clock places the origin of the genus at 8.14 ± 1.63 Myr. The most ancient fossil record of *Tatera* in Africa occurs in a layer dated between the end of Miocene and the early Pliocene (Tong, 1989), suggesting that the upper bound of the value obtained may represent an overestimation. Older fossils may still be found, but it should be stressed that the use of a molecular clock may result in an overestimation (Rodriguez-Trelles et al., 2002; Wray, 2001).

The separation between clade A and clade B occurred at 7.32 ± 0.81 Myr, i.e., during Early Pliocene. Within clade A, *T. nigricauda* has a late Miocene origin (6.33 ± 0.7 Myr), while *T. vicina*, *T. robusta*, and the taxon represented by KE102 originated more recently (2.30–1.53 Myr), at the turn of Pliocene and Pleistocene. This period was characterized by an intense speciation also for clade B, with the appearance of *T. kempi*, *T. leucogaster*, *T. valida*, and the DRC taxon.

4. Discussion

4.1. Taxonomy

Specimen recognition through "bar codes" (Blaxter, 2004) and comparison with type specimens and larger museum series led to the clear identification of six species and the occurrence of two further taxa or MOTUs that, for the moment, cannot be properly assigned to any recognized taxon.

Our results provide strong evidence that *T. vicina* should not be synonymized with *T. robusta* and that the former constitutes a separate valid species. Until now (Musser and Carleton, 1993) populations from the central and northern Tanzanian savannahs have all been attributed to *T. robusta*. However, a significant geographic discontinuity within this taxon was reported by Bates in 1988. He recognized a distinct race in central Tanzania. *T. vicina* and *T. robusta* share the same 2*n* and NFa and have a relatively low genetic divergence when compared to the other species pairs (see Table 2). However, this amount of genetic differentiation may be sufficient to characterize distinct species and is similar to what was found by Bradley and Baker (2001) in a set of genetic distances computed from the cytochrome *b* sequences in four different rodent genera, where the average percentage in sequence divergence ranges from

0 to 2.9% at the intraspecific level, and from 2.7 to 19.23% between sister species. The values found between *T. vicina* and *T. robusta* (Table 2) correspond to the second category, supporting our conclusion that these are two distinct and valid species, which have been separated since the Plio-Pleistocene (Fig. 5). The distribution of *T. vicina* (type locality: Kitui, Ukambani, Kenya) approximately extends from the southern limit of the Somali-Maasai biome (8°S) to southern Kenya (1°S), with some overlapping with *T. leucogaster* in the South and *T. nigricauda* in the North (see Fig. 1 and Table 1). Consequently, the extended range of the real *T. robusta* (see Musser and Carleton, 1993) needs to be carefully checked and recalculated.

KE102 from Rongai, Kenya, seems to represents a further separate species related to *T. vicina* and *T. robusta*. It should be noted (Bates, 1988) that *T. phillipsi* occurs in the same area and along the Rift Valley up to Ethiopia (its range is restricted to the Rift). Although we think this specimen should be assigned to *T. phillipsi*, we suggest caution when using genetic distances for species identification (Ferguson, 2002), particularly if there is no comparison with an extended data set including type locality specimens.

The same can be said for the samples from DRC (KIK1703, KIK1704). They are phylogenetically close to *T. valida* and *T. leucogaster*, although separated by high genetic distances. For the moment no taxonomic hypothesis for these specimens can be made. Therefore, these MOTUs were provisionally named as *Tatera* sp.

Finally, we suggest that clade A (including the East African species *T. nigricauda*, *T. robusta*, *T. vicina*, and KE102) represents the real *robusta* group, following the definition of Davis (1975). Furthermore, there is no support for the inclusion of *T. leucogaster* in this group as stated by Davis (1975). We suggest this species should be included in the *afra* group (also *sensu* Davis, 1975).

4.2. Systematics and zoogeography

The separation of the *robusta* group (clade A) from the other species of clade B occurred about 7 Myr ago, approximately following the expansion of arid savannah habitats during the rifting. Since then, forest fragmentation has led to the creation of the typical East African savannah biome, found today.

The apparently stable karyotype of the *robusta* group does not support any suggestion of chromosomal speciation within this assemblage (see below). Speciation may instead have been driven by the repeated events of savannah contractions that characterized the Rift Valley area since those times. The ages of the rifting activity that determined the main features of the current landscape of East and Central Africa (Griffiths, 1993) match our estimate for a late Miocene divergence between *T. nigricauda* and the other "*robusta*" species (Fig. 5). Bates (1985, 1988) suggested that *T. nigricauda* evolved during a particularly protracted pluvial period in the areas south of the River Tana. The subsequent formation of the Eastern Rift Valley, with its low precipitation rates, then constituted an efficient barrier so blocking its expansion. The differing distribution pattern of the other *robusta* taxa (i.e., *T. robusta* and *T. vicina*) could reflect a higher ecological tolerance to arid conditions (Bates, 1988).

Speciation of *T. robusta* and *T. vicina* (and possibly, of the taxon represented by KE102) occurred later during the end of the Pliocene and Early Pleistocene (Fig. 5). Habitat fragmentation in the Afar and East African plateau during this period (Partridge et al., 1995) was most probably the cause of their origin, triggered by savannah expansion/contraction, volcanism, and tectonic activity. These phenomena were common until 2 million years ago (Partridge et al., 1995) and produced restricted and isolated areas of suitable habitats for these species. Furthermore, the fact that different subclades can be recognized within *T. nigricauda* and *T. vicina* (Fig. 3) supports the idea that the same ecological and geographical factors that drove speciation within the *robusta* lineage have been extended to very recent times and produced the current population fragmentation.

The other East African species, *T. leucogaster*, does not share any recent evolutionary history with the *robusta* group. However, it is phylogenetically close to other species of clade A (*T. valida* and *T. kempi*). Moreover, its karyotype is similar to those of *T. brantsii* and *T. afra* (Qumsiyeh, 1986), two species endemic of southern Africa. This would suggest a southern African origin for *T. leucogaster*, its current range being the result of its expansion northwards to the limits of the southern Tanzanian savannahs. This geographical limit is caused by the arid conditions of the Somali-Maasai biome and/or by the competition with *T. vicina*. In fact, *T. leucogaster* was only found in the contact area between the Somali-Maasai and Zambezian biomes (central-southern Tanzania), an area characterized by higher rainfall compared to the arid savannahs of the North.

Recent data on rodents (Faulkes et al., 2004) and bovids (Arctander et al., 1999; Flagstad et al., 2001) highlight the role of the rifting activity and consequential climatic changes from Late Miocene to Pleistocene over the diversification of African savannah faunas. For example, Faulkes et al. (2004) showed how vegetation changes due to the formation of the Rift Valley affected speciation and distribution of the mole-rats Bathyergidae. They also underlined how different environmental stress arising from this geological activity resulted in different speciation patterns, even within the single genus *Cryptomys*. Here we suggest a similar pattern for the evolution of *Tatera*. In fact, we suggest that the rifting activity and the consequential climatic changes played a fundamental role in the diversification within the eastern African *Tatera* lineages.

4.3. Chromosomal evolution

Karyotypic evolution in *Tatera* has included numerous and repeated complex rearrangements such as inversions, centric fusions and fissions, translocations and heterochromatic additions (Qumsiyeh and Schlitter, 1991). Qumsiyeh (1986) and Qumsiyeh et al. (1991), on the basis of G-band analysis, suggested $2n=52$ for the ancestral karyotype for gerbils and $2n=44$ for *Tatera*. If this were to be true, then the $2n=36$ karyotype of the *robusta* lineages would represent a derived form. Qumsiyeh et al. (1987) also proposed a phylogeny based on chromosomal rearrangements. It is not possible to compare all the G-banded karyotypes (presented here and available from the literature) due to the different quality of the chromosomes and banding resolution. Therefore, we have avoided any inaccurate chromosomal phylogenies but we hypothesize a possible scenario of chromosomal evolution.

In accordance with Qumsiyeh and Schlitter (1991), we suggest that the $2n=36$ of the *robusta* group and $2n=40$ of *T. leucogaster* originated from an ancestral $2n=44$ karyotype. This ancestral condition has been preserved by *T. brantsii* and *T. afra* in the southern part of the continent. The $2n=36$ evolved in East Africa approximately 7 Myr after three Robertsonian fusions (1.30, 2.31, 29.32) and has been preserved in the form of the typical *robusta* karyotype of the arid savannahs of the Somali-Maasai. It would be surprising if the limited chromosomal variation found (Qumsiyeh et al., 1987; and this paper) caused a reduction in gene flow and favoured speciation. Therefore, the high levels of genetic divergence found (Table 2) would seem to suggest that speciation has proceeded independently from karyotype evolution, possibly favoured by geographic barriers, such as mountain ranges, lakes and the rift depressions, and/or in strict allopatry during phases of savannah contraction.

On the other hand, on the western side of the Rift Valley extensive chromosomal variation—typical of other genera of the subfamily Gerbillinae (see, for a list of karyotypes, Qumsiyeh and Schlitter, 1991)—became predominant. From the $2n=44$ ancestral karyotype, the $2n=40$ of *T. leucogaster* originated after two Robertsonian fusions (1.2, 7.8) and spread into the moist Zambezian savannahs; likewise the other karyotypes originated during the expansion in West Africa, with fissions being predominant ($2n=46$–48, *T. kempi*; $2n=50$, *T. guineae*; $2n=52$, *T. gambiana* Thomas, 1910, but in synonymy with *T. kempi*, Musser and Carleton, 1993).

It is well known that rodents usually display high intra- and interspecific chromosomal polymorphism (see for example, the African rodent *Arvicanthis*; Capanna et al., 1996; Castiglia et al., 2003; Corti, 2001; Volobouev et al., 2002a, for a discussion). For this reason it has been suggested that chromosomal rearrangements have played a role in speciation (see King, 1993, and references therein). Because of meiotic problems in the structural heterozygote hybrids, chromosomal rearrangements can drastically reduce gene flow (see Capanna, 1982, for Robertsonian rearrangements in *Mus*; and King, 1993, for a general discussion). Although an experimental study in the wild or in the laboratory has not yet been attempted regarding the reproductive fitness of the hybrids, one may hypothesize that chromosomal changes had a direct role in the speciation within the west African taxa, following a stasipatric-like (White, 1978) or alloperipatric (Capanna and Redi, 1994) model. One should note, however, that there are also cases where limited negative heterotic effects in structural heterozygotes have been shown, such as for the pericentric inversions in the African rodent *M. erythroleucus* (Volobouev et al., 2002b), but also for Robertsonian fusions in *Mus* (Castiglia and Capanna, 2000).

While the role of chromosomal rearrangements in favouring speciation has been extensively discussed, the two different evolutionary strategies within *Tatera*, i.e., the "unusual" karyotypic stability (the *robusta* group, clade A) or the extensive chromosomal reordering (clade B), should be better investigated as they may depend strictly on the structural properties of the chromosome itself.

There has been considerable debate concerning the role of heterochromatin (when and when not detected by C-banding) as a molecular drive for structural rearrangements of the karyotype (reviewed by Redi et al., 2001), such as during the Robertsonian formation of metacentrics (Garagna et al., 1995). For example, repetitive DNAs intragenomic movements are associated to heterochromatin transfer among homologous and non homologous chromosomes and are related to speciation (see Garagna et al., 1995; Hamilton et al., 1990, for Rodents and Wichman et al., 1991, for the Equids). Colangelo et al. (2001) found in *T. kempi* evident heterochromatic blocks at the centromere of all acrocentrics and less evident blocks in most of the biarmed chromosomes. Qumsiyeh (1986) obtained similar results in *T. afra*, *T. brantsii*, and *T. leucogaster*, suggesting repetitive DNAs families may have been involved, at least, in Robertsonian processes. However, this still does not reveal or explain the structural and functional causes at molecular level that have preserved the $2n=36$ karyotype for a long time from any form of rearrangement in one case, and have continued allowing chromosomal rearrangements in the other. Therefore, these structural and functional causes must be better investigated.

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References

Arctander, P., Johansen, C., Coutellec-Vreto, M.-A., 1999. Phylogeography of three closely related African bovids (Tribe Alcelaphini). Mol. Biol. Evol. 16, 1724–1739.

Bates, P.J.J., 1985. Studies of gerbils of genus *Tatera*: the specific distinction of *Tatera robusta* (Cretzschmar, 1826), *Tatera nigricauda* (Peters, 1978) and *Tatera phillipsi* (De Winton, 1898). Mammalia 49, 37–52.

Bates, P.J.J., 1988. Systematic and zoogeography of *Tatera* (Rodentia: Gerbillinae) of north-east Africa and Asia. Bonn. Zool. Beitr. 39, 265–303.

Benazzou, T., Viegas-Pequignot, E., Prod'Homme, M., Lombard, M., Setter, F., Dutrillaux, B., 1984. Chromosomal phylogeny of Gerbillidae. III. Species study of the genera *Tatera*, *Taterillus*, *Psammomys* and *Pachyuromys*. Ann. Genet. 27, 17–26.

Blaxter, M.L., 2004. The promise of a DNA taxonomy. Phil. Trans. R. Soc. Lond. B 259, 669–679.

Bradley, R.D., Baker, R.J., 2001. A test of genetic species concept: cytochrome *b* sequences and mammals. J. Mamm. 82, 960–973.

Capanna, E., 1982. Robertsonian numerical variation in animal speciation: *Mus musculus*, an emblematic model. In: Barigozzi, C. (Ed.), Mechanisms of Speciation. A. Liss, New York, pp. 155–177.

Capanna, E., Redi, C.A., 1994. Chromosomes and microevolutionary process. Boll. Zool. 61, 285–294.

Capanna, E., Afework, B., Capula, M., Castiglia, R., Civitelli, M.V., Codja, J.T.Cl., Corti, M., Fadda, C., 1996. A multidisciplinary approach to the systematics of the genus *Arvicanthis*, Lesson 1842 (Rodentia, Murinae). Mammalia 60, 677–696.

Castiglia, R., Capanna, E., 2000. Contact zones between chromosomal races of *Mus musculus domesticus.* 2. Fertility and segregation in laboratory-reared and wild mice multiple heterozygous for Robertsonian rearrangements. Heredity 85, 147–157.

Castiglia, R., Corti, M., Tesha, P., Scanzani, A., Fadda, C., Capanna, E., Verheyen W, 2003. Cytogenetics of the genus *Arvicanthis* (Rodentia, Muridae). 3. Comparative cytogenetics of *A. neumanni* and *A. nairobae* from Tanzania. Genetica 118, 33–39.

Codjia, J.T.Cl., Chrysostome, C., Civitelli, M.V., Capanna, E., 1984. Les chromosome des Rongeurs du Bénin (Afrique de l'Ouest): 1. Cricetidae. Rend. Fis. Acc. Lincei 5 (9), 277–287.

Colangelo, P., Civitelli, M.V., Capanna, E., 2001. Morphology and chromosomes of *Tatera* Lataste 1882 (Rodentia Muridae Gerbillinae) in West Africa. Trop. Zool. 14, 243–253.

Corti, M., 2001. Rodent taxonomy at the end of the 20th century. Delectatur musica. Bull. Inst. R. Sci. Nat. Belg. 71, 17–33.

Davis, D.H.S., 1949. The affinities of the South African Gerbils of the genus *Tatera*. Proc. Zool. Soc. Lond. 144, 1002–1018.

Davis, D.H.S., 1965. Classification problems of African Muridae. Zoologica Africana I (I), 125–145.

Davis, D.H.S., 1966. Contribution to the revision of the genus *Tatera* in Africa. In: Proceedings of the Colloquium on African Rodents. Brussels-Tervuren, 1964. Ann. Mus. R. Afr. Cent. 144, 49–65.

Davis, D.H.S., 1975. Part 6.4, Genera *Tatera* and *Gerbillurus*. In: Meester, J., Setzer, H.W. (Eds.), The Mammals of Africa: An Identification Manual. Parts 1–15. 1971–1977. Smithsonian Inst. Press, Washington.

Denys, C., Lecompte, E., Granjon, L., Baylac, M., Cordeiro, P., Cornette, R., Dobigny, G., Fichet-Calvet, E., Hugot, J.-P., Meslage, C., Millien-Parra, V., Petrillo, P., Volobouev, V., Welz, M., 2003. Integrative systematics: the importance of combining techniques for increasing knowledge of African Murinae. In: Singleton, G.R., Hinds, L.A., Krebs, C.J., Spratt, D.M. (Eds.), Rats, Mice and People: Rodent Biology and Management. Australian Centre for International Agricultural Research, Camberran.

Dobigny, G., Ducroz, J.-F., Robinson, T.J., Volobouev, V., 2004. Cytogenetics and cladistics. Syst. Biol. 53, 470–484.

Ducroz, J.F., Volobouev, V., Granjon, L., 1998. A molecular perspective on the systematics and evolution of the genus *Arvicanthis* (Rodentia, Muridae): inferences from complete cytochrome *b* gene sequences. Mol. Phyl. Evol. 10, 104–117.

Fadda, C., Castiglia, R., Colangelo, P., Corti, M., Machang'u, R., Makundi, R., Scanzani, A., Tesha, P., Verheyen, W., Capanna, E., 2001. The rodent fauna of Tanzania: a cytotaxonomic report from the Maasai Steppe 1999. Rend. Fis. Acc. Lincei 12 (9), 29–49.

Faulkes, C.G., Verheyen, E., Verheyen, W., Jarvis, J.U.M., Bennett, N.C., 2004. Phylogeographic patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). Mol. Ecol. 13, 613–629.

Ferguson, J.W.H., 2002. On the use of genetic divergence for identifying species. Biol. J. Linn. Soc. 75, 509–516.

Flagstad, Ø., Syvertsen, P.O., Stenseth, N.C., Jakobsen, K.S., 2001. Environmental change and rates of evolution: the phylogeographic pattern within the hartebeest complex as related to climatic variation. Proc. R. Soc. Lond. 268, 667–677.

Garagna, S., Broccoli, D., Redi, C.A., Searle, J.B., Cooke, H.J., Capanna, E., 1995. Robertsonian metacentrics in the house mouse lose telomeric sequences but retain some minor satellite DNA in the pericentromeric area. Chromosoma 103, 685–692.

Granjon, L., Dobigny, G., 2003. The importance of cytotaxonomy in understanding the biogeography of African rodents: Lake Chad murids as an example. Mammal Rev. 33, 77–91.

Griffiths, J.C., 1993. The geological evolution of East Africa. In: Colvett, J., Wasser, S.K. (Eds.), Biogeography and Ecology of the Rain Forest of Eastern Africa. Cambridge University Press, Cambridge, pp. 9–21.

Hamilton, M.J., Honeycutt, R.L., Baker, R.J., 1990. Intragenomic movement, sequence amplification and concerted evolution in satellite DNA in harvest mice, *Reithrodontomys*: evidence from in situ hybridisation. Chromosoma 99, 321–329.

Hsu, T.C., Patton, J.L., 1969. Bone marrow preparation for chromosomes studies. In: Comparative Mammalian Cytogenetics. Springer, Berlin, pp. 454–460.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. 32, 128–144.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

King, M., 1993. Species Evolution, the Role of Chromosome Change. Cambridge University Press, Cambridge.

Kumar, S., 1996. PHYLTEST: a program for testing phylogenetic hypothesis. Version 2.0. Pennsylvania State University.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis Software, Arizona State University, Tempe, Arizona, USA.

Matthey, R., 1969. Chromosomes de Gerbillinae genres *Tatera* e *Taterillus*. Mammalia 33, 522–528.

Matthey, R., Petter, F., 1970. Etude cytogenetique et taxonomique de 40 *Tatera* et *Taterillus* provenant de Haute-Volta et de Republique Centrafricaine (Rogeurs, Gerbillidae). Mammalia 34, 585–597.

Musser, G.G., Carleton, M.D., 1993. Family Muridae. A taxonomic and geographic reference. In: Wilson, D.E., Reeder, D.M. (Eds.), Mammal Species of the World. Smithsonian Instit. Press, Washington/London, pp. 560–562.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR, version 2.0. Univ. of Hawaii Press, Honolulu.

Partridge, T.C., Wood, B.A., deMenocal, P.B., 1995. The influence of global climate changes and regional uplift on large-mammalian evolution in East and Southern Africa. In: Vrba, E.S. (Ed.), Paleoclimate and Evolution with Emphasis on Human Evolution. Yale University Press, New Haven, CT, pp. 331–356.

Pavlinov, I.J., 1999. Current concepts of Gerbillid phylogeny and classification. In: IRD (Ed.), African Small Mammals. Proceedings of the 8th International Symposium on African Small Mammals, Paris, pp. 141–149.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Quérouil, S., Hutterer, R., Barriére, P., Colyn, M., Peterhans, J.C.K., Verheyen, E., 2001. Phylogeny and evolution of African shrews (Mammalia: Soricidae) inferred from 16S rRNA sequences. Mol. Phylogenet. Evol. 20, 185–195.

Qumsiyeh, M.B., 1986. Phylogenetic studies of the rodent family Gerbillidae: I. Chromosomal evolution in the southern African complex. J. Mamm. 67, 680–692.

Qumsiyeh, M.B., Hamilton, M.J., Schlitter, D.A., 1987. Problems in using Robertsonian rearrangements in determining monophyly: examples from the genera *Tatera* and *Gerbillurus*. Cytogenet. Cell Genet. 44, 198–208.

Qumsiyeh, M.B., Schlitter, D.A., 1991. Cytogenetic data on the rodent family Gerbillidae. Occas. Papers Mus. Texas Tech Univ. 144, 1–20.

Qumsiyeh, M.B., Meredith, J.H., Hamilton, M.J., Dempster, E.R., Baker, R.J., 1991. Cytogenetics and systematics of the rodent genus *Gerbillurus*. J. Mamm. 72, 89–96.

Redi, C.A., Garagna, S., Zacharias, H., Zuccotti, M., Capanna, E., 2001. The other chromatin. Chromosoma 110, 136–147.

Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. Trends Ecol. Evol. 8, 129–132.

Rodriguez-Trelles, F., Tarrio, R., Ayala, J., 2002. A methodological bias toward overestimation of molecular evolutionary time scales. Proc. Natl. Acad. Sci. USA 99, 8112–8115.

Seabright, M.A., 1971. A rapid banding technique for human chromosomes. Lancet 2, 971–972.

Singleton, G.R., Hinds, L.A., Leirs, H., Zhang, Z., 1999. Ecologically-based management of rodent pest. ACIAR Monograph No. 59, 494 p., Camberran.

Staplerat, 2003. Protecting staple crops in eastern Africa: integrated approaches for ecologically based field rodent pest management. Final report. Contract Number ICA4-CT-2000-30029, an European Union Shared-Cost Project.

Swofford, D.L., 1998. PAUP*: Phylogenetic Analysis using Parsimony (* and Other Methods). Version4. Sinauer Associates, Sunderland, Massachusetts.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Tranier, M., 1974. Dimorphisme chromosomique dans une population de *Tatera* du sud du Tchad (Rongeurs, Gerbillidae). Mammalia 38, 224–233.

Tong, H., 1989. Origine et évolution des Gerbillidae (Mammalia, Rodentia) en Afrique du Nord. Mem. Soc. Geol. Fr. 155, 120.

Viegas-Pequignot, E., Benazzou, T., Dutrillaux, B., Petter, F., 1982. Complex evolution of sex chromosomes in Gerbillidae (Rodentia). Cytogenet. Cell Genet. 34, 158–167.

Volobouev, V.T., Ducroz, J.-F., Aniskin, V.M., Britton-Davidian, J., Castiglia, R., Dobigny, G., Granjon, L., Lombard, M., Corti, M., Sicard, B., Capanna, E., 2002a. Chromosomal characterization of *Arvicanthis* species (Rodentia, Murinae) from western and central Africa: implications for taxonomy. Cytogenet. Gen. Res. 96, 250–260.

Volobouev, V.T., Aniskin, V.M., Lecompte, E., Ducroz, J.F., 2002b. Patters of karyotype evolution in complexes of sibling species within three genera of African muroid rodents inferred from the comparison and molecular data. Cytogenet. Gen. Res 96, 261–274.

White, M.J.D., 1978. Modes of Speciation. W.H. Freeman Co., San Francisco, CA pp. 454.

Wichman, H.A., Payne, C.T., Ryder, O.A., Hamilton, M.J., Maltbie, M., Baker, R.J., 1991. Genomic distribution and heterochromatic sequences in Equids: implications to rapid chromosomal evolution. J. Hered. 82, 369–377.

Wray, G.A., 2001. Dating branches on tree of life using DNA. Genome Biol. 3, 1–7.

Yang, Z., Yolder, A.D., 1999. Estimation of the transition/transversion rate bias and species sampling. J. Mol. Evol. 48, 274–283.