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Evolutionary systematics in African gerbilline rodents of the genus *Gerbilliscus*: Inference from mitochondrial genes

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Abstract

Gerbilliscus has recently been proposed as an endemic African rodent genus distinct from the Asian *Tatera*. A molecular phylogeny of the genus, including nine species from southern, western and eastern Africa, is presented here based on the analysis of the cytochrome *b* and 16S mitochondrial genes. With an adequate taxonomic sampling over a wide geographic range, we here provide a clear picture of the phylogenetic relationships between species and species groups in this genus. Three distinct clades were resolved, corresponding to major geographical subdivisions: an eastern clade that possibly diverged first, then a southern and a western clades which appeared later. We suggest two possible hypotheses concerning the dispersal of the genus across Africa, considering also the patterns of karyotypic variation. Finally, we discuss the taxonomic status of *G. gambianus* and the relationships between *Gerbillurus* and *Gerbilliscus*, as previous studies have suggested that the former should be included in the latter. Our data seem to support the synonymy of the two taxa and suggest that *Gerbillurus* and *Gerbilliscus* lineages diverged from a common ancestor appeared in eastern Africa.

Keywords: African rodents; *Gerbilliscus*; *Gerbillurus*; Phylogeny; Cytochrome *b*; 16S; mtDNA

1. Introduction

The advance of molecular techniques applied to systematics has provided a particularly useful tool, which can improve our knowledge of the taxonomy and evolution of rodents at any taxonomic level (Barome et al., 1998; Ducroz et al., 2001; Fadda et al., 2001; Steppan et al., 2004, 2005; Lecompte et al., 2005). Concerning one of the most speciose rodent subfamilies, the Gerbillinae, recent phylogenetic reconstructions using mtDNA (Chevret and Dobigny, 2005; Colangelo et al., 2005) showed how the use of molecular phylogenetics can shed some light on questions where morphometrics and/or cytogenetics gave conflicting or ambiguous answers.

Morphological characters are under constant pressure of adaptive selection which can lead to convergent evolution of morphological traits, even in taxa that do not share a recent common evolutionary history (Fadda and Corti, 2001; Lecompte et al., 2005). On the other hand, although the use of comparative cytogenetics often makes it possible to identify sibling species (Taylor, 2000; Granjon and Dobigny, 2003), phylogenetic reconstructions based on chromosomal rearrangements may fail to distinguish some homoplasies and ill define ancestral states (Qumsiyeh et al., 1987; Dobigny et al., 2004). Moreover, in particular circumstances, it may be difficult to establish whether different chromosomal forms are effectively reproductively isolated or not while the combination of molecular and chromo-

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somal data could add useful information on the real level of isolation of two chromosomal races and their taxonomic status (for an example, see the case of *Mastomys erythroleucus* in Volobouev et al., 2001, 2002).

In this paper, we focus on the African murid rodent genus *Gerbilliscus* Thomas, 1897, a taxon considered, just few years ago, to be a subgenus of *Tatera* Lataste, 1882 (Musser and Carleton, 1993; Pavlinov, 2001). *Gerbilliscus* Thomas, 1897, which includes all the African species of the former *Tatera* genus, has recently been elevated to genus rank by Musser and Carleton (2005), as a consequence of the morphological differences between these two taxa (summarized in Pavlinov, 2001). As a result, the Asian *Tatera indica* (Hardwicke, 1807) remains the only species of this genus (Musser and Carleton, 2005). The validity of the separation of Asian and African species into two distinct genera has been recently strengthened by molecular analyses (Chevret and Dobigny, 2005), but the systematics of the new genus *Gerbilliscus* remains unclear. In particular, chromosomal (Qumsiyeh et al., 1987, 1991), allozymic (Qumsiyeh et al., 1987), and molecular (Chevret and Dobigny, 2005) data suggest there are intricate relationships among the species of *Gerbilliscus* and *Gerbillurus* Shortridge, 1942 species. Furthermore, the value of the *Gerbilliscus* "*afra*" and "*robusta*" groups proposed by Davis (1966, 1975) for the whole Africa, based on morphological considerations, and confirmed for West African species via morphometric analyses (Granjon, 2005) needs to be tested by characters that are supposed not to be influenced by environmental factors. Moreover, recent molecular data (Colangelo et al., 2005) have suggested the possible occurrence of sibling species within the genus *Gerbilliscus*. These include *G. gambianus* (Thomas, 1910), still considered a synonym of *G. kempi* Wroughton, 1906 in Musser and Carleton (2005), but unambiguously characterized by chromosomal data as a valid biological species [Hubert et al. (1973), following Matthey and Petter (1970)].

To address these questions, we here present the results of a molecular phylogeny of the genus *Gerbilliscus*, including specimens of nine species from southern [*G. leucogaster* (Peters, 1852), *G. brantsii* (Smith, 1836), and *G. afra* (Gray, 1830)], western [*G. gambianus*, *G. guineae* Thomas, 1910, and *G. kempi*] and eastern [*G. vicinus* (Peters, 1878), *G. nigricaudus* (Peters, 1878), and *G. robustus* (Cretzschmar, 1826)] Africa, based on the analysis of the cytochrome *b* and 16S mitochondrial genes. Through an adequate taxonomic sampling and a wide geographic coverage, we provide a clear picture of the phylogeny of the genus *Gerbilliscus* and offer a better resolution of the evolutionary relationships between southern and western species in comparison to previous studies (Colangelo et al., 2005). Chevret and Dobigny (2005) using different mitochondrial and nuclear genes investigated the relationships among the genera of the subfamily Gerbillinae and suggested a closer affinity between the genera *Gerbilliscus* and *Gerbillurus*. However, in their dataset the species of *Gerbilliscus* from eastern Africa were not included, and which show a high chromosomal homology with two species of *Gerbillurus* (i.e. *G. paeba* and *G. tytonys*) as described by Qumsiyeh et al. (1987, 1991). To further investigate the taxonomic status of the genus *Gerbilliscus* and its relationship with the genus *Gerbillurus* we have also included in the analyses available cytochrome *b* data on *Gerbillurus* and *Desmodillus* Thomas and Schwann, 1904, which were proposed by Chevret and Dobigny (2005) to constitute the tribe Taterillini together with *Gerbilliscus*, cytochrome *b* and 16S sequences of *T. indica* as *Gerbilliscus* was formerly included in the genus *Tatera*, and cytochrome *b* and 16S sequences of *Gerbillus nigeriae* and *Taterillus gracilis* that are two representatives of the tribe Gerbillini (Chevret and Dobigny, 2005).

2. Materials and methods

2.1. DNA extraction, amplification, sequencing and alignment

We performed a phylogenetic analysis on 22 specimens from Mali, Chad, Benin, Burkina-Faso, Ethiopia, Kenya, Tanzania, and South Africa (Fig. 1; Table 1). All the specimens from Mali, Burkina-Faso, and Chad were karyotyped for unambiguous determination (L. Granjon, unpubl. data). Species names from Ethiopia, Kenya, and Tanzania samples were attributed on the basis of morphological comparison with type specimens (W. Verheyen, unpubl. data).

Partial cytochrome *b* sequences (495 bp) for six specimens were provided in a previous work (Colangelo et al., 2005) and were completed in this work (see Table 1).

Tissues are stored in the collections of the Museo di Anatomia Comparata of the University of "La Sapienza" (Rome, Italy), the Muséum National d'Histoire Naturelle (Paris, France), the eThekwini Natural Science Museum (Durban, South Africa), and the Royal Museum for Central Africa (Tervuren, Belgium). Museum codes for reference specimens are indicated in Table 1.

DNA was extracted from ethanol-preserved tissues using NucleoSpin Tissue kit (Macherey-Nagel).

The cytochrome *b* and 16S mitochondrial genes were amplified by polymerase chain reaction (PCR) using a MJ Research PTC-150HB MiniCycler. A 1140 bp fragment of cytochrome *b* was amplified combining primers L14723 (5′-ACC AAT GAC ATG AAA AAT CAT CGT T-3′) and H15915 (5′-TCT CCA TTT CTG GTT TAC AAG AC-3′) (Ducroz et al., 1998) as follows: a first cycle of 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. A 16S fragment was amplified combining 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) as follows: a first cycle of 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.

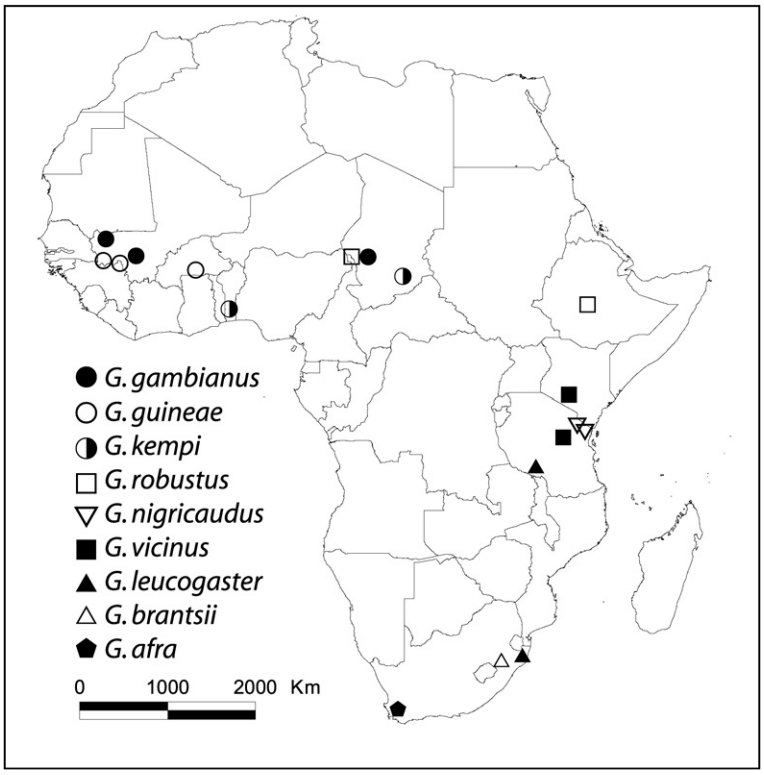

Fig. 1. Localities of the specimens used for the phylogenetic analysis (for geographical coordinates, see Table 1).

The PCRs 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 NucleoSpin Extract II PCR purification kit (Macherey-Nagel) and prepared for automated sequencing using the same primers utilized for amplification. In some cases to obtain a complete cytochrome *b* sequence internal primers L15408 (5′-ATA GAC AAA TCC CAT TCC A-3′) and H15553 (5′-TAG GCA AAT AGG AAA TAT CAT TCT GGT-3′) were also used (Ducroz et al., 1998).

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

2.2. Saturation analysis and genetic distances

Possible saturation for transitions and transversions was evaluated following the approach of Hassanin et al. (1998) where the pairwise number of observed differences is plotted against patristic distances calculated using the Paup4b10 program (Swofford, 1998). The analysis was performed for each different substitution type at each codon position thus generating 18 different datasets. For each dataset were calculated the consistency index (CI) that give information on the level of homoplasy present in the dataset (CI = 1 means absence of homoplasy) and the slope (*S*) of the linear regression of the plots which provides a measure of the deviation from the linearity of the substitution obtained (*S* = 1 means absence of saturation).

Genetic divergence between the cytochrome *b* and 16S sequences (Kimura two-parameter distances), and the number of variable and parsimonious informative sites was computed using the program MEGA3.1 (Kumar et al., 2004).

2.3. Phylogenetic analyses and molecular clock

Partition-homogeneity test (1000 randomizations) was performed to check for eventual incongruence between cytochrome *b* and 16S datasets as implemented in Paup4b10 (Swofford, 1998).

A first analysis was done to reconstruct phylogenetic relationships within the genus *Gerbilliscus* by combining 16S and cytochrome *b* gene sequences. This was done through maximum parsimony (MP), neighbor-joining (NJ), using the Paup4b10 program (Swofford, 1998), and Bayesian analysis (BA), using the MrBayes 3.1 program (Huelsenbeck and Ronquist, 2001).

MP was done using a heuristic search with tree–bisection reconnection and a random addition (10 replications) of sequences. NJ, ML, and Bayesian analysis were done under the assumption of a GTR model with gamma-distributed rate variation across sites (Γ) and a proportion of invariable sites (I) according to the results obtained using a hierarchical likelihood ratio test as implemented in the program Modeltest3.7 (Posada and Crandall, 1998). The robustness of the obtained topologies was tested via 500 bootstrap replications for ML and 1000 replications for NJ. Bayesian analysis was done allowing different parameter

Table 1
List of the specimens, species (with their geographic origin) included in the analysis, with the EMLB accession

Specimen code and museum collection	Species	Country	Locality name, with latitude and longitude	EMBL accession	
				Cytochrome *b*	16S
KE138[c]	*G. vicinus*	Kenya	Nairobi (01°16′S–36°49′E)	AM409376[b]	AJ878522[a]
T50031[f]	*G. vicinus*	Tanzania	Matongolo (05°46′S–36°28′E)	AM409375[b]	AJ878525[a]
M4190[d] (=CG 2004-689)	*G. gambiana*	Mali	Maréna (14°38′N–10°36′W)	AM409386	AM409230
LAC19[d] (=CG 2004-596)	*G. gambiana*	Chad	Karal (12°50′N–14°45′E)	AM409387	AM409231
M4955[d] (=CG 2004-696)	*G. gambiana*	Mali	Samanko (12°32′N–8°05′W)	AM409385	AM409229
M4723[d] (=CG 2004-673)	*G. guineae*	Mali	Gainsoa (12°27′N–10°15′W)	AM409380	AM409224
M4902[d] (=CG 2004-679)	*G. guineae*	Mali	Balamansala (12°10′N–08°46′W)	AM409381	AM409225
M4905[d]	*G. guineae*	Burkina-Faso	Nazinon (11°30′N–01°24′W)	AM409379	AM409223
M4137[d] (=CG 2004-577)	*G. kempi*	Chad	P.N. Zakouma (10°50′N–19°47′E)	AM409383	AM409227
M4143[d] (=CG 2004-585)	*G. kempi*	Chad	P.N. Zakouma (10°42′N–19°28′E)	AM409382	AM409226
B94[c]	*G. kempi*	Benin	Settò (07°31′N–02°51′E)	AM409384	AM409228
SWT56[f]	*G. leucogaster*	Tanzania	Chunya (08°36′S–35°05′E)	AM409391	AM409235
DM7523[e]	*G. leucogaster*	South Africa	Mkuzi Game Reserve (27°35′S–32°13′E)	AM409389	AM409233
DM7524[e]	*G. leucogaster*	South Africa	Mkuzi Game Reserve (27°35′S–32°13′E)	AM409390	AM409234
DM7895[e]	*G. brantsii*	South Africa	Boschoek Farm, KwaZulu Natal (29°21′–30°05′)	AM409392	AM409236
DM7896[e]	*G. brantsii*	South Africa	Boschoek Farm, KwaZulu Natal (29°21′–30°05′)	AM409393	AM409237
TWF18[e]	*G. afra*	South Africa	Zevenwacht Farm, Elsenberg Research Station, 50 km NE Cape Town (33°55′–18°40′)	AM409388	AM409232
T50496[f]	*G. nigricaudus*	Tanzania	Lwami (03°41′S–37°32′E)	AM409377[b]	AJ878520[a]
T50217[f]	*G. nigricaudus*	Tanzania	Mkomazi (04°39′S–38°05E)	AM409378[b]	AJ878521[a]
LAC 18[d] (=CG 2004-595)	*G. robustus*	Chad	Karal (12°50′N–14°45′E)	AM409374	AM409222
ET107[c]	*G. robustus*	Ethiopia	Zeway (05°15′N–34°35′E)	AM409372[b]	AJ878516[a]
ET127[c]	*G. robustus*	Ethiopia	Zeway (05°15′N–34°35′E)	AM409373[b]	AJ878517[a]
BKF1[d]	*Taterillus gracilis*	Burkina-Faso	Goden (12°25′N–01°21′E)	AM409394	AM409238
T-877	*Tatera indica*	Pakistan	Unknown locality	AJ430563[a]	AM409239
M4912[d] (=CG 2004-692)	*Mastomys erythroleucus*	Mali	Samanko (12°32′N–08°05′W)	AM409395	AM409240
TZ54	*Acomys spinosissimus*	Tanzania	Zoissa (05°40′S–36°25′E)	AM409396	AM409241
	Gerbillurus paeba	South Africa	Univ. Natal, Durban	AJ430557[a]	
	Gerbillurus tytonis	South Africa	Namibian desert	AJ430559[a]	
	Gerbillurus setzeri	South Africa	Western Cape	AJ430558[a]	
	Gerbillus nigeriae	Mauritania	Kiji	AF141226[a]	AF141257[a]
	Desmodillus auricularis	South Africa	Kalahari Gemsbok	AJ851272[a]	

Specimen codes and the museum collection where they are stored are indicated.
[a] Sequences available at EMBL.
[b] Partial sequences (495 bp) published by Colangelo et al. (2005) and completed in this work.
[c] Museo di anatomia Comparata, Università di Roma 'La Sapienza'.
[d] Muséum national d'Histoire naturelle, Paris.
[e] eThekwini Natural Science Museum, Durban.
[f] Royal Museum for Central Africa, Tervuren.

estimations for cytochrome *b* and 16S. Two simultaneous searches of 500,000 generations were performed using Metropolis coupling Markov Chain Monte Carlo (MCMC) algorithm (four chains each) sampling trees every 100 generations. The first 200 trees were discarded because the two runs converged after 20,000 generations (average standard deviation of split frequencies <0.01).

A second analysis was performed using only the cytochrome *b* and including the sequences of *Gerbillurus paeba*, *G. tytonis*, *G. setzeri*, and *Desmodillus auricularis*, all available in the EMBL nucleotide sequence database (see Table 1). The phylogenetic relationship between the genera *Gerbilliscus* and *Gerbillurus* was tested through MP, NJ, maximum likelihood (ML), and Bayesian analysis. MP was done using TBR and random addition of samples (10 replications). A weighting scheme was applied according to the results of the saturation analysis. ML analysis was performed assuming a GTR + Γ + I model of evolution using the Paup4b10 program (Swofford, 1998). The model of evolution was chosen using the Modeltest3.7 program (Posada and Crandall, 1998). The distance matrix constructed under the assumption of a GTR + Γ + I model was used to obtain a NJ tree. The robustness of the obtained topologies was tested through bootstrap resampling (500 replications for MP and ML and 1000 for NJ).

Finally, a Bayesian analysis was performed using a GTR + Γ + I model allowing different parameter estimation for each codon position. Two simultaneous runs of 500,000 generations each were done by sampling trees every 100 generations. The two runs converged after 120,000 generation thus the first 1200 trees were discarded.

For all the analyses, sequences of the other Gerbillinae species *Tatera indica* (cytochrome *b* from EMBL), *Gerbillus nigeriae* (cytochrome *b* and 16S from EMBL), and *Taterillus gracilis* (Thomas, 1892) were added, while the Murinae *M. erythroleucus* (Temminck, 1853) and the Deomyinae

Acomys spinosissimus Peters, 1854 were used as outgroups (Table 1). The program MrBayes 3.1 allows only one outgroup sequence, thus for bayesian analysis only *M. erythroleucus* was used.

Divergence times among lineages were estimated using the r8s 1.71 program (Sanderson, 2003) which allows the estimation of divergence times even in the absence of a clock like rate of substitution among lineages by using a semiparametric approach known as penalized likelihood (PL). This method combines a maximum likelihood estimation of rates among the branches with a roughly penalty allowing rates to changes along the branches. The contribution of the two components is determined by the smoothing parameter (λ). The appropriate λ value was calculated using a cross-validation approach, as described by Sanderson (2002), exploring values from 1 to 10,000. The branch lengths of the tree obtained using MrBayes 3.1 (Huelsenbeck and Ronquist, 2001) and cytochrome *b* only were used to compute the chronogram and divergence estimates. The outgroup (*M. erythroleucus*) was pruned from the tree and divergence dates and their confidence intervals for each node were estimated using two different calibration points: first, the most recent common ancestor (MRCA) between *Acomys* and the subfamily Gerbillinae was set at 18 mya, second, the MRCA of Gerbillinae genera included in our analysis was set from 9 to 12.5 mya. The calibration points were chosen according to those previous estimations based on molecular data (mitochondrial and nuclear genes) that showned a good agreement with fossil records. The Deomyinae–Gerbillinae divergence was set at 18 mya following Steppan et al. (2004) and Chevret and Dobigny (2005), who estimated the split between the two subfamilies between 17.4 and 20 mya, and between 15.64 and 19.26 mya, respectively. For the MRCA of the Gerbillinae genera analyzed the estimation suggested by Chevret and Dobigny (2005), based on different nuclear and mitochondrial genes (i.e. between 9.04 and12.56 mya) was used for the divergence between the *Taterillus–Gerbillus* clade and the *Gerbilliscus–Gerbillurus–Desmodillus–Tatera* clade.

3. Results

The saturation analysis shows high level of saturation for transition at 3rd codon position. The main contribution is due to CT changes (CI = 0.23; $S = 0.10$), while AG changes appear less saturated (CI = 0.52; $S = 0.39$). A high level of saturation in CT changes has also been found at 1st codon position (CI = 0.30; $S = 0.16$), probably due to the fact that CT substitutions in this position give different codons for leucine. The survey of saturation plots for the other substitutions at all the three codon positions shows a lower level or absence of saturation, according with results reported in previous studies (Hassanin et al., 1998; Chevret and Dobigny, 2005). When sequences of the genera *Gerbillurus* and *Desmodillus* (that are excluded from the analysis combining cytochrome *b* and 16S) are removed, the saturation analysis leads to analogous results for CT changes at first (CI = 0.29; $S = 0.14$) and third (CI = 0.27; $S = 0.14$) codon positions.

It is difficult to define an *a priori* weight for the homoplasic changes (Hassanin et al., 1998) because removal of those changes that are supposed to introduce noise in phylogenetic analysis could also eliminate important phylogenetic information for some parts of the tree. Consequently, we applied a weighting scheme to MP to reduce the influence only of the most saturated changes observed at first and third codon positions, by down-weighting CT changes according to the product of CI × S (Hassanin and Douzery, 1999).

The first analysis was conducted excluding the taxa for which 16S sequences were not available. For this dataset ($n = 27$) the cytochrome *b* sequences (1140 bp) show 534 variable sites, 455 of which are parsimoniously informative; the 16S sequences were 470 bp long after alignment and show 159 variable sites, 113 of which are parsimoniously informative.

The partition-homogeneity test suggested the absence of significant incongruence between 16S and cytochrome *b* ($P = 0.19$). Therefore, we analyzed the two datasets combined in a unique 1610 bp sequence. The resulting trees show similar topologies independently of the method used (Fig. 2).

All the analyses produced the same topology except for *T. indica* that occurs in a poorly supported ambiguous position suggesting a basal politomy in the subfamily Gerbillinae while the monophyly of the genus *Gerbilliscus* is strongly supported by the bayesian *a posteriori* probability (1.00) and bootstrap value (96) in NJ, MP (tree length = 2331, consistency index = 0.45) and ML analyses (Fig. 2). Within *Gerbilliscus*, all the taxa considered are monophyletic and grouped in three main clades well defined geographically and well supported by bayesian posterior probabilities and bootstrap replications (Fig. 2): an eastern clade that includes *G. robustus*, *G. vicinus*, and *G. nigricaudus*, a southern clade, including *G. brantsii*, *G. afra*, and *G. leucogaster* and, finally, a western clade with *G. kempi*, *G. gambianus*, and *G. guineae* (Fig. 2).

The eastern clade is basal compared to the others and the pattern of phylogenetic relationships among its species is in agreement with what has previously been found in a previous study based on partial cytochrome *b* fragment (495 bp) and 16S (Colangelo et al., 2005). The sample of *G. robustus* from Chad clusters together with the Ethiopian ones (Fig. 2), but the specimens from the two origins show a high level of genetic differentiation (K2P = 0.082) when compared to those observed in the other species of the genus (Colangelo et al., 2005).

Within the southern clade, *G. brantsii* and *G. afra* cluster together with the lowest level of interspecific genetic divergence recorded in the genus (Table 2), suggesting that these are the more recently appeared species. These two species are phylogenetically closely related to *G. leucogaster*.

Finally, within the western clade the two taxa *G. gambianus* and *G. kempi*, still often considered synonymous (Musser and Carleton, 2005), form two distinct and

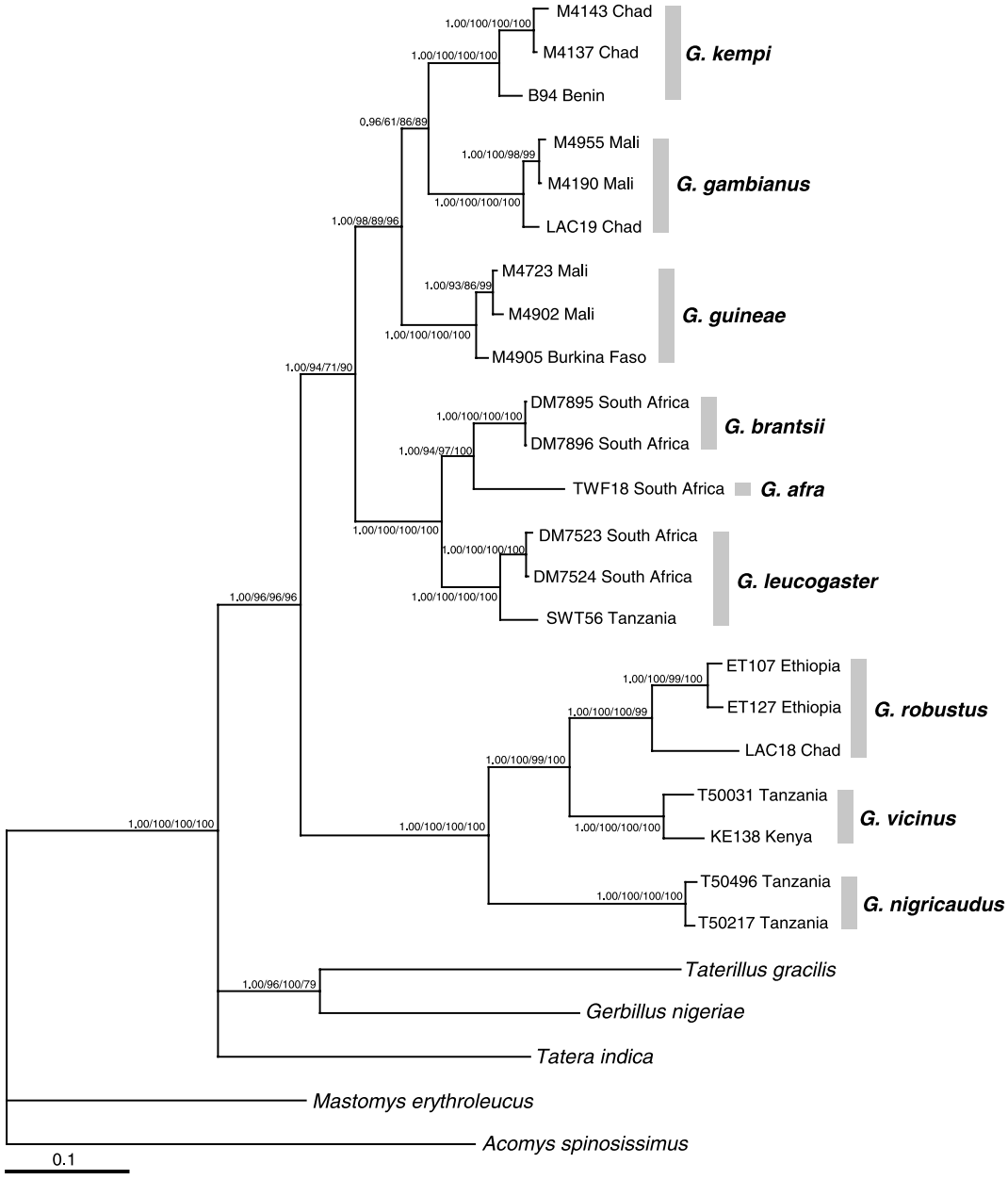

Fig. 2. Bayesian 50% majority rule consensus tree obtained combining cytochrome *b* and 16S. Numbers are Bayesian posteriori probability, MP, ML, and NJ bootstrap values.

Table 2
Genetic divergence among *Gerbilliscus* species based on K2P distances using 16S (above the diagonal) and cytochrome *b* (below the diagonal)

	ROB	VIC	NIG	GUI	KEM	GAM	AFR	BRA	LEU
ROB		0.043	0.076	0.084	0.087	0.088	0.093	0.102	0.109
VIC	0.165		0.067	0.084	0.092	0.082	0.093	0.103	0.110
NIG	0.221	0.202		0.094	0.109	0.117	0.105	0.100	0.118
GUI	0.246	0.226	0.242		0.043	0.040	0.050	0.060	0.053
KEM	0.236	0.257	0.226	0.141		0.045	0.066	0.084	0.069
GAM	0.218	0.231	0.240	0.147	0.137		0.055	0.077	0.060
AFR	0.238	0.241	0.234	0.179	0.195	0.194		0.030	0.026
BRA	0.234	0.232	0.215	0.156	0.168	0.170	0.096		0.047
LEU	0.238	0.254	0.235	0.166	0.168	0.191	0.135	0.111	

ROB, *G. robustus*; VIC, *G. vicinus*; NIG, *G. nigricaudus*; GUI, *G. guineae*; KEM, *G. kempi*; GAM, *G. gambiana*; AFR, *G. afra*; BRA, *G. brantsii*; LEU, *G. leucogaster*.

strongly supported monophyletic clades with a level of genetic divergence comparable to that observed between both of them and *G. guineae* (Table 2), a long standing well-accepted species.

The second analysis was conducted using only the cytochrome *b* sequences and included specimens of the genus *Gerbillurus* and *Desmodillus*. For this dataset the cytochrome *b* shows 558 variable sites, 472 of which are parsimoniously informative.

Here again, the monophyly of the subfamily Gerbillinae is strongly supported in all the trees but the lower number of characters used with respect to the previous analyses apparently is not sufficient to resolve the relationships among the genera of the subfamily (Fig. 3). However, all the topologies obtained suggest that the genus *Gerbilliscus*,

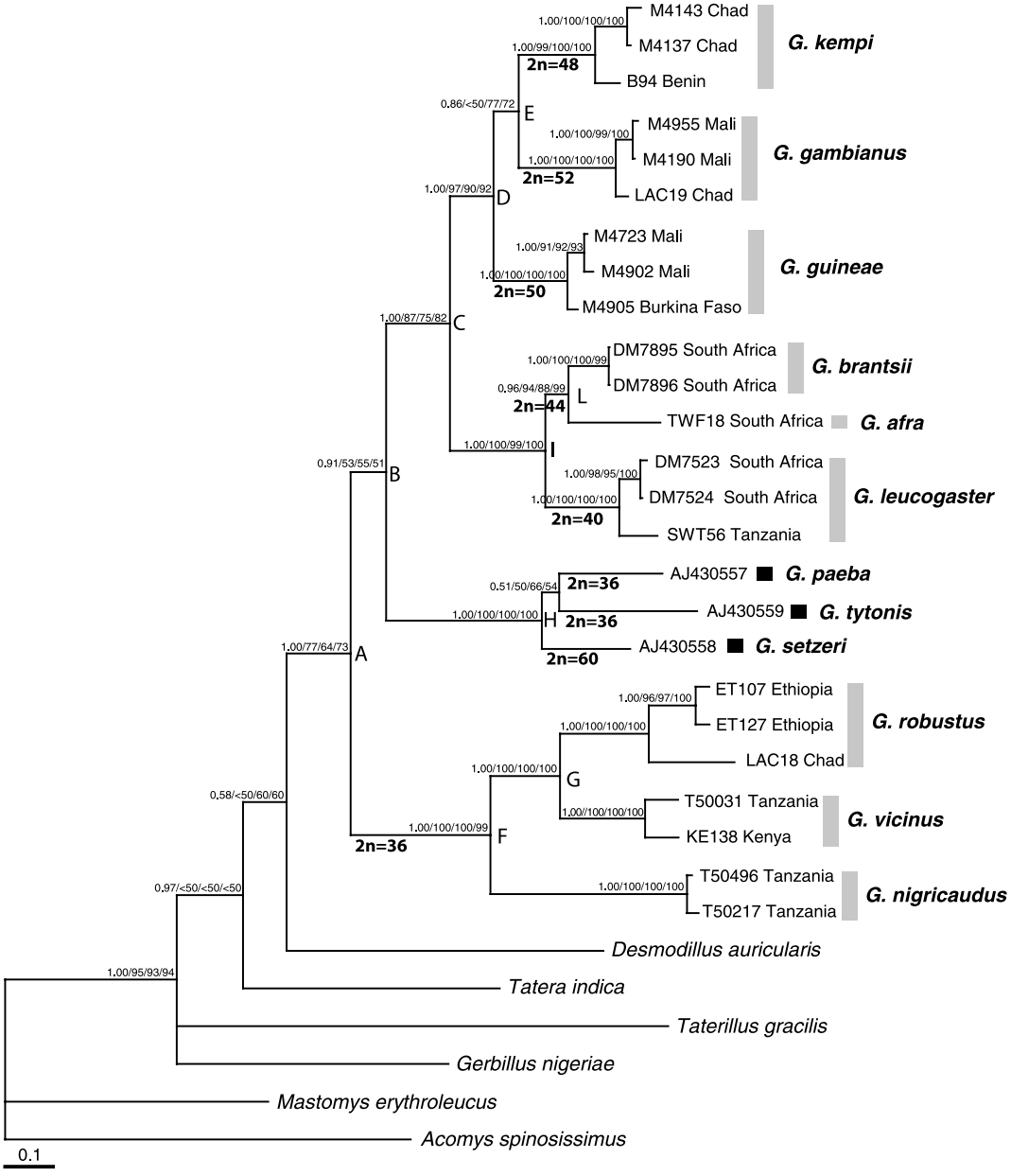

Fig. 3. Bayesian 50% majority rule consensus tree (GTR + Γ + I model). Numbers above branches are Bayesian posteriori probabilities, MP, ML and NJ bootstrap values. Diploid numbers for each lineage are reported (below branches). Letters for A–L indicate nodes for which age were estimated (see Table 3).

as it is actually defined (Musser and Carleton, 2005), is not monophyletic. Actually, the genus *Gerbillurus* clusters together with the southern–western species of *Gerbilliscus*, while the eastern *Gerbilliscus* species group remains the basal clade. The hypothesis that *Gerbilliscus* and *Gerbillurus* should be consider a unique genus, already proposed by Chevret and Dobigny (2005), is here strongly supported by bayesian inference and well supported by NJ and MP (bootstrap values 73 and 75, respectively) while it is weakly supported by ML (bootstrap value 64). Nevertheless, despite the fact that in all the phylogenetic reconstructions the branching order of *Gerbillurus* is the same, its relationships with the *Gerbilliscus* species are only well supported using bayesian inference,but weakly supported by bootstrap replications in MP, NJ and ML (Fig. 3). Low bootstrap values are also recognized in MP parsimony analysis for the support to a common ancestry between *G. kempi* and *G. gambiana*.

Concerning the divergence time estimates, the cross-validation procedure suggests an optimal value for the smoothing parameter λ equal to 1, suggesting a heterogeneous substitution rate along the branches. This is congruent with the likelihood ratio test ($p < 0.01$) that rejects the hypothesis of a molecular clock in presence of a homogeneous substitution rate. Divergence age and their confidence estimated using PL are reported in Table 3. We found that the split between the eastern *Gerbilliscus* species (the "*robustus*" lineage) and the other *Gerbilliscus* and *Gerbillurus* species

Table 3
Estimated age of divergence for nodes in Fig. 3

Nodes		Age
A	*Gerbilliscus* origin	8.54 (8.26–8.81)
B	*Gerbillurus*/western–southern *Gerbilliscus* clades split	7.54 (7.24–7.85)
C	Southern/western *Gerbilliscus* lineages split	5.47 (5.17–5.7)
D	Western clade origin	3.89 (3.58–4.17)
E	*G. gambianus*/*G. kempi* split	3.21 (2.97–3.21)
F	Eastern clade origin	5.47 (5.16–5.7)
G	*G. vicinus*/*G. robustus* split	3.81 (3.56–4.06)
H	*Gerbillurus* origin	3.43 (3.19–3.68)
I	Southern clade origin	2.83 (2.60–3.07)
L	*G. brantsii*/*G. afra* split	2.08 (2.87–2.31)

Age and confidence interval are reported in millions of year.

occurred around 8.5 mya, while the *Gerbillurus* and western–southern *Gerbilliscus* lineage split around 7.5 mya. The separation between the western and southern *Gerbilliscus* lineages occurred around two million years later (5.5 mya), in the same period when in eastern Africa occurred the split between *G. nigricaudus* from the lineage that led to *G. vicinus* and *G. robustus*. The diversification within the other lineages (western, southern and *Gerbillurus*) occurred during middle-late Pliocene (see Table 3).

4. Discussion

Our results shed light on the systematics and taxonomy of the genus *Gerbilliscus* as currently understood (Musser and Carleton, 2005). First, our analyses suggest the occurrence of three main lineages characterized by specific genetic peculiarities and evolutionary patterns. These lineages seem to follow a geographical pattern, but do not match the morphological groups of Davis (1966, 1975), who distinguished an "*afra*" group (including *G. afra*, *G. brantsii*, *G. gambianus*, and *G. kempi*) and a "*robusta*" group (including *G. guineae*, *G. leucogaster*, *G. nigricaudus*, *G. robustus*, and *G. validus*). The "*robustus*" group here found on molecular grounds is one of mainly East African affinities, with species having parapatric distributions. It is characterized by a high karyotype stability ($2n = 36$; Colangelo et al., 2005), but shows a high degree of inter and intraspecific molecular divergence (Colangelo et al., 2005; this study, see Table 2). This pattern could be explained by the ecological heterogeneity characterizing East African arid savannas, an environment shaped by the Pliocene and Pleistocene climatic and ecological fluctuations (deMenocal, 2004) and the geological and volcanic activity of the Rift Valley area (Partridge et al., 1995).

The second group, including *G. afra*, *G. brantsii*, and *G. leucogaster*, comprises species of mainly southern African affinities, characterized by a low level of karyotype diversity (Qumsiyeh, 1986). The two South African species, *G. afra* and *G. brantsii*, share the same karyotype ($2n = 44$, NFa = 66) and show an allopatric distribution while *G. leucogaster* (sympatric with *G. brantsii* in the southern part of its distribution range) has a different karyotype with $2n = 40$ and NFa = 66 (Qumsiyeh, 1986). On chromosomal and allozymic grounds, Qumsiyeh et al. (1987) found a close association between *G. afra* and *G. brantsii* (as we found here), as well as between *G. nigricaudus* and *G. leucogaster* (here separated by a large genetic distance). However, it should be noted that in the latter case the two karyotype are differentiated by a pericentric inversion and a translocation and that the attribution of the karyotype with $2n = 40$ to *G. nigricaudus* by Qumsiyeh et al. (1987), which was based only on one specimen, remains uncertain and needs further investigations (see Colangelo et al., 2005). Dempster and Perrin (1994), Dempster (1996) investigated pre- and post-mating isolation mechanisms in *G. afra*, *G. brantsii* and *G. leucogaster* and found an efficient behavioral pre-mating isolation between sympatric species (*G. brantsii* and *G. leucogaster*) but not allopatric species (*G. brantsii* and *G. afra*). This would support the evolution of reinforcement due to selection against wasted gamete-production by hybrids (Dempster and Perrin, 1994). However, it should be noted that, during laboratory cross-breeding experiments, allopatric *G. brantsii* and *G. afra* maintain a degree of post-mating isolation due to male sterility at F1 (Dempster, 1996). This pattern of asymmetric post-mating isolation in absence of extensive chromosomal rearrangements was interpreted by Taylor (2000) as a clear example of Haldane's (1922) rule.

Finally, the third group includes all the western African species that are partly sympatric (*G. guineae* is sympatric with both *G. gambianus* and *G. kempi* over a part of their ranges, but the latter two species are apparently allopatric). This group is characterized by a relatively high level of karyotype variability (Benazzou et al., 1984; Colangelo et al., 2001; Gautun et al., 1986; Hubert et al., 1973; Tranier, 1974) and moderate interspecific genetic divergence (Table 2). Our results confirm that *G. gambianus* (considered a synonym of *G. kempi* by Musser and Carleton, 2005) should be considered a valid species, genetically and chromosomally (Hubert et al., 1973) well differentiated from both *G. kempi* and *G. guineae*.

A second main issue emerging from these molecular analyses concerns the relationships between the three *Gerbilliscus* clades and the genus *Gerbillurus*. Previous cytogenetical and allozymic analyses (Qumsiyeh et al., 1987, 1991) provided strong evidence of the paraphyly of the genus *Gerbilliscus*. Chevret and Dobigny (2005) suggested that the two genera should be placed in synonymy and referred to as *Gerbilliscus*. Our results, based on a more comprehensive species sampling, strongly support the same hypothesis. It appears plausible that the common ancestor of *Gerbilliscus* and *Gerbillurus* showed the same chromosomal diploid number of $2n = 36$ that nowadays characterizes the three species here included in the *Gerbilliscus* eastern clade (Colangelo et al., 2005), as well as *Gerbillurus paeba*, considered by some authors as the ancestor of the *Gerbillurus* lineage (Perrin et al., 1999; and reference herein). Our analyses, according with previous results of Chevret and Dobigny (2005), do not support a basal position of *G. paeba* within

the *Gerbillurus* clade, but rather suggest that the $2n = 36$ karyotype has been retained in the lineage that led to *G. paeba* and *G. tytonis*, whereas the other *Gerbillurus* species underwent chromosomal rearrangements. A similar process interested the southern–western *Gerbilliscus* clade that went through chromosomal evolution towards an increase in diploid number (see Fig. 3).

At present *Gerbillurus* is endemic to South West arid savanna regions, an area separated from the east African savannas by a portion of Zambezian biome which is characterized by a wetter climate. However, it has been hypothesized that faunal exchanges between the northern Somali-Maasai savannas and the South West arid regions occurred repeatedly from late Miocene up to Pleistocene along an arid corridor now restricted to a discontinuous narrow line by the Mopane woodlands (Grubb et al., 1999; Foley, 1999). The ancestor of *Gerbillurus* and the eastern *Gerbilliscus* species group may have occupied these areas and a successive humid period may have caused an expansion of the Zambezian woodlands isolating the "*robustus*" and the *Gerbillurus* lineages. It is likely that at that time the genus *Gerbilliscus* started to colonize less arid areas such as the Zambezian and western African savannas, giving rise to the southern and western species complexes.

It is difficult to hypothesize the route of colonization then followed by western and southern species. The basal position of the "*robustus*" species complex suggests an eastern African origin of the genus, nonetheless two alternative patterns of dispersal through the African continent can be hypothesized: the first from east to south and subsequently to north through a corridor between eastern arid savannas and the western Congolian rain forest; the second implies that the dispersal happened almost at the same time and independently for each lineage along two different routes, starting from east toward south and west, respectively. The successive diversification of the southern and western species complex would have occurred when the ancestors of the two lineages already reached their present locations. For the first hypothesis to be taken in consideration one would expect a general trend in diploid number increment from $2n = 36$ to $2n = 52$. If this were to be the case, western species should present more chromosomal homologies with southern species than with eastern ones. There are no available comparisons between the southern and western karyotypes. Therefore, only further comparative cytogenetic studies will make it possible to identify chromosomal homologies between lineages. On the other hand, the interspecific genetic divergence (Table 2) and the molecular clock estimates suggest that the radiation that led to the western and southern species complex began roughly at the same time, thus strengthening the hypothesis of two different and independent colonization routes.

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