

Evolutionary systematics and biogeography of endemic gerbils (Rodentia, Muridae) from Morocco: an integrative approach

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Gerbils of the genus *Gerbillus* represent an important part of small mammal diversity in arid regions of North Africa, but their taxonomy and evolutionary systematics and biogeography are still poorly known. Based on the recent collection of a series of gerbils in southern Morocco, including at the type localities of three endemic species, we gathered morphological, chromosomal and molecular data on a number of specimens that we compared with other available results. These data provide new insights into the geographical distribution of the endemic species known to date and suggest that an additional and previously undetected species may exist within this area. Multivariate analyses of morphometrical skull data validated the criteria used in the description of some of these species, while confirming the morphological similarity of a set of medium to large-sized species with hairy feet that can be considered sibling species. Karyotypes were useful for the specific assignation of a number of specimens. Molecular phylogenetics and divergence time estimates allow us to draw the first picture of the evolutionary relationships between endemic *Gerbillus* species from Morocco and related species of North Africa, and to estimate their respective timing of diversification. Most of the lineage emergence events took place during the Pleistocene, a period characterised by a global aridification of North Africa, but more importantly by important climatic and eustatic variations that may have played a role in species differentiation and subsequent expansion. This integrative approach proved to be essential in such species group including morphologically similar species. It also enables to propose testable hypotheses as to possible scenarios of emergence and evolution of these species along the western margin of the Sahara desert.

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Introduction

Integrative taxonomy has recently been coined to define ‘the science that aims to delimit the units of life’s diversity from multiple and complementary perspectives (behaviour, comparative morphology, development, ecology, phylogeography, population genetics, etc.)’ (Dayrat 2005). Later the same year, Will *et al.* (2005) also pleaded for integrative taxonomy against ‘the gloomy and narrow prospect for taxonomy’ represented by DNA bar-coding. Since that date, a number of studies dealing with a variety of taxa have addressed various questions related to systematics within the explicit frame of integrative taxonomy (e.g. Mengual *et al.* 2006; Schlick-Steiner *et al.* 2010 in insects, Haase *et al.* 2007 in gastropods, Fonseca *et al.* 2008 in nematodes, Padial & de la Riva 2009 in amphibians, Miralles *et al.* 2011 in squamates, Andres-Sanchez *et al.* 2009 in plants). In rodents, Lanzone *et al.* (2007) and Leite *et al.* (2008) also used this approach in studies of South American rodents. However, many taxonomists and systematists did use for long an integrative taxonomy approach without naming it this way. This is especially true in rodents where polytypic and sibling species have been identified and studied for decades, first using combinations of anatomy, biochemical genetics, cytogenetics, morphology and reproductive biology (e.g. Matthey & Petter 1970; Benado *et al.* 1979; Bonhomme *et al.* 1984), and more recently by combining all or some of these disciplines with DNA sequencing (see Vié *et al.* 1996; Ducroz *et al.* 1997; Steppan 1998; Patton *et al.* 2000; Lecompte *et al.* 2003; D’Elia & Pardinás 2004; among others). As underlined by Dayrat (2005), this evolution does not mean the end of morphological methods. Indeed, studies on morphological or biometrical characters should rather gain from the confrontation with results obtained via alternative methods. Of special interest in this respect are the findings, the confirmation and/or the quantification of morphological criteria on samples first diagnosed using other methods (e.g. Granjon 2005; Nicolas *et al.* 2008).

Here, we apply the principles of integrative taxonomy to a group of rodent species of the genus *Gerbillus* Desmarest, 1804 (Muridae, Gerbillinae), focusing our attention on species from Morocco. The genus *Gerbillus* is one of the most speciose among rodents, with 38 species currently recognised (Musser & Carleton 2005). The major part of this diversity is found in arid and semi-arid regions within and around the Sahara desert (Granjon & Denys 2006). Species assemblages within the genus can be distinguished by a suite of anatomical and morphological features (Lay 1983), but chromosomal data appear essential for species-specific diagnose within these groups. One of them gathers medium- to large-sized species with hairy feet, including *Gerbillus hesperinus* Cabrera, 1936, *G. boogstrali*

Lay 1975; *G. latastei* Thomas and Trouessart, 1903, *G. occidentuus* Lay 1975; *G. perpallidus* Setzer, 1958, *G. pyramidum* Geoffroy, 1803, and *G. tarabuli* Thomas, 1902. The karyotypes of these species have been established (Jordan *et al.* 1974; Lay 1975; Lay *et al.* 1975; Viegas-Pequignot *et al.* 1982, 1984; Granjon *et al.* 1999; Aniskin *et al.* 2006), and a detailed comparison of G-banding and C-banding patterns in four of them (*G. occidentuus*, *G. perpallidus*, *G. pyramidum* and *G. tarabuli*) has shown an impressive amount of chromosomal rearrangements between these species (Aniskin *et al.* 2006). Whereas *G. tarabuli* and, to a lesser extent, *G. pyramidum* have very large distribution areas over northern Africa, *G. perpallidus* is endemic from Egypt, *G. latastei* is only known from Tunisia and Libya to date, and *G. hesperinus*, *G. boogstrali* and *G. occidentuus* are considered endemic from Morocco (Musser & Carleton 2005). Unfortunately, since the description of the latter two by Lay (1975), very few data have been published on these species. Benazzou & Genest-Villard (1980) confirmed the presence of *G. hesperinus* (described in 1936 by Cabrera as a subspecies of *G. hirtipes*, syn. *G. gerbillus* (Olivier, 1801), Cockrum & Setzer 1976) in the immediate vicinity of the type locality of the species (Essaouira = Mogador). Following the capture of *G. occidentuus* near Tarfaya, Aulagnier & Thévenot (1986) proposed that its distribution extends from the south of Anti-Atlas (where lies Aoreora, the type locality of the species) to the Saquia el-Hamra, a wadi reaching Laâyoune (Western Sahara). Finally, Zyadi (1988) found *G. boogstrali* in various localities near and west of the type locality (ca. 7 km south of Taroudant), thus confirming the hypothesis of Lay (1975) that this species may occupy sandy habitats of the lower Sous valley.

Lay (1975) made some hypotheses about the emergence and differentiation of these endemic species from Morocco, in relation to fluctuations of eustatic sea level during the Pliocene and early Pleistocene, and with the role of the High Atlas and Anti-Atlas mountains as barriers between these species ranges. However, until now, no data about the phylogenetic relationships or about possible dates of divergence between species in this group were available to test these scenarios. Chevret & Dobigny (2005) provided data on five species belonging to the genus *Gerbillus* (including members of the subgenera *Gerbillus* and *Dipodillus* Lataste, 1881) in the wider context of the Gerbillinae subfamily. A recent study by Abiadh *et al.* (2010a) comprised *G. tarabuli* and the related endemic *G. latastei* from Tunisia, in a phylogenetic analysis based on cytochrome *b* gene sequences of six species of *Gerbillus* from Tunisia. The same set of species was also submitted to morphometric analyses (Abiadh *et al.* 2010b). We recently collected a series of gerbils in southern Morocco, including at the type localities of the three endemic

species mentioned earlier. On this sample as well as on additional specimens from north-western Africa, overall belonging to eight species, we gathered morphological, chromosomal and molecular data. These data not only provide new insights into the geographical distribution of the endemic species known to date, but they also suggest that an additional and previously undetected species may exist within this area. Morphological analyses confirmed the value of the criteria originally used in the description of some of these species, and chromosomal data clearly diagnosed a number of specimens. Molecular results allow us to draw the first picture of the evolutionary relationships and timing of diversification between endemics from Morocco and other *Gerbillus* species of wider distribution. Altogether, our data significantly improve our knowledge on gerbil biogeography and systematics in an area of high endemism for this lineage.

Materials and methods

We here follow Abiadh *et al.* (2010a) who, based on the results of Chevret & Dobigny (2005) and contrary to Musser & Carleton (2005), considered *Dipodillus* as a sub-genus of (and not a separate genus from) *Gerbillus*.

Sample collection

Seventy-five *Gerbillus* specimens have been collected in Morocco including the southern region of Western Sahara (Table 1). Most of these specimens (labelled LG) were collected in November 2008, while five (labelled MAK) were caught in April 2008, and one came from earlier collection and was kept in the cryopreserved cell collection held by Vitaly Volobouev at the Museum National d'Histoire Naturelle, Paris (no. 1981-019). Their geographic origin is illustrated on Fig. 1. To the exception of the latter one, all these specimens are preserved as skins and skulls in the collections of the UMR022 (CBGP-IRD) in Dakar (Senegal), and of the Institute of Vertebrate Biology of the Czech Academy of Sciences in Brno (Czech Republic). The type localities of the three endemic species of Morocco, namely Essaouira (*G. hesperinus*, site no. 11 in Fig. 1), 7 km south of Taroudant (*G. boogstrali*, site no. 9 in Fig. 1) and Aoreora (*G. occiduus*, site no. 6 in Fig. 1) were sampled.

Among these 75 specimens, 11 were subjected to simultaneous chromosomal, molecular and morphometrical analyses, whereas 39 additional ones were both sequenced and measured, 13 others were sequenced and karyotyped and nine were both karyotyped and measured (Table 1).

Additionally, 55 specimens from other African countries (Algeria, Egypt, Libya, Mali, Mauritania, Niger, Tunisia, and Senegal) were used (Table 1). Their specific identities were ascertained via chromosomal and/or molecular analyses (Abiadh *et al.* 2010a; J.F. Cosson, G. Dobigny,

L. Granjon, and N. Nesi, unpubl. data), except for 11 specimens from Aoujeft (Mauritania) used in morphological analyses (see below), which were tentatively named *Gerbillus cf. tarabuli* based on external morphological characteristics (Granjon & Duplantier 2009).

Molecular phylogenetics and divergence time analyses

In total, 104 cytochrome *b* sequences were included in the molecular analyses. Among them, 58 were obtained from Moroccan gerbils in the frame of this study and 44 from gerbils of other countries came from recent other studies (in particular, 29 sequences from Nesi 2007 and 12 sequences from Abiadh *et al.* 2010a). The latter belong to the species *G. campestris* Loche 1867 ($N = 1$), *G. gerbillus* ($N = 1$), *G. latastei* ($N = 12$), *G. nanus* Blanford, 1875 ($N = 1$), *G. nigeriae* Thomas and Hinton, 1920 ($N = 2$), *G. tarabuli* ($N = 19$), *G. pyramidum* ($N = 7$) and *G. perpallidus* ($N = 1$; Table 1). One *Sekeetamys calurus* (Thomas, 1892) sequence retrieved from Genbank (AJ851276) was used as a closely related outgroup (Chevret & Dobigny 2005), whereas *Taterillus gracilis* (Thomas, 1892) (Genbank accession number AM409394) was used as a more distantly related outgroup species in the phylogenetic analyses.

For the Moroccan specimens, genomic DNA was isolated from ethanol-preserved tissue using the 'DNeasy Blood and Tissue' Kit (Qiagen, Courtaboeuf, France). Complete mitochondrial cytochrome *b* gene (1140 bp) was amplified using the primers H15915 (5'-TTC CAT TTC TGG TTT ACA AGA C-3') and L14723 (5'-ACC AAT GAC ATG AAA AAT CAT GGT T-3') on a MJ Research PTC-200 thermal cycler. Each 25 μ L reaction contained 14.4 μ L of milliQ water, 2.5 μ L of 10 \times buffer supplemented with 1.5 mM of MgCl₂ (Qiagen), 0.1 mM of dNTP, 1 μ M of each primer, 0.5 U of *Taq* polymerase (Qiagen) and 2 μ L of sample extraction containing approximately 50 ng of DNA. Cycling conditions were denaturation at 94 °C for 3 min, followed by 37 cycles at 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min. PCR products were purified and sequenced in both directions with primers H15915 and L14723 by Macrogen (Seoul, Korea).

The sequences obtained were corrected and aligned using BioEdit (Hall 1999). Alignment procedure was trivial as no gaps were recovered in the molecular dataset. Pairwise Kimura two-parameter (K2P) genetic distances were computed using MEGA 4 (Tamura *et al.* 2007). The best-fit model of evolution was then determined using the Bayesian information criterion (BIC; Schwarz 1978), as implemented in jModelTest v0.1.1 (Posada 2008). The General time reversible (GTR) + I + G model (Yang 1994; Gu *et al.* 1995) was indicated as the best-fit model for the cytochrome *b* gene and was further used in the Bayesian

Table 1 List of specimens studied, from Morocco and other countries, with their geographic origin and use

Individual no.	Identification	Geographic origin	Geographical coordinates	Use	2n/Nfa	Genebank accession no.
<i>Morocco</i>						
LG85	<i>Gerbillus campestris</i>	5 km N Aglou (7)	29°50'N/9°47'W	S		JN021401
LG78	<i>Gerbillus campestris</i>	Souss Massa NP (8)	30°04'N/9°39'W	S		JN021400
LG29	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG30	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG31	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG32	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG33	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG34	<i>Gerbillus campestris</i>	3 km S Essaouira (11)	31°28'N/9°45'W	M		
LG120	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021405
LG121	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021406
LG122	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021407
LG123	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021408
LG124	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021409
LG125	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M		JN021410
LG126	<i>Gerbillus gerbillus</i>	12 km N Tarfaya (4)	27°58'N/12°47'W	S, M, K	43/74	JN021411
LG111	<i>Gerbillus gerbillus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M		JN021402
LG112	<i>Gerbillus gerbillus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M		JN021403
LG113	<i>Gerbillus gerbillus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M		JN021404
V.V.1981-019*	<i>Gerbillus hesperinus</i>	S Essaouira	31°28'N/9°45'W	S, K*	58/78	JN652803
LG65	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021412
LG66	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021413
LG67	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021417
LG68	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	K*	72/84	
LG71	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021418
LG72	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021414
LG73	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021419
LG74	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021415
LG75	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021420
LG76	<i>Gerbillus hoogstrali</i>	8 km S Oued Souss (9)	30°25'N/8°54'W	S, M		JN021416
LG90	<i>Gerbillus</i> sp.	Aglou (7)	29°48'N/9°50'W	S, M		JN021447
LG91	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021448
LG92	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021449
LG93	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021442
LG94	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021450
LG95	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021443
LG96	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021451
LG97	<i>Gerbillus</i> sp.	3 km N Aglou (7)	29°49'N/9°49'W	S, M		JN021444
LG87	<i>Gerbillus</i> sp.	5 km N Aglou (7)	29°50'N/9°47'W	S, M		JN021445
LG77	<i>Gerbillus</i> sp.	Souss Massa NP (8)	30°04'N/9°39'W	S, M		JN021441
LG79	<i>Gerbillus</i> sp.	Souss Massa NP (8)	30°04'N/9°39'W	S, M		JN021446
LG102	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	M, K	40/76	
LG103	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021421
LG104	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021422
LG105	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	M		
LG106	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021424
LG107	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021423
LG108	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021425
LG109	<i>Gerbillus occiduus</i>	Aoreora (6)	28°50'N/10°51'W	S, M		JN021426
MAK7	<i>Gerbillus occiduus</i>	Boujdour (3)	26°08'N/14°30'W	S		JN652804
MAK8	<i>Gerbillus occiduus</i>	Boujdour (3)	26°08'N/14°30'W	S		JN652805
LG127	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	
LG128	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	
LG129	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	–	JN021427
LG129/1/2	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	
LG130	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	

Table 1 (continued)

Individual no.	Identification	Geographic origin	Geographical coordinates	Use	2n/Nfa	Genebank accession no.
LG131	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021428
LG132	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021429
LG133	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021433
LG134	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021434
LG135	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	
LG136	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	40/76	
LG137	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	M, K	–	
LG138	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	–	JN021439
LG139	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021430
LG140	<i>Gerbillus occiduus</i>	Dakhla (2)	23°55'N/15°46'W	S, M, K	40/76	JN021431
LG110	<i>Gerbillus occiduus</i>	Tan Tan/El Ouatia (5)	28°29'N/11°14'W	S, M, K	40/76	JN021432
LG117	<i>Gerbillus occiduus</i>	7 km N Tarfaya (4)	27°58'N/12°50'W	S, M		JN021437
LG118	<i>Gerbillus occiduus</i>	7 km N Tarfaya (4)	27°58'N/12°50'W	M, K	40/76	
LG119	<i>Gerbillus occiduus</i>	7 km N Tarfaya (4)	27°58'N/12°50'W	S, M		JN021438
LG114	<i>Gerbillus occiduus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M		JN021435
LG115	<i>Gerbillus occiduus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M, K	40/75?	JN021440
LG116	<i>Gerbillus occiduus</i>	14 km N Tarfaya (4)	27°57'N/12°46'W	S, M		JN021436
MAK6	<i>Gerbillus tarabuli</i>	Bir Anzarane (1)	23°53'N/14°32'W	S		JN652798
MAK21	<i>Gerbillus tarabuli</i>	Oued Rheris (10)	31°17'N/4°19'W	S		JN652799
MAK23	<i>Gerbillus tarabuli</i>	Oued Rheris (10)	31°17'N/4°19'W	S		JN652800
<i>Other countries</i>						
Ge.camp1**	<i>Gerbillus campestris</i>	Air, Niger	17°N/8°E	S		JN652801
BM113	<i>Gerbillus gerbillus</i>	Touajil, Mauritania	22°08'N/12°41'W	M		
Ge. Gerb1**	<i>Gerbillus gerbillus</i>	Achegour, Niger	19°01'N/11°43'E	S		JN652802
56HM***	<i>Gerbillus latastei</i>	Hammamet, Tunisia	36°20'N/10°30'E	S	74/97	GU356559
69HM***	<i>Gerbillus latastei</i>	Hammamet, Tunisia	36°20'N/10°30'E	S	74/97	GU356553
57HM***	<i>Gerbillus latastei</i>	Hammamet, Tunisia	36°20'N/10°30'E	S	74/97	GU356561
12BH***	<i>Gerbillus latastei</i>	Bouhedma, Tunisia	34°29'N/09°39'E	S	74/100	GU356552
11BH***	<i>Gerbillus latastei</i>	Bouhedma, Tunisia	34°29'N/09°39'E	S	74/100	GU356562
22BH***	<i>Gerbillus latastei</i>	Bouhedma, Tunisia	34°29'N/09°39'E	S	74/100	GU356556
65LB***	<i>Gerbillus latastei</i>	Labaied, Tunisia	35°11'N/09°16'E	S	74/94	GU356551
64LB***	<i>Gerbillus latastei</i>	Labaied, Tunisia	35°11'N/09°16'E	S	74/96	GU356558
90F***	<i>Gerbillus latastei</i>	Faouar, Tunisia	33°10'N/08°17'E	S	74/102	GU356557
91F***	<i>Gerbillus latastei</i>	Faouar, Tunisia	33°10'N/08°17'E	S	74/99	GU356554
101F***	<i>Gerbillus latastei</i>	Faouar, Tunisia	33°10'N/08°17'E	S	74/102	GU356560
108F***	<i>Gerbillus latastei</i>	Faouar, Tunisia	33°10'N/08°17'E	S	74/102	GU356555
	<i>Gerbillus nanus</i>	Nouackchott, Mauritania		S		AJ851270
	<i>Gerbillus nigeriae</i>	Niamey, Niger		S		AJ430555
	<i>Gerbillus nigeriae</i>	Kiji, Mauritania		S		AF141226
Ge. Perp1**	<i>Gerbillus perpallidus</i>	Egypt		S		JN652806
JEZlib2**	<i>Gerbillus pyramidum</i>	Jezero Gabroon, Lybia	27°03'N/14°26'E	S		JN652808
MENmal**	<i>Gerbillus pyramidum</i>	Ménaka, Mali	15°54'N/02°25'E	S, K	38/72	JN652812
CHAmal1**	<i>Gerbillus pyramidum</i>	Oued Chacheguerène, Mali	19°43'N/00°01'W	S, K	38/72	JN652809
AKJmau7**	<i>Gerbillus pyramidum</i>	Akjoujt, Mauritania	16°44'N/14°22'W	S, K	38	JN652811
SOUmau**	<i>Gerbillus pyramidum</i>	Souegya, Mauritania	20°16'N/13°07'W	S		JN652810
Ge. Pyra1**	<i>Gerbillus pyramidum</i>	Fachi, Niger	18°07'N/11°35'E	S		JN652807
GOUnig**	<i>Gerbillus pyramidum</i>	Gougaram, Niger	18°33'N/07°47'E	S, K	38/72	JN652813
BENalg1**	<i>Gerbillus tarabuli</i>	Beni-Abbès, Algeria	30°04'N/02°05'W	S		JN652820
BENalg2**	<i>Gerbillus tarabuli</i>	Beni-Abbès, Algeria	30°04'N/02°05'W	S		JN652819
BENalg3**	<i>Gerbillus tarabuli</i>	Beni-Abbès, Algeria	30°04'N/02°05'W	S		JN652821
INAmal1**	<i>Gerbillus tarabuli</i>	Inabog, Mali	19°21'N/00°14'W	S, K	40/74	JN652832
KABmal2**	<i>Gerbillus tarabuli</i>	Kabara, Mali	16°43'N/02°59'W	S, K	40/74	JN652828
KREmal1**	<i>Gerbillus tarabuli</i>	Kreb in Karoua, Mali	19°21'N/00°11'E	S, K	40/74	JN652831
TIDmal1**	<i>Gerbillus tarabuli</i>	Tidermène, Mali	17°01'N/02°07'E	S, K	40/74	JN652829
TOMmal2**	<i>Gerbillus tarabuli</i>	Tombouctou, Mali	16°45'N/02°59'E	S, K	40/74	JN652830
AGNmau**	<i>Gerbillus tarabuli</i>	Agneitir, Mauritania	19°20'N/16°17'W	S		JN652815

Table 1 (continued)

Individual no.	Identification	Geographic origin	Geographical coordinates	Use	2n/Nfa	Genebank accession no.
AKJmau**	<i>Gerbillus tarabuli</i>	Akjoujt, Mauritania	16°44'N/14°22'W	S, K	40	JN652826
MOUmau1**	<i>Gerbillus tarabuli</i>	El Mounane, Mauritania	17°13'N/16°07'W	S		JN652827
AYOmau1**	<i>Gerbillus tarabuli</i>	Ayôun el Atrôus, Mauritania	16°35' N/09°35'W	S		JN652818
AYOmau2**	<i>Gerbillus tarabuli</i>	Ayôun el Atrôus, Mauritania	16°35' N/09°35'W	S		JN652816
AYOmau3**	<i>Gerbillus tarabuli</i>	Ayôun el Atrôus, Mauritania	16°35' N/09°35'W	S		JN652817
Ge.tara1**	<i>Gerbillus tarabuli</i>	Nguigmi, Niger	14°15'N/13°06'E	S		JN652814
TEGnig2**	<i>Gerbillus tarabuli</i>	Teguidda'n Tessoumt, Niger	17°27'N/06°42'E	S, K	40/74	JN652825
TOUnig**	<i>Gerbillus tarabuli</i>	Toukounous, Niger	14°31'N/03°18'E	S		JN652823
RICsen**	<i>Gerbillus tarabuli</i>	Richard-Toll, Senegal	16°28'N/15°45'W	S		JN652824
AGAnig**	<i>Gerbillus tarabuli</i>	Agadez, Niger	17°00'N/07°56'E	S, K	40/74	JN652822
BM81	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM82	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM83	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM86	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM87	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM89	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM92	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM95	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM98	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
BM99	<i>Gerbillus cf. tarabuli</i>	Aoujeft, Mauritania	20°00'N/13°03'W	M		
Outgroups						
	<i>Taterillus gracilis</i>			S		AM409394
	<i>Sekeetyamys calurus</i>			S		AJ851276a

S, cytochrome *b* gene sequencing; M, morphometry (skull measurements); K, karyology (K*: from cell culture); 2n/Nfa, diploid and autosomal fundamental numbers ('-' indicates uninterpretable karyotype).

*Karyotype in Viegas-Pequignot *et al.* (1984).

†Sequence data from Nesi (2007).

‡Data from Abiadh *et al.* (2010a).

analyses. Both the phylogenetic relationships and divergence time estimates were inferred using BEAST v1.6.1 package (Drummond & Rambaut 2007). BEAST uses Markov Chain Monte Carlo (MCMC) to approximate time-measured phylogenies and simultaneously infer their credibility intervals. For the molecular dating analyses, we used a Bayesian relaxed clock (BRC) approach, which accounts for rate variation across lineages and assumes that substitution rates are uncorrelated across the tree (there is thus no *a priori* correlation between a lineage rate and that of its ancestor). The age of the most ancient known fossil of *Gerbillus* (late Pliocene; Winckler *et al.* 2010) was used to assign a minimum age for the crown group that encompasses all sampled *Gerbillus* representatives. For this specific constraint, the most conservative age [lower boundary of late Pliocene; 3.6 millions years (Myr)] was preferentially chosen. In a conservative way, the upper bound (7.03 Myr) of the estimate of divergence time between *Gerbillus* and *Meriones* Illiger, 1811 (Abiadh *et al.* 2010a,b), was used as maximum age for the *Gerbillus* node. Thus, we set the *Gerbillus* node age prior to an uniform distribution comprised between 3.6 and 7.03 Myr. To account for the fact that our trees mostly describe intra-specific relation-

ships, we used a coalescent model tree prior with a constant population size. Two distinct runs were carried out, each one with four independent chains of 50 000 000 generations, default priors (with the exception of the tree priors) and trees sampled every 1000 generations. Results were visually inspected using TRACER v1.5 to ensure proper mixing of the MCMC. After applying a conservative burn-in of 12 500 000 generations for each run, both the median age estimates and the 95% higher posterior densities (95% HPD) were directly estimated using TreeAnnotator 1.6.1 (Drummond & Rambaut 2007). The robustness of nodes under BI was estimated using clade posterior probabilities (CPP, expressed in %) with the same burn-in period of 12 500 000 generations.

Chromosomal analyses

Twenty specimens of *Gerbillus* from Morocco were brought back alive to the laboratory at the Institut de Recherche pour le Développement, Dakar, where they were karyotyped following the slightly modified air-drying technique (Evans *et al.* 1963). Animals were yeast stimulated overnight and injected with an anti-mitotic solution (vinblastin sulphate 10 mg in 40 mL NaCl 8.5%) 45 min

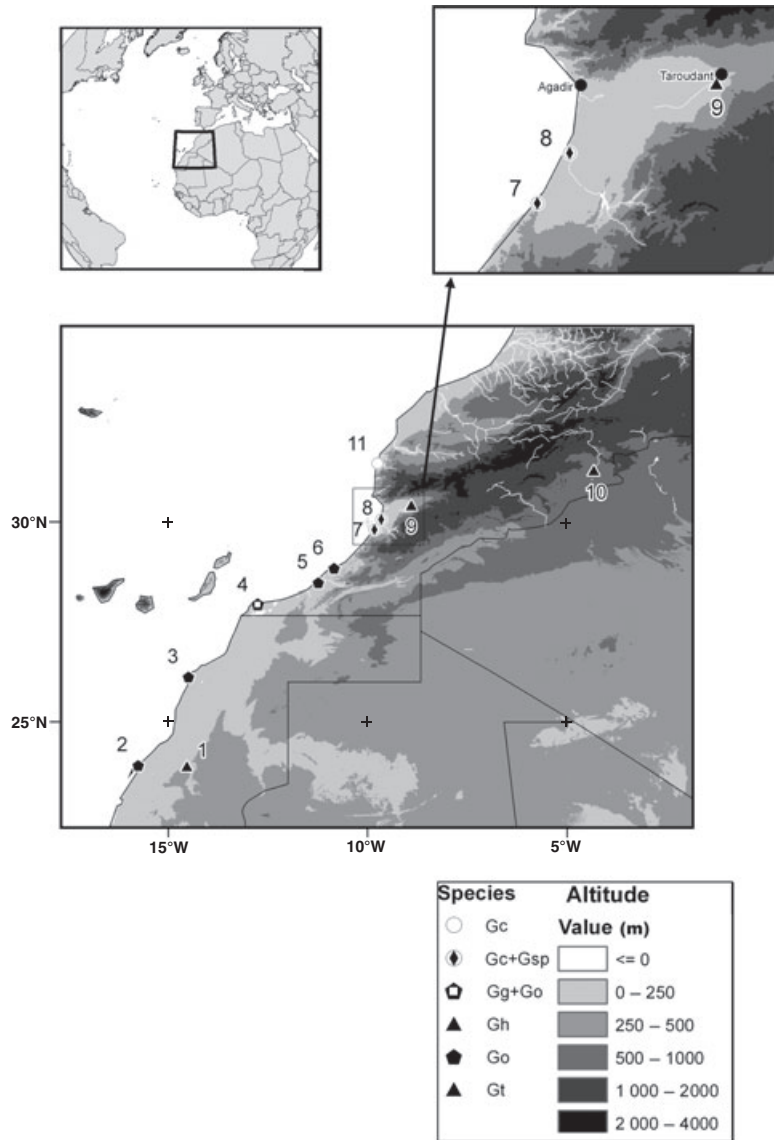


Fig. 1 Map of Morocco showing the sampling sites (numbers 1–11 refer to localities of Table 1), and the gerbil species occurrences (Gc, *Gerbillus campestris*; Gho, *Gerbillus hoogstrali*; Ghe, *Gerbillus hesperinus*; Gg, *Gerbillus gerbillus*; Go, *Gerbillus occiduus*; Gt, *Gerbillus tarabuli*; Gsp, *Gerbillus* sp.). The insert focus on the Sous-Massa area, between the High Atlas (north) and Anti-Atlas (south) mountain ranges.

before sacrifice. Bone marrow was extracted and incubated for 20 min at 37 °C in 8 mL KCl 0.075 M. Fixation involved methanol and acetic acid 3:1 v/v. Metaphasic suspensions were then deposited on slides, stained using 4% Giemsa and observed under a Leitz Wetzlar (Germany) microscope. For each specimen, at least 10 high-quality metaphases were captured using the Genus software (Applied Imaging, Genetix, Gateshead, UK), and karyotypes were prepared from the clearest ones.

Additionally, intercostal tissue from one specimen was collected on culture medium and sent to the Museum National d’Histoire Naturelle, Paris. The karyotype of this particular individual was established from fibroblast culture using the same procedure as described earlier. The karyotypes of specimens originating from other countries

than Morocco (Table 1) came from Abiadh *et al.* (2010a) or were obtained previously (G. Dobigny, J.M. Duplantier and L. Granjon, unpubl. data).

Morphological analyses

Seventy-seven adult specimens of gerbils were subjected to morphological analyses (Table 1). Most of them originated from Morocco (6 *G. campestris*, 10 *G. gerbillus*, 9 *G. hoogstrali*, 11 *Gerbillus* sp., 30 *G. occiduus*), the remaining ones coming from N. Mauritania (1 *G. gerbillus*, 10 *G. cf tarabuli*). Of special interest was the confrontation of *G. hoogstrali*, *Gerbillus* sp., *G. occiduus* and *G. cf tarabuli*, all belonging to the medium to large-sized, hairy-footed species of North Africa. The following 11 skull measurements (Fig. 2) were taken to the nearest 0.01 mm using a digital calliper:

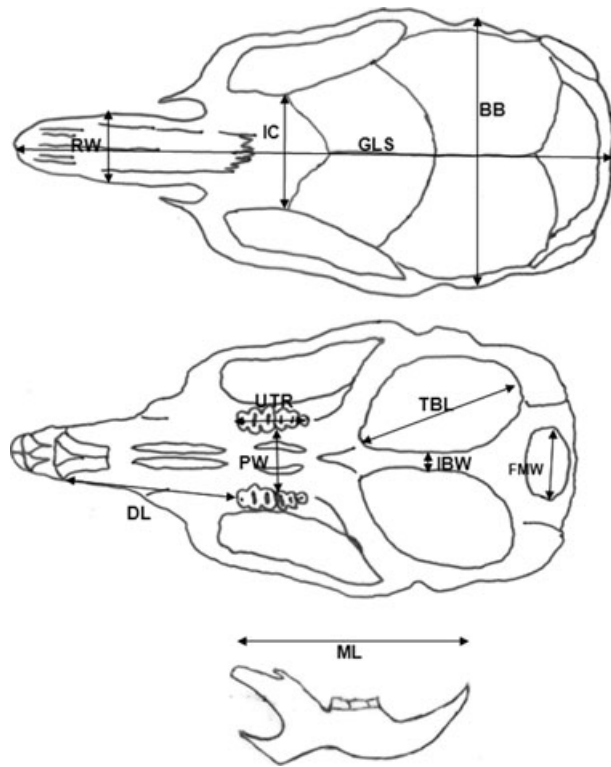


Fig. 2 Dorsal, ventral view and mandible of a *Gerbillus* skull, showing the 11 measurements taken for morphometrical analyses.

greatest length of skull (GLS), breadth of braincase (BB), interorbital constriction (IC), rostral width (RW), diastema length (DL), palatal width (PW), tympanic bulla length (TBL), upper tooth row length (UTR), foramen magnum width (FMW), inter-bullae width (at the basioccipital-basisphenoid junction; IBW) and mandible length (ML).

T-tests with Bonferroni adjustments were performed using MYSTAT 12 (2007) for measurement mean comparisons.

These measurements were subjected to principal component analyses (PCA) and discriminant analyses (DA) using Systat 8.0 (1998). Both PCA and DA used non-transformed data, and PCA was run using the correlation matrix. A jackknifed classification matrix was produced to assess predication accuracy based on the discriminant functions generated by the DA. A hierarchical classification was performed on canonical scores of group means obtained in DA, using single linkage method on Euclidian distances.

Results

For clarity reasons, specimens will be referred to after the species names that were eventually attributed to them following the confrontation of morphometric, cytogenetic and molecular data.

Molecular phylogenetics and divergence time analyses

One hundred and four complete (1140 bp) cytochrome *b* sequences were considered in the phylogenetic analyses. Within the genus *Gerbillus*, K2P genetic distances range between 0.018 (between *G. tarabuli* and *G. occiduus*) and 0.162 (between *Gerbillus nanus* and *G. nigeriae*; Table 2).

Overall the phylogenetic relationships of major clades (see Fig. 3) were unambiguous under BI as most nodes beyond the intraspecific level were supported by CPP > 95%. All members of genus *Gerbillus* are recovered in a highly supported clade (CPP of 100%). The species *Gerbillus nanus* is found in a sister position to all remaining sampled *Gerbillus* individuals. All specimens from *Gerbillus* sp. clustered together in a sister position to a large clade that comprises *G. tarabuli* and *G. occiduus* (CPP of 100%

Table 2 Kimura 2-parameter genetic distances between the clades identified in the phylogenetic analysis

	<i>Taterillus gracilis</i>	<i>Gerbillus campestris</i>	<i>Gerbillus gerbillus</i>	<i>Gerbillus hesperinus</i>	<i>Gerbillus hoogstrali</i>	<i>Gerbillus latastei</i>	<i>Gerbillus nanus</i>	<i>Gerbillus nigeriae</i>	<i>Gerbillus occiduus</i>	<i>Gerbillus perpalidus</i>	<i>Gerbillus pyramidum</i>	<i>Gerbillus sp.</i>	<i>Gerbillus tarabuli</i>
<i>Taterillus gracilis</i>													
<i>Gerbillus campestris</i>	0.235												
<i>Gerbillus gerbillus</i>	0.240	0.149											
<i>Gerbillus hesperinus</i>	0.227	0.131	0.132										
<i>Gerbillus hoogstrali</i>	0.231	0.146	0.130	0.026									
<i>Gerbillus latastei</i>	0.236	0.136	0.129	0.080	0.091								
<i>Gerbillus nanus</i>	0.218	0.147	0.150	0.142	0.157	0.161							
<i>Gerbillus nigeriae</i>	0.242	0.145	0.137	0.113	0.127	0.113	0.162						
<i>Gerbillus occiduus</i>	0.232	0.151	0.125	0.095	0.100	0.102	0.157	0.132					
<i>Gerbillus perpalidus</i>	0.232	0.124	0.123	0.081	0.081	0.083	0.144	0.105	0.076				
<i>Gerbillus pyramidum</i>	0.233	0.125	0.128	0.078	0.085	0.078	0.139	0.110	0.073	0.031			
<i>Gerbillus sp.</i>	0.241	0.139	0.129	0.090	0.096	0.094	0.147	0.114	0.040	0.067	0.065		
<i>Gerbillus tarabuli</i>	0.233	0.146	0.131	0.095	0.100	0.100	0.154	0.128	0.018	0.072	0.070	0.040	
<i>Sekeetamys calurus</i>	0.244	0.162	0.170	0.179	0.174	0.182	0.168	0.186	0.177	0.163	0.170	0.170	0.179

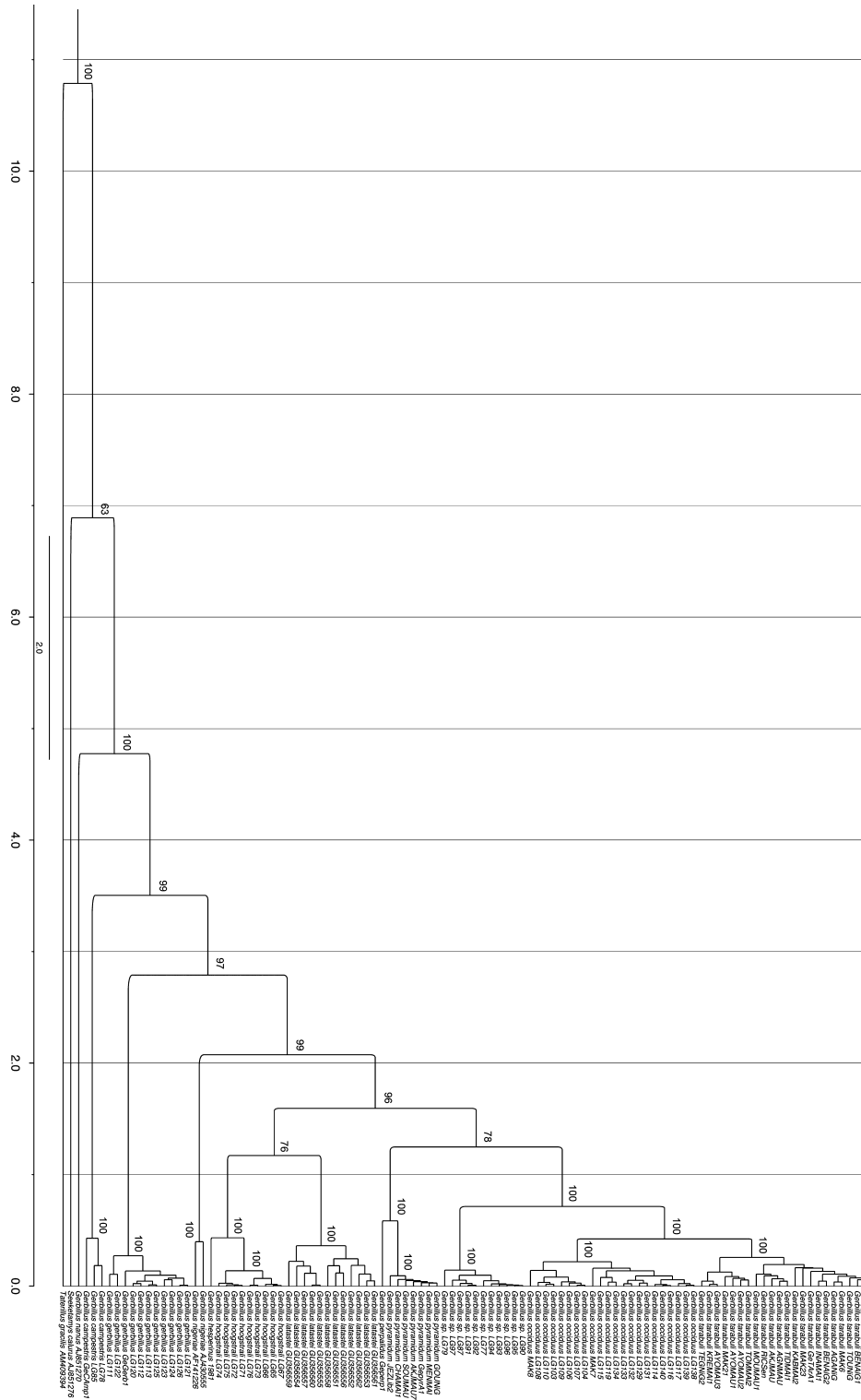


Fig. 3 Time calibrated phylogeny inferred using BEAST, depicting the phylogenetic relationships between all studies *Gerbillus* specimens. Numbers at nodes represent clade posterior probabilities (CPP).

for all nodes). *Gerbillus perpallidus* and *G. pyramidum* together form the sister group of this assemblage, with a moderate support (CPP of 78%). A group of three species (*G. latastei*, *G. hesperinus* and *G. hoogstrali*) is found as a well-supported sister group (CPP of 96%) of this *G. tarabuli*/*G. occiduus*/*Gerbillus* sp./*G. pyramidum*/*G. perpallidus* clade. Then, a highly supported clade (CPP of 99%) corresponding to *G. nigeriae* confidently represents the sister taxon of the eight latter species, followed by *Gerbillus gerbillus* and *G. campestris*, also with high support (CPP of 97% and 99%, respectively).

Estimates of divergence times are presented in Table 3. The age of the common ancestor of the genus *Gerbillus* is estimated around 4.77 Myr (95% HPD: 3.60–6.81 Myr). Quite recent ages (comprised between 0.09 and 0.43 Myr) are recovered for all nodes directly leading to individual *Gerbillus* species (see Table 3). The two most recent common ancestors would be those of *G. tarabuli* and *G. occiduus* on the one hand, and the one of *G. hesperinus* and *G. hoogstrali* on the other hand (0.42 and 0.43 Myr, respectively).

Karyology

Fifteen (including one from the type locality of *G. occiduus*, Aoreora, Morocco) of the 20 specimens whose karyotype

was obtained from bone marrow displayed a diploid number of $2n = 40$ chromosomes. All the chromosomes observed were meta/submetacentric, including the X and Y sex chromosomes, thus yielding an autosomal fundamental number (aFN) of 76, and a fundamental number (FN) of 80 (Fig. 4A). This karyotypic morphology very closely matches that of *Gerbillus occiduus* as published by Lay (1975) from specimens of the type locality. In another specimen with $2n = 40$, the two smallest autosome pairs seemed to comprise three metacentrics and one acrocentric (aFN = 75). This individual was furthermore molecularly characterised as *G. occiduus*, which implies that some chromosomal polymorphism may exist in this species. The karyotype of three specimens could not be determined with safety; however, they were subsequently identified by molecular analysis as belonging to the species *Gerbillus occiduus* (on the basis of an incomplete cytochrome *b* sequence for the specimen LG137). The karyotype of one of them (LG129) was not interpretable, but the one of its captivity-born young was obtained, showing the typical *G. occiduus* complement.

One male specimen clearly had $2n = 43$, with three pairs of small acrocentric autosomes (aFN = 74) and three

Table 3 Divergence time estimates (Myr) obtained through a Bayesian relaxed clock analysis of the molecular dataset

Ancestor of	Median	95% HPD
<i>Taterillus gracilis</i> and <i>Gerbillus gerbillus</i>	10.79	3.80–23.42
<i>Sekeetamys calurus</i> and <i>Gerbillus gerbillus</i>	6.89	4.01–11.79
<i>Gerbillus nanus</i> and <i>Gerbillus gerbillus</i>	4.77	3.60–6.81
<i>Gerbillus campestris</i> and <i>Gerbillus gerbillus</i>	3.50	1.88–5.62
<i>Gerbillus tarabuli</i> and <i>Gerbillus gerbillus</i>	2.79	1.36–4.54
<i>Gerbillus tarabuli</i> and <i>Gerbillus nigeriae</i>	2.07	1.03–3.53
<i>Gerbillus tarabuli</i> and <i>Gerbillus hesperinus</i>	1.59	0.78–2.75
<i>Gerbillus tarabuli</i> and <i>Gerbillus perpallidus</i>	1.25	0.56–2.22
<i>Gerbillus latastei</i> and <i>Gerbillus hesperinus</i>	1.17	0.51–2.10
<i>Gerbillus tarabuli</i> and <i>Gerbillus</i> sp.	0.71	0.29–1.34
<i>Gerbillus perpallidus</i> and <i>Gerbillus pyramidum</i>	0.58	0.17–1.23
<i>Gerbillus hesperinus</i> and <i>Gerbillus hoogstrali</i>	0.43	0.12–0.93
<i>Gerbillus tarabuli</i> and <i>Gerbillus occiduus</i>	0.42	0.18–0.79
<i>Gerbillus campestris</i>	0.43	0.09–1.00
<i>Gerbillus gerbillus</i>	0.27	0.08–0.61
<i>Gerbillus hoogstrali</i>	0.14	0.04–0.32
<i>Gerbillus latastei</i>	0.36	0.14–0.71
<i>Gerbillus nigeriae</i>	0.40	0.06–1.00
<i>Gerbillus occiduus</i>	0.22	0.09–0.42
<i>Gerbillus pyramidum</i>	0.09	0.02–0.23
<i>Gerbillus tarabuli</i>	0.26	0.11–0.49
<i>Gerbillus</i> sp.	0.14	0.03–0.31

For clarity, ages are only reported for major nodes of the phylogeny. The age estimates for *Gerbillus* species are figured on the bottom part of the Table. For each node, the median age and the 95% higher posterior densities (95% HPD) are reported.



Fig. 4 A–B. Karyotypes of *Gerbillus occiduus* (specimen LG118)—A, and of *Gerbillus hoogstrali* (specimen LG68)—B.

submetacentric sex chromosomes of various sizes (not shown). This karyotype corresponds to the one of *Gerbillus gerbillus*, as presented in Lay et al. (1975) and Wahrman et al. (1988).

The karyotype obtained after cell culture was from a male specimen caught close to the type locality of *G. hoogstrali*. It was characterised by $2n = 72$ chromosomes, with aFN = 84 resulting from the presence of one large meta-centric pair, six small submetacentric pairs (sometimes

with very small short arms) and 28 acrocentric pairs. Sex chromosomes consisted of a large submetacentric and a medium-sized metacentric elements (FN = 88; Fig. 4B). This perfectly fits to the karyotype of one male *G. hoogstrali* from the type locality of the species (Lay 1975). However, Lay (1975) proposed a FN = 80 for this specimen, probably considering that four of the small pairs that we here considered as submetacentric were rather telocentric (see his Fig. 3).

Morphological analyses

A PCA was run on 77 individuals belonging to six different species. All 11 variables correlated positively with the first principal component (PC1) that, in turn, reflects an important part of the total variance (45.9%), thus suggesting that PC1 represents a size axis (Table 4). The variables TBL, FMW, and IC and IBW were the most strongly (negatively) correlated variables on PC2, PC3 and PC4, respectively (Table 4). On the PC1 × PC2 graph, representing 59.5% of the total variance, specimens of *G. gerbillus* and *G. campestris* appeared rather well differentiated (Fig. 5A). On the PC3 × PC4 graph (19.6% of total variance), specimens of *G. cf. tarabuli* were well-separated along both axes, whereas *G. occiduus* and *G. hoogstrali*/*Gerbillus* sp. were partly discriminated along PC3 and PC4 axes, respectively (Fig. 5B). Based on these observations, some skull measurements appear to be very significantly

Table 4 Variable loadings on the first four principal components (PC) of the principal component analysis run on the 77 *Gerbillus* individuals × 11 skull measurements data matrix (loadings <0.500 in bold, see text)

	PC1	PC2	PC3	PC4
Greatest length of skull	0.889	-0.002	0.061	0.264
Breadth of braincase	0.707	-0.456	0.102	0.056
Foramen magnum width	0.276	0.134	-0.824	-0.118
Diastema length	0.892	0.151	0.032	0.063
Upper tooth row length	0.625	-0.416	-0.398	-0.013
Palatal width	0.690	0.270	0.467	0.150
Interorbital constriction	0.633	-0.122	0.232	-0.626
Tympanic bulla length	0.367	-0.846	0.059	0.009
Mandible length	0.628	0.228	-0.373	0.167
Inter-bullae width	0.660	0.387	0.017	-0.516
Rostral width	0.803	0.268	0.067	0.294

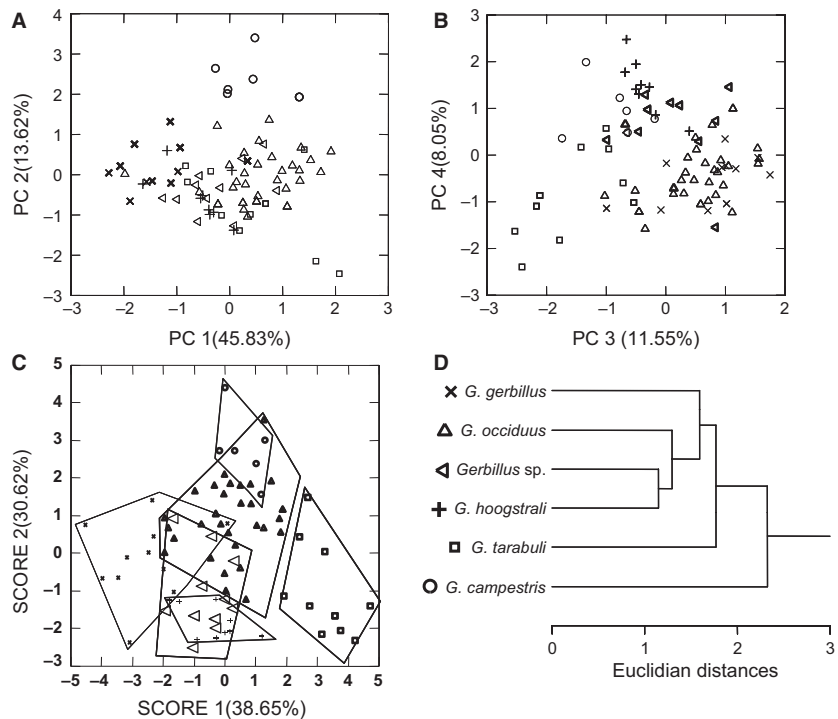


Fig. 5 A–D. Scatterplots of multivariate analyses performed on 11 skull measurements on a sample of 77 individuals of *Gerbillus*: PC1 × PC2 and PC3 × PC4, respectively, of the principal component analysis—A and B. Discriminant vectors 1 and 2 of the Discriminant Analysis—C; and hierarchical classification using euclidian distance between mean scores of the 6 specific samples in the DA—D.

different between some species and all the others: a small TBL characterises *G. campestris* relative to the five other species (*t*-tests; $P < 0.006$), whereas *G. cf tarabuli* has a large FMW when compared with all other species but *G. campestris* (*t*-tests; $P < 0.011$). *Gerbillus occiduus* is characterised by a large IC (*t*-tests, $P = 1.529 \cdot 10^{-11}$, and $P = 3.9 \cdot 10^{-4}$) and IBW (*t*-tests, $P = 6.49 \cdot 10^{-9}$, and $P = 4.45 \cdot 10^{-7}$ when compared with *G. hoogstrali* and *Gerbillus* sp., respectively (i.e. the two other endemic species from Morocco).

To maximise the differentiation of these species, a DA was performed using the same set of individuals (Fig. 5C). The corresponding jackknifed classification matrix showed an overall 78% of well-classified individuals over the whole sample. Among them, all *G. campestris* appeared well classified, while only 45% (5/11) of the *Gerbillus* sp. was correctly assigned (three of them were assigned to *G. hoogstrali*, two to *G. occiduus*, and one to *G. gerbillus*). The other species showed between 78% (*G. hoogstrali*) and 83% (*G. occiduus*) of well-classified individuals. The hierarchical classification shows that *Gerbillus* sp. and *G. hoogstrali* are the most similar species, followed by *G. occiduus*. Again, *G. campestris* appears clearly as the most phenetically divergent species based on the skull measurements considered (Fig. 5D).

Discussion

On Fig. 6 are summarised the main features of the species here studied, in particular, their phylogenetic relationships based on cytochrome *b* sequence data, their biogeographic distribution, karyotypic description and main morphological characteristics.

Integrative taxonomy in North African medium to large-sized hairy-footed gerbils

At the scale of the whole *Gerbillus* genus, our molecular results confirmed those of Chevret & Dobigny (2005) and Abiadh *et al.* (2010a) as to the relative phylogenetic position of *G. nanus* and *G. campestris*. These results strongly suggest that *campestris* should not be considered as belonging to the genus *Dipodillus* while *nanus* and all other species here studied are maintained in the genus *Gerbillus* (as in Musser & Carleton 2005). Rather, one should either consider the possibility that the whole group be splitted into three genera, or more conservatively, into three subgenera that would be *Dipodillus* (for *campestris* and allied species), *Hendecapleura* Lataste, 1894 (for *nanus* and allied species) and *Gerbillus* (all other species, see Musser & Carleton 2005 for further comments). *Gerbillus campestris* was also clearly differentiated on a morphometrical basis (as in Abiadh *et al.* 2010b). *Gerbillus*

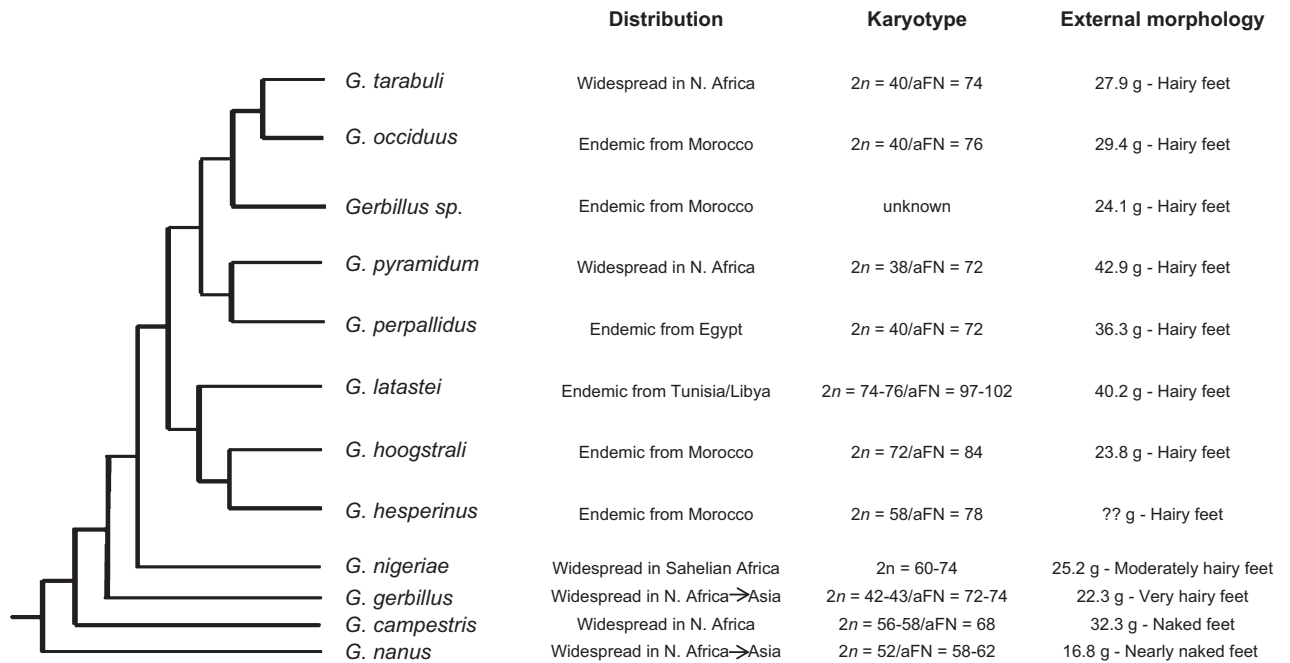


Fig. 6 Main phylogenetic, biogeographic, chromosomal and morphological characteristics of the *Gerbillus* species studied. Weight data compiled from Osborn & Helmy (1980), Granjon & Duplantier (2009), and original data. Note that no weight data seem to exist for *G. hesperinus*, but body measurements presented in Aulagnier & Thévenot (1986) suggest it is of similar size as *G. hoogstrali* and *G. occiduus*.

gerbillus appeared well differentiated from other lineages whatever the method used (even if some overlap remains in morphometric analyses on the basis of the 11 variables retained here), thus confirming that this species may represent a quite distinct lineage of hairy-footed gerbils (Chevret & Dobigny 2005).

At the specific scale, all the specimens studied here could be unambiguously assigned to a species, be it already well-known or potentially new. This was made possible via the combined use of, mainly, chromosomal and molecular data. Morphometric results left a space of uncertainty, especially at the borders between overlapping morphological groups. However, the analyses conducted here were also very instructive *a posteriori* in pointing towards some potentially diagnostic characters between species.

Karyotypes obtained in the present study, when compared with reference data (Lay 1975; Lay *et al.* 1975; Viegas-Pequignot *et al.* 1982; Aniskin *et al.* 2006), enabled us to unambiguously diagnose the corresponding specimens as *Gerbillus gerbillus*, *G. occiduus* or *G. hoogstrali*. Uncertainties concerned three specimens that were all clearly shown to belong to the *G. occiduus* clade through cytochrome *b* analysis. Phylogenetic analyses of cytochrome *b* sequences revealed well-differentiated clades that proved to correspond to distinct biological species as demonstrated by their correspondence with diagnostic karyotypes. This also concerns *G. tarabuli* whose species-specific molecular clade was well-supported (100% CPP) and includes several individuals that show the typical $2n = 40$, $NFa = 72$ karyotype of *G. tarabuli* (Granjon *et al.* 1999; Aniskin *et al.* 2006).

The molecular clade corresponding to *G. hoogstrali* was highly supported (CPP of 100%), and the karyotype obtained from a specimen caught in one of the same sites as the sequenced specimens once again confirmed the specific identity of this group of individuals. *Gerbillus hoogstrali* ($2n = 72$, $aFN = 84$) appears as the sister species of *G. hesperinus* ($2n = 58$, $aFN = 78$, Lay 1975; Viegas-Pequignot *et al.* 1984) in what should be considered to date as an endemic Moroccan clade. This clade would be the sister group of *G. latastei* ($2n = 74-76$, $aFN = 97-102$, Jordan *et al.* 1974; Volobouev *et al.* 1995; under *G. aureus* Setzer, 1956), currently considered as an endemic species from Tunisia and Libya. Interestingly, these species with relatively high diploid numbers represent the sister group of a set of species characterised by much smaller $2n$ as summarised in Aniskin *et al.* (2006): $38/72$, $40/72$, $40/74$ and $40/76$ for *G. pyramidum*, *G. perpallidus*, *G. tarabuli* and *G. occiduus*, respectively.

Unfortunately, no chromosomal data could be obtained for specimens of the *Gerbillus* sp. lineage belonging to this

$2n = 38-40$ species group. This lineage, which is very well supported in all analyses, was not expected because its representatives come from the distribution area of *G. hoogstrali* (as depicted by Aulagnier & Thévenot 1986; and Zyadi 1988; reviewed in Musser & Carleton 2005). The possibility that this sample be referred to an existing species appears doubtful, as all hairy-footed *Gerbillus* species from north-western Africa have been taken into account at least in molecular analyses. No obvious morphological feature enabled us to distinguish between these specimens and those from *G. hoogstrali* studied here. Multivariate analyses of skull measurements show the two sets of individuals to be largely overlapping (even with DA), and the hierarchical classification based on phenetic distances confirmed that *Gerbillus* sp. and *G. hoogstrali* are the most similar species in terms of skull morphology. This phenetic similarity may be interpreted as the result of common adaptive pressures associated with the same geographic and environmental context that is the sandy areas of the Sous Valley. It may also reflect the conservation of a common 'primitive' skull morphology in these two species among the group of molecularly related species to which they belong. Such morphological conservatism/convergence has already been documented in sympatric small mammals (see for instance, Fadda & Corti 2001; and Rychlik *et al.* 2006).

In a more general manner, the overall morphometric similarity of the three species *G. hoogstrali*, *G. occiduus* and *Gerbillus* sp. confirms their status of sibling species. This is a widely encountered situation within the Gerbillinae subfamily, where groups of cryptic species have been identified in a number of genera (*Taterillus* Thomas, 1910; Dobigny *et al.* 2002; *Gerbillus* Thomas, 1897; Granjon 2005). Abiadh *et al.* (2010b) also found an important overlap of skull shape between *G. tarabuli* and *G. latastei*, an endemic species from Tunisia and Libya. The multivariate analyses, however, confirmed the validity of criteria used originally in the description of some of the species, and especially large interorbital constriction (IC) and interbulbar length (at the basioccipital-basisphenoid junction, IBW) in *Gerbillus occiduus*, especially when compared to *Gerbillus hoogstrali* (Lay 1975).

Distribution and conservation implications

When plotted on a map, the specimens here studied bring new information as far as the diversity and distribution of Moroccan gerbils are concerned (Fig. 1). First, *G. occiduus* appeared as much more widely distributed than previously thought, ranging all along the Atlantic coast from Aoreora, its type locality, in the north, to Dakhla (southern part of Western Sahara) in the south. This represents an extension of ca. 450 km to the south for the distribution

of this poorly known species (Aulagnier & Thévenot 1986; Musser & Carleton 2005). As a nomenclatural consequence, this raises the possibility that *G. occiduus* is in fact a junior synonym of *G. riggenbachi* Thomas 1903 described from a site situated just on the Tropic of Cancer in the Rio de Oro (southern part of Western Sahara) that is immediately south of Dakhla (Thomas 1903). *Gerbillus riggenbachi* is currently considered a synonym of *G. tarabuli* (Granjon *et al.* 1999; Musser & Carleton 2005).

Gerbillus boogstrali was claimed to occupy all favourable habitats from its type locality (7 km south of Taroudant, 1 km south of the Sous River) to the coastal sandy areas between the Sous and Massa Rivers (Zyadi 1988). Here, two species were found in the Sous Valley on the basis of molecular results. One does correspond to *G. boogstrali*, which was only caught in one site 8 km south of the Sous River at the longitude of Taroudant. It was not found in the immediate vicinity of the type locality as described in Lay (1975), where the only gerbil found was *G. campestris*. This may be due to the environmental changes that have occurred in the last decades in this area, which is now mostly occupied by agricultural areas (fields and orchards). In the coastal area (entrance of the Sous-Massa National Park and surroundings of Aglou), another species, here referred to as *Gerbillus* sp., occurs. As a result, it is probable that (i) at least two instead of one endemic *Gerbillus* species occupy this relatively small area, estimated to cover <20 000 km² by Aulagnier & Hutterer (2008) and that (ii) the *G. boogstrali* population would be far less important than previously thought. This would imply a change in IUCN red list category from vulnerable to endangered, given the possible population reduction that this species may have experienced in the last 10–20 years. In the meantime, would its distribution area confirmed to be restricted to the coastal sandy plains between the Sous and Massa Rivers, the potentially new endemic *Gerbillus* sp. may also face serious threats.

Last but not least, we failed to trap *G. hesperinus* in the vicinity of its type locality, Essaouira, despite an effort of ca. 200 trap-nights in sandy areas immediately south of the city and 150 trap-nights 20 km farther south in various habitats including coastal sand dunes. The last specimen captured in the sand dunes south of Essaouira dates back to the early 80s and it is the one which karyotype is figured in Viegas-Pequignot *et al.* (1984) and which cytochrome *b* gene sequence has been studied here. This endemic species was said to occupy <500 km² in two distinct populations on both sides of Essaouira and was thus considered as Endangered (Aulagnier 2008). It may be currently even more threatened by current habitat loss associated with human infrastructure building.

Evolutionary systematics

The relationships inferred by the analyses of the cytochrome *b* sequences are the first for this group of North African medium-sized hairy-footed gerbil species (but see Abiadh *et al.* 2010a on a small sample of species from Tunisia only). Chevret & Dobigny (2005) recently presented a phylogenetic analysis of the subfamily Gerbillinae and estimated the emergence of the genus *Gerbillus* at ca. 4.12 ± 0.9 Myr. Our own estimation for the emergence of the *Gerbillus* group at 4.77 Myr (range 3.60–6.81) is not inconsistent with this date. Abiadh *et al.* (2010a), partly basing their calibration on the results obtained by Chevret & Dobigny (2005), dated the emergence of *Gerbillus* at 2.8 Myr (range 1.7–4.04). This date is somewhat more recent than ours, likely because the information on fossil ages from Winkler *et al.* (2010) was not available at the time of their publication. From there, the lineage leading to *G. campestris* would have differentiated at ca. 3.5 Myr, followed by the ones leading to *G. gerbillus* and *G. nigeriae* at 2.79 and 2.07 Myr, respectively. These events would have taken place between Upper Pliocene and Lower Pleistocene, a period characterised by both gradual climatic cooling and aridification (deMenocal 2004).

The other events would all have taken place during the Pleistocene, a period characterised by the onset of marked climatic oscillations, with repeated glacial – interglacial cycles and an overall trend towards a more arid climate (deMenocal 2004). In the Sahara desert, these climatic variations translated in the alternation between very dry and rather wet periods (Rognon 1993). Two major lineages (encompassing *G. hesperinus*, *G. boogstrali* and *G. latastei* on the one hand, and *G. occiduus*, *G. perpallidus*, *G. pyramidum*, *G. tarabuli* and *Gerbillus* sp. on the other hand) then differentiated at 1.59 Myr. Subsequent split between lineages occurred between 1.25 and 0.42 Myr, in a period that corresponds to an intensification of glacial cycles coinciding with a phase of maximum climate variability in Africa (especially West Africa: deMenocal 2004; Trauth *et al.* 2009).

The most striking finding consists in the very recent differentiation between sister species *G. tarabuli*/*G. occiduus*, and *G. boogstrali*/*G. hesperinus* that would both have occurred at ca. 0.42–0.43 Myr. In the first instance, the very small genetic distance (0.018 K2P) greatly contrasts with the huge amount of chromosomal rearrangements that has been found between the karyotypes of *G. tarabuli* and *G. occiduus*, with no less than 49 structural differences (Aniskin *et al.* 2006). This would lead to a conservative estimate of rate of chromosomal change of more than 100 rearrangement per Myr, thus making them undoubtedly one of the most rapidly evolving species group of mammals ever documented from a chromosomal point of view (see Dobigny *et al.* 2005 and Trifonov *et al.* 2008 for

examples in various mammalian orders). In rodents, this rate largely exceeds those estimated so far in the genera *Taterillus* (Dobigny *et al.* 2005), *Gerbillus* (Aniskin *et al.* 2006), *Acomys* I. Geoffroy, 1838 (Volobouev *et al.* 2002), *Mastomys* Thomas, 1915 (Volobouev *et al.* 2002), and *Gerbilliscus* (Volobouev *et al.* 2007), with respectively 45, 10, 7, 6 and 2 rearrangements per million of years.

As to the processes that may have taken place to promote the differentiation of these lineages, one may propose some refinements to the biogeographic hypothesis proposed by Lay (1975). One possible scenario is that endemic hairy-footed gerbils of Morocco differentiated along the Atlantic coast of north-western Africa during periods of marine transgressions that should have corresponded with interglacials. Then, favourable habitats were restricted in space, both because of an elevation of the coast line and because of the climate that was probably more humid than during glacial periods. Subsequently, these differentiated populations would then have dispersed northwards across the current Western Sahara region during glacial periods of the Pleistocene. Such periods, generally characterised by marine regressions and a drier climate, would have left coastal corridors along which gerbils have colonised favourable habitats of wide extension. There, subsequent marine transgressions could then have trapped them north of (*G. hesperinus*), south of (*G. occiduus*), or between (*G. boogstrali*, and now *Gerbillus* sp.) the High Atlas and Anti-Atlas mountain ranges. This scenario is especially probable in the case of the differentiation of *G. occiduus*; according to our date estimates, and given the respective distribution range of *G. tarabuli* and *G. occiduus* as they are currently perceived, we speculate that these two lineages differentiated from each other from populations that were isolated by a marine transgression that occurred around 420 000 years ago, between northern Mauritania and southern Western Sahara. Such marine transgressions are known to have occurred repeatedly along the Atlantic Moroccan coast all along the Pleistocene (Aumassip & Chaïd-Saoudi 2004; Chabli *et al.* 2005). The population that then evolved allopatrically into *G. occiduus* would have further dispersed northwards to occupy its current range along the Atlantic coast of southern Morocco, thanks to the marine regression accompanying a subsequent glacial period (as happened during the Würm, see Plaziat *et al.* 2008). At the same time, demographic and spatial expansion of Mauritanian *G. tarabuli* populations would have occurred all over arid North Africa, and the two species would have become sympatric again, as exemplified by their co-occurrence in southern Western Sahara (see Fig. 1), but they were already highly differentiated at least by their respective karyotypes which clearly insure postzygotic reproductive isolation.

Similar processes may have taken place at the same time, but more to the North, to explain the differentiation between the sister species *G. hesperinus* – *G. boogstrali*. Earlier (i.e. ca.710 000 years ago), *Gerbillus* sp. may also have differentiated from the *G. occiduus* – *G. tarabuli* lineage according to a similar scenario. Indeed, regular climatic variations of the Pleistocene have undoubtedly rendered the scenario described earlier plausible at various occasions (Lisiecki & Raymo 2005; Trauth *et al.* 2009). As a result, *G. boogstrali* and *Gerbillus* sp. probably differentiated and then reached the area they currently occupy between the High Atlas and Anti-Atlas Mountains at two distinct periods. This implies that they may have disappeared from the areas where they formerly occur, maybe due to competition with other gerbil species. The alternative hypothesis is one of successive *in situ* differentiation of populations trapped in the Sous and Massa River valley area.

These scenarios of differentiation/colonisation of Morocco by medium- to large-sized hairy-footed species of *Gerbillus* are not in contradiction with paleontological data. Indeed, all the species described from Upper Pliocene to Middle Pleistocene in Morocco or Algeria were considered close to, or even related with, modern species of the *campestris* (i.e. *G. campestris*, *G. dasyurus* or *G. simoni*) or the *nanus* group, but never to species allied to *G. tarabuli*/*G. pyramidum*. This concerned *G. minutus* Tong 1989 and *G. oebrae* Tong 1989 from the Moroccan site of Irhoud Ocre (Lower Pleistocene, Jaeger 1970; Tong 1989), *G. grandis* Tong 1989 from the Thomas I deposit (Morocco, Middle Pleistocene, Jaeger 1975; Tong 1989), *G. jebileti* Tong 1989 from the Irhoud Derbala Virage site (Morocco, Middle Pleistocene, Jaeger 1970; Tong 1989), *G. bibersoni* Geraads 1995 from the Moroccan site of Ahl al Oughlam (Plio-Pleistocene, Geraads 1995) and *G. major* Tong, 1986 and *G. cingulatus* Tong, 1986 from the Middle Pleistocene site of Tighennif (Algeria, Jaeger 1969; Tong 1985). No information on affinity or relatedness with modern species was given for *G. abdallabi* Tong 1989 from the Middle Pleistocene of Sidi Abdallah Rehamna (Morocco, Jaeger 1975; Tong 1989) and *G. robustus* Tong 1989 from the lower Pleistocene of Irhoud Ocre (Morocco, Jaeger 1970; Tong 1989), so the possibility that these fossil taxa be allied with hairy-footed species of the *tarabuli-pyramidum* group can not entirely be ruled out. However, awaiting further evidence, the hypothesis of recurrent events of differentiation/colonisation of Morocco by currently endemic or wide-ranging species of medium- to large-sized gerbils with hairy feet thanks to geological and climatic variations of the Pleistocene seems likely.

Conclusion

Based on an integrative approach combining the results of molecular sequencing, chromosomal analyses and multi-

variate treatments of morphometrical data, we were able to safely assign all specimens of a sample of gerbil species including a number of morphologically very similar ones to a distinct species. Among them, a species that could well be new to science was evidenced, for which chromosomal data are still lacking. Another species (*G. occiduus*, endemic from Morocco) proved to have differentiated from the widely distributed *G. tarabuli* only very recently, as have two other endemic Moroccan species *G. hoogstraali* and *G. hesperinus*. A barcoding-like approach may have yielded ambiguous results as to the distinction between these species (see Will *et al.* 2005), but the addition of chromosomal data clearly removed any doubt on the specific status of these two taxa, as in other instances (see for rodent examples Vié *et al.* 1996 in *Isothrix*, or Dobigny *et al.* 2005 in *Taterillus*). These cases represent good arguments in favour of an active role of chromosomal change in speciation processes (Rieseberg 2001). Isolation of small populations can offer opportunities to fix more easily chromosomal rearrangements that further may represent barriers to the reproduction and gene flow, thus promoting rapid speciation. Such a situation is likely to have happened for gerbil populations at the western margin of the Sahara, and especially in Morocco where mountain ranges come to the sea across arid habitats. Further sampling and more detailed analyses of genetic variations in these North African *Gerbillus* species will undoubtedly help us to better understand these processes of emergence of biological diversity in the context of recent climatic and environmental changes in these arid areas.

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