Molecular Ecology (2004) **13**, 613–629 doi: 10.1046/j.1365-294X.2004.02099.x

# Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae)

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## Abstract

**African mole-rats are subterranean Hystricomorph rodents, distributed widely throughout sub-Saharan Africa, and displaying a range of social and reproductive strategies from solitary dwelling to the 'insect-like' sociality of the naked mole-rat, *Heterocephalus glaber*. Both molecular systematic studies of Rodentia and the fossil record of bathyergids indicate an ancient origin for the family. This study uses an extensive molecular phylogeny and mitochondrial cytochrome *b* and 12s rRNA molecular clocks to examine in detail the divergence times, and patterns of speciation of the five extant genera in the context of rift valley formation in Africa. Based on a value of 40–48 million years ago (Myr) for the basal divergence of the family (*Heterocephalus*), we estimate divergence times of 32–40 Myr for *Heliophobius*, 20–26 Myr for *Georychus*/*Bathyergus* and 12–17 Myr for *Cryptomys*, the most speciose genus. While early divergences may have been independent of rifting, patterns of distribution of later lineages may have been influenced directly by physical barriers imposed by the formation of the Kenya and Western Rift, and indirectly by accompanying climatic and vegetative changes. Rates of chromosomal evolution and speciation appear to vary markedly within the family. In particular, the genus *Cryptomys* appears to have undergone an extensive radiation and shows the widest geographical distribution. Of the two distinct clades within this genus, one exhibits considerable karyotypic variation while the other does not, despite comparatively high levels of sequence divergence between some taxa. These different patterns of speciation observed both within the family and within the genus *Cryptomys* may have been a result of environmental changes associated with rifting.**

*Keywords*: African mole-rats, Bathyergidae, mitochondrial DNA, phylogeography, Rift Valley, taxonomy

*Received 6 November 2003; revision received 14 November 2003; accepted 14 November 2003*

## Introduction

African mole-rats (family Bathyergidae) are well known for the highly social behaviour exhibited by the naked mole-rat, *Heterocephalus glaber*, and the Damaraland mole-rat, C*ryptomys damarensis* (for recent review see Bennett & Faulkes 2000). The Batherygidae are the most species-rich of the four families that constitute Phiomorpha, the African clade of the Hystricognath suborder of rodents. A second clade within the suborder, reciprocally monophyletic to the Phiomorpha, includes the South American Caviomorpha (Nedbal *et al*. 1994). For an endemic African rodent family, mole-rats have a comparatively wide distribution throughout sub-Saharan Africa. All species are highly adapted to a strictly subterranean lifestyle and occur in physically and climatically divergent habitats. They inhabit a variety of soil types that may vary from coarse sands to fine clays, and can be found in areas ranging in altitudes, rainfall patterns and vegetation type. The common factor linking these disparate habitats is the presence of geophytes, the roots, corms and tubers of plants that form the staple diet of all species (including all their water requirements), for which mole-rats excavate extensive burrow systems to locate and harvest.

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While some mole-rats are strictly allopatric in their distribution, others can occur in sympatry, and it is thought that these patterns may be influenced, at least in part, by social system and patterns of dispersal (Bennett & Faulkes 2000). Three of the five genera within the family, *Bathyergus*, *Georychus* and *Heliophobius*, are strictly solitary in nature. In general, these solitary mole-rats are larger in body size and restricted to mesic regions, where precipitation is greater than 400 mm per annum. All species that have been studied to date in the two other genera in the family, *Cryptomys* and *Heterocephalus*, are characteristically social and exhibit varying degrees of cooperative breeding (Bennett & Faulkes 2000; Faulkes & Bennett 2001). These social species are found in both mesic and arid regions. However, there are some exceptions to this general rule. The social genera are particularly widespread and generally have greater distributional ranges than the solitary species, with the possible exception of *Heliophobius* (Jarvis & Bennett 1990, 1991). The ranges of some of these social species extend into areas of very low rainfall, which is sporadic and unpredictable (sometimes less than 200 mm per annum). The possible relationship between the patterns of rainfall, its subsequent effect on geophyte distribution and social evolution in the Bathyergidae has received much discussion (Jarvis *et al*. 1994; Faulkes *et al*. 1997a; Bennett & Faulkes 2000; Burda *et al*. 2000).

Although the social behaviour of mole-rats was not reported until relatively recently (Jarvis 1981; Bennett & Jarvis 1988; Jarvis & Bennett 1993), descriptions of African mole-rats date back to the 18th century (for *Bathyergus suillus*), and since then a large number of holotypes have been recorded, particularly around the late 19th and early 20th centuries. Morphological synapomorphies and previous molecular genetic studies support unambiguously the early taxonomic division at the generic level. However, phenotypic convergence resulting from adaptation to the subterranean niche has made intrageneric classification based on morphology problematic, especially in the genus *Cryptomys*, and despite a large body of descriptive literature no phylogenetic analysis has been attempted. This is due at least in part to a lack of consistency in the published morphometric data and sample sizes that were often small. It is now known that there is high variation in some of the more easily quantifiable traits used previously to describe new types, such as body size, pelage colour and the presence of a white head spot. This variation can occur within a species and even within a single social group or colony (Filippucci *et al*. 1994; J. U. M. Jarvis & N. C. Bennett, unpublished). DNA sequence data are now helping to resolve some of the taxonomic problems that have prevailed for many years.

Earlier molecular phylogenetic studies have looked at several loci (both mitochondrial and nuclear) and varying numbers of taxa in the family (Allard & Honeycutt 1992; Nedbal *et al*. 1994; Faulkes *et al*. 1997a; Walton *et al*. 2000; Huchon & Douzery 2001). In this study we greatly extend the sampling regime to include new taxa and populations, and intraspecific comparisons across species ranges to examine: (i) patterns of sequence divergence with respect to chromosomal evolution and speciation and (ii) phylogeographical trends within the family, with particular reference to the formation of the African Rift Valley, a major geographical feature that cuts through the distributional range of the Bathyergidae.

## Materials and Methods

### *Sampling, PCR and sequencing*

Samples were collected from a range of locations across sub-Saharan Africa between 1987 and 2002 (Table 1, Fig. 1). From the total of 128 samples, data from 105, representing 32 new geographical locations, are previously unpublished. Tissue (muscle or skin biopsies) was fixed in either 95% ethanol or a buffer of 20% dimethyl sulphoxide in saturated NaCl (approx. 6 M), and then stored at –20 °C prior to DNA extraction. Genomic DNA was extracted from the tissue samples using a standard protocol, and PCR amplification of the 12s rRNA and/or cyt *b* gene carried out using primers and protocols described previously for African mole-rats by Faulkes *et al*. (1997a,b). Sequencing was carried out in both directions using combinations of primers to obtain complementary partially overlapping strands (20–100% overlap). An Amersham Megabace 1000 automated sequencer was used to separate fragments produced using the Dynamic ET Terminator Sequencing kit (Amersham, UK).

### *Analysis of mitochondrial DNA sequences*

Sequences were compiled for analysis and aligned manually using MacClade version 3 (Madison & Madison 1992) and phylogenetic relationships and genetic distances between haplotypes determined using PAUP* version 4.0b8 (Swofford 2001). 12s rRNA and cyt *b* data were analysed both separately and in combination. Maximum parsimony was performed using both the branch and bound and heuristic search options both with all characters having an equal weighting, and with characters weighted a posteriori according to their rescaled consistency index. Gaps were treated as missing data. Bootstrap analysis using these parsimony criteria was conducted with 100 replicates of the data set. Prior to maximum likelihood analysis, MODELTEST 3.06 (Posada & Crandall 1998) was used to establish the evolutionary model most appropriate for the data, and these parameters then used in PAUP*.

For the phylogenetic analysis, only representative haplotypes from the different geographical locations were included for both reasons of clarity and to prevent computing

**Table 1** Number of individuals sequenced (*n*) at the cyt *b* locus for each species, with their approximate geographical locations; *denotes that 12s rRNA was also sequenced for an individual in this sample, and its respective Accession no.

| Species (reference) | *n* | Country and location | GenBank Accession nos |
|---|---|---|---|
| *H. glaber* | 1 | Ethiopia, Diredawa (9°35′ N, 41°49′ E) | AY425847 |
| (Rüppell 1842) | 1 | Ethiopia, Dembalawachu (4°53′ N, 38°6′ E) | U87521 |
| | 3 | Kenya, Lerata (0°37′ N, 37°39′ E) | U87522–U87524 |
| | 1 | Kenya, Mtito Andei (2°41′ S, 38°9′ E) | U87525, AY425847* |
| *C. damarensis* | 4 | Namibia, Dordabis (22°58′ S, 17°41′ E) | AF012225–AF012228 |
| (Ogilby 1838) | 1 | Namibia, Rundu (17°48′ S, 19°32′ E) | AY425858 |
| | 1 | Zimbabwe, Bulawayo (20°9′ S, 28°38′ E) | AY425857 |
| | 4 | Botswana, Okavango Delta (19°32′ S, 23°11′ E) | U87526, AY425839*, AF012220, AF012223 AF012224 |
| | 2 | Botswana, Maun (19°59′ S, 23°21′ E) | AF012221–AF012222 |
| | 9 | South Africa, Hotazel (27°17′ S, 23°0′ E) | AY425848–AY425856 |
| *C. h. hottentotus* | 7 | South Africa, Steinkopf (29°17′ S, 17°45′ E) | AF012240, AY425898–AY425903 |
| (Lesson 1826) | 1 | South Africa, Klawer (31°48′ S, 18°38′ E) | AF012238 |
| | 15 | South Africa, Somerset West (34°4′ S, 18°50′ E) | AF012239, AY425841*, AY425891–AY425897, AY425904–AY425910 |
| *C. h. pretoriae* | 4 | South Africa, Pretoria (25°47′ S, 28°13′ E) | AY425879–AY425882 |
| (Roberts 1913) | 4 | South Africa, Johannesburg (26°11′ S, 28°4′ E) | AY425875–AY4278 |
| | 2 | South Africa, Krugersdorp (26°6′ S, 27°43′ E) | AY425883, AY425884 |
| | 2 | South Africa, Hekpoort (25°52′ S, 27°37′ E) | AF012218*, AF012236, AY425874 |
| *C. h. mahali* | 4 | South Africa, Patryshoek, Pretoria (25°40′ S, 28°2′ E) | AY425870–AY425873 |
| (Roberts 1913) | | | |
| *C. h. nimrodi* | 1 | Zimbabwe, Hillside (20°55′ S, 28°38′ E) | AF012237, AF012219* |
| (De Winton 1896) | 5 | Zimbabwe, Bulawayo (20°9′ S, 28°38′ E) | AY425886–AY425890 |
| | 1 | Zimbabwe, Limpopo Valley (22°30′ S, 28°40′ E) | AY425885 |
| *C. h. natalensis* | 1 | South Africa, Kokstad (31°32′ S, 29°38′ E) | AF012235, AY425840* |
| (Roberts 1913) | 1 | South Africa, Komatiepoort (25°25′ S, 31°57′ E) | AY425869 |
| *C. h. anselli* | 1 | Zambia, Lusaka (15°19′ S, 28°27′ E) | AF012216*, AF012233 |
| (Burda *et al*. 1999) | | | |
| *C. mechowi* | 3 | Zambia, Chingola (12°31′ S, 27°51′ E) | AF012214*, AF012230, AY425867 AY425868 |
| (Peters 1881) | | | |
| | 3 | Zambia, Kapiri Mposhi (13°58′ S, 28°40′ E) | AY425864–AY425866 |
| | 1 | Dem. Rep. Congo, Kinshasa (4°22′ S, 15°27′ E) | AF012231 |
| *C. bocagei* | 1 | Angola, Lubango (14°56′ S, 13°27′ E) | AF012213*, AF012229 |
| (De Winton 1897) | | | |
| *C. whytei* | 3 | Zambia, Mbala (9°50′ S, 31°24′ E) | AY425860–AY425862 |
| (Thomas 1897) | 1 | Tanzania, Suma (9°10′ S, 33°40′ E) | AY425859 |
| *C. darlingi* | 1 | Malawi, Mzuzu (11°27′ S, 34°3′ E) | AY425863 |
| (Thomas 1895) | 1 | Zimbabwe, Goromonzi (17°52′ S, 31°30′ E) | AF012215*, AF012232 |
| *Cryptomys* species | 1 | Zambia, nr Kalomo (16°45′ S, 27°0′ E) | AF012217*, AF012234 |
| *G. capensis* | 1 | South Africa, Cape Town (33°48′ S, 18°28′ E) | AY425844*, AF012243 |
| (Pallas 1778) | 1 | South Africa, Virginia Farm (29°40′ S, 29°57′ E) | AY425920 |
| *B. suillus* | 1 | South Africa, Stilbaai (34°12′ S, 22°23′ E) | AY425842*, AF012242 |
| (von Schreber 1782) | 2 | South Africa, Cape Town (33°48′ S, 18°28′ E) | AY425911, AY425912 |
| | 1 | South Africa, Rondawel (30°47′ S, 17°53′ E) | AY425913 |
| *B. janetta* | 2 | South Africa, De Riet (30°8′ S, 17°26′ E) | AY425843*, AF012241, AY425919 |
| (Thomas & Schwann 1904) | 2 | South Africa, Rondawel (30°47′ S, 17°53′ E) | AY425915, AY425916 |
| | 2 | South Africa, Kamieskroon (30°12′ S, 17°56′ E) | AY425917, AY425918 |
| | 1 | Namibia, Boesmanberg (27°45′ S, 16°20′ E) | AY425914 |
| *H. argenteocinereus* | 4 | Kenya, Athi Plains (1°43′ S, 37°0′ E) | AY425845*, U87527, AY425921–AY425923 |
| (Peters 1852) | | | |
| | 1 | Tanzania, Bagamoyo Village (5°07′ S, 38°25′ E) | AY425846*, AY425931 |
| | 1 | Tanzania, Amani (5°0′ S, 38°40′ E) | AY425930 |
| | 1 | Tanzania, Nguru Forest (6°40′ S, 37°35′ E) | AY425928 |
| | 1 | Tanzania, Msembe (6°44′ S, 37°38′ E) | AY425929 |
| | 3 | Tanzania, Dakawa (6°26′ S, 37°34′ E) | AY425924, AY425925, AY425933 |
| | 3 | Tanzania, Morogoro (6°50′ S, 37°39′ E) | AY425926, AY425936, AY425937 |
| | 1 | Tanzania, Mbete (6°52′ S, 37°41′ E) | AY425932 |
| | 7 | Tanzania, Mlali (6°57′ S, 37°41′ E) | AY425927, AY425934, AY425935, AY425938–AY425941 |
| | 2 | Malawi, Rumphi (11°2′ S, 34°52′ E) | AY425942, AY425943 |

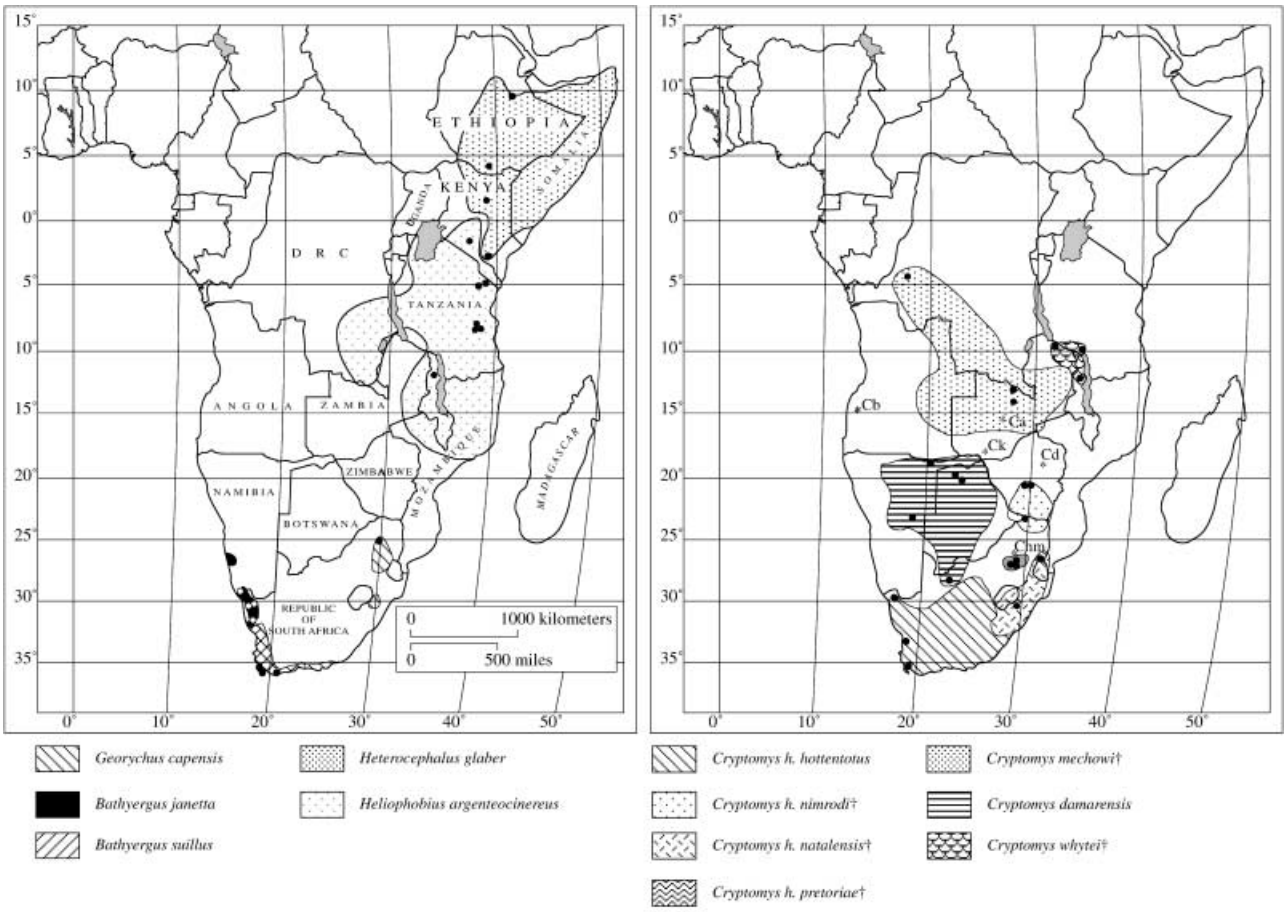


**Fig. 1** Sampling map showing the species ranges (shaded areas), and the relative locations of the sampling sites for that species (filled circles within respective shaded areas). †Denotes approximate species distributions. Sampling localities for species for which ranges are unknown are indicated by * as follows: Cb, *Cryptomys bocagei*; Ca, *C. anselli*; Cd, *C. darlingi*; Chm, *C. h. mahali*; Ck, *Cryptomys* collected near Kalomo, Zambia.

time becoming prohibitive. As outgroups, we obtained the published sequences for two other Old World Hystricomorph rodents, the Cape porcupine, *Hystrix africaeaustralis* (Nedbal *et al*. 1994) and the cane rat, *Thryonomys swinderianus* (Mouchaty *et al*. 2001). Previously published sequences were obtained from NCBI with Accession nos U87521–U87527 and AF012213–AF012243 (Faulkes *et al*. 1997a). New sequences have been deposited in NCBI with Accession nos AY425839–AY425944 (Table 1).

### *Molecular clock calibration and estimation of divergence times*

Evidence for rate heterogeneity was investigated in both 12s rRNA and cyt *b* sequences using the relative rate test option in MEGA version 2.1 (Kumar *et al*. 2001), and by likelihood ratio tests of the data with and without a molecular clock enforced during maximum likelihood analysis in PAUP*. Divergence times within the family were estimated from Tamura–Nei (+ gamma) corrected distances for both loci separately and combined, with substitutions restricted to transversions only for the 12s rRNA/cyt *b* combined data set. Internal nodes were dated from linearized trees constructed from the output of neighbour-joining analysis in MEGA version 2.1 (Kumar *et al*. 2001**).** The molecular clock rate was calibrated by assuming divergence dates of 40 and 48 Myr for the basal node of the bathyergid phylogeny. These times were obtained from the study of Huchon & Douzery (2001), who used the occurrence of the first caviomorph fossil in the Tinguiririan of South America (31–37 Myr; Wyss *et al*. 1993) to estimate divergence times of all the hystricognath families. The 40 Myr estimate for the common ancestor of the Bathyergidae was deduced from the analysis of codon positions 1 and 2 of the von Willebrand factor gene, while the 48 Myr estimate was deduced from the amino acid sequences of the same gene.

To calculate genetic distances within and between taxa in PAUP*, cyt *b* sequences were used, as this data set was complete for all samples and of sufficient variability to measure differences down to the within-species level.

## Results

### *Phylogenetic relationships*

Maximum parsimony analysis using the heuristic search option in PAUP* on the 12s rRNA/cyt *b* combined sequences produced 891 045 rearrangements, and gave 24 trees of equal length (3207 steps). From the total of 1975 characters, 1017 were constant, 749 were parsimony informative and 125 uninformative. In a second round of parsimony analysis, 619 characters were re-weighted a posteriori with their respective rescaled consistency index, while 1356 retained a character weighting of one. After 109 796 rearrangements within the heuristic search option in PAUP*, the number of equally parsimonious trees was reduced from 24 to just two (tree length: 3211). A consensus tree produced from the re-weighted character set is shown in Fig. 2a. Most nodes increased in bootstrap support following re-weighting, implying that any uncertainty was due to homoplasy in the data set. The addition of new sequences from previously investigated species and inclusion of new taxa into the bathyergid phylogeny did not alter the overall topology of the phylogeny from that previously published from analysis of both mitochondrial and nuclear genes (e.g. Allard & Honeycutt 1992; Faulkes *et al*. 1997a,b; Walton *et al*. 2000; Huchon & Douzery 2001). All genera formed monophyletic clades, with the genus *Cryptomys* splitting into two clear subclades, one containing *C. mechowi* and the other *C. h. hottentotus* (hereafter referred to as the '*C. mechowi*' and '*C. hottentotus*' subclades, respectively). *Heterocephalus* and *Heliophobius* were the two basal lineages, respectively, and *Cryptomys* the more derived (Fig. 2a). Therefore, the overall phylogeographical trend within the family is from east Africa into southern and central Africa.

Following Akaike information criterion (AIC) tests of different models of evolution using MODELTEST, the one found to best fit the combined 12s rRNA/cyt *b* data corresponded to the GTR + G + I model of sequence evolution. Thus, the parameters chosen for maximum likelihood analysis were unequal base frequencies (A: 0.3770; C: 0.2447; G: 0.1022; T: 0.2761), proportion of invariable sites (I) = 0.4239 and for variable sites (G), a gamma distribution shape parameter of 1.1398. Hierarchical likelihood ratio tests with MODELTEST produced a slightly different result and found a Tamura–Nei + G + I model to be the best fit to the data (A: 0.3786; C: 0.2368; G: 0.0999; T: 0.2847; I = 0.4239; G = 1.1398). The latter model is supported in MEGA and was thus was chosen to calculate neighbour-joining and linearized trees. Both models produced maximum likelihood trees with the same topology, as described below.

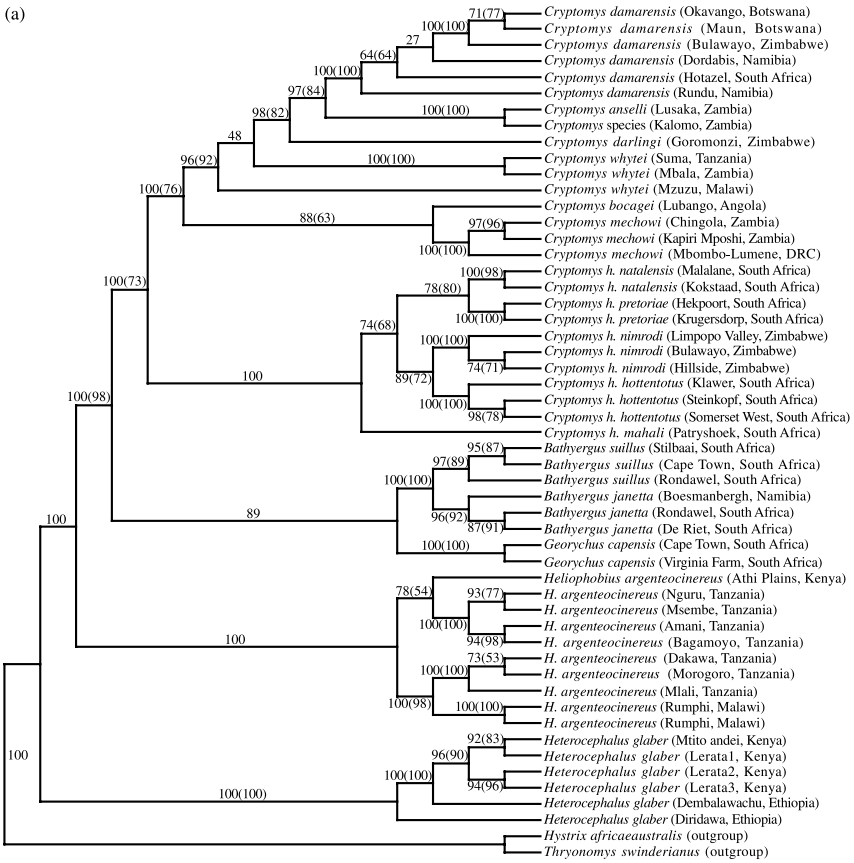


**Fig. 2** Phylogenetic relationships of 51 bathyergid mtDNA haplotypes and outgroup species *Hystrix africaeaustralis* and *Thryonomys swinderianus*, constructed using combined 12s rRNA and cyt *b* sequences. (a) 50% majority rule consensus tree generated from maximum parsimony analysis using the heuristic search option in PAUP*. Tree length = 3211, consistency index 0.64, retention index 0.83, rescaled consistency index 0.53. Numbers above each branch refer to the percentage bootstrap values following 100 replications, after weighting sites with the rescaled consistency index and without weighting (in parentheses). (b) Phylogram generated by the maximum likelihood option in PAUP* using a GTR + G + I model of sequence evolution (see text), tree score: –ln L = 16014.33, tree length = 3213, consistency index 0.45, retention index 0.77, rescaled consistency index 0.35. *H. arg* = *Heliophobius argenteocinereus*.

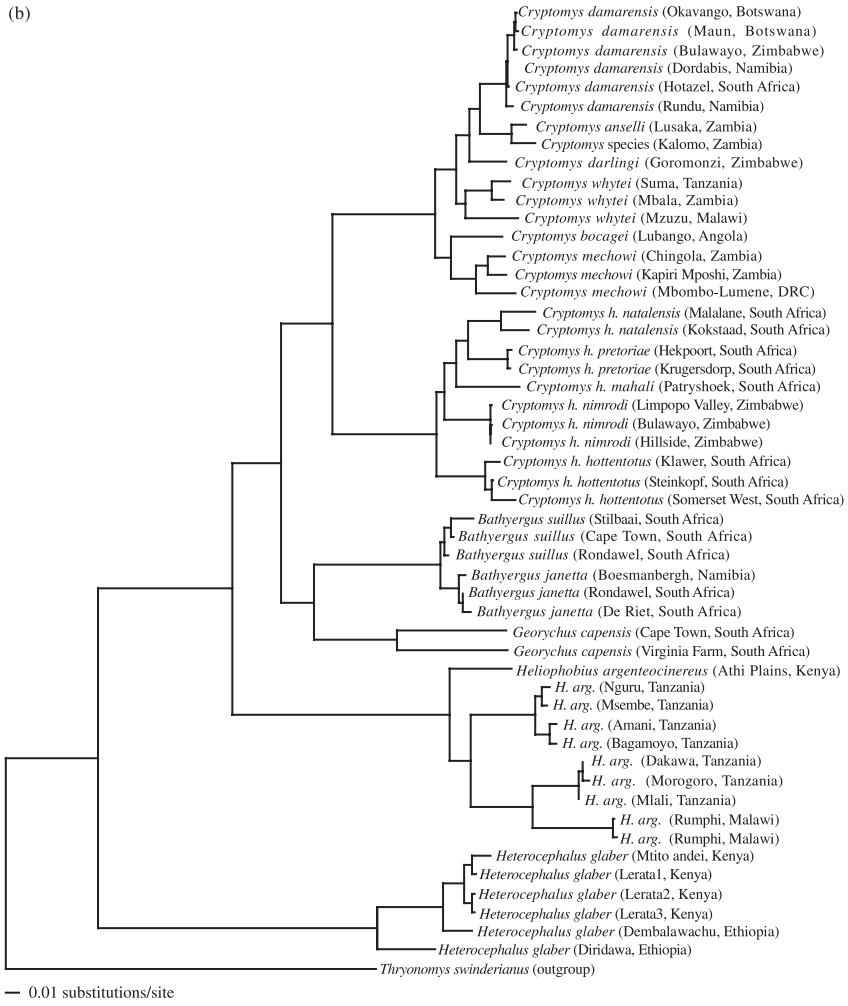


**Fig. 2** *Continued*

The maximum likelihood tree was just two steps longer than the most parsimonious tree (tree length 3213 vs. 3211, respectively) and supported the maximum parsimony analysis at all the nodes characterizing the five genera within the family (Fig. 2b), with small differences within clades. The *Heliophobius* clade differed in the placement of the Kenyan haplotype, with maximum likelihood analysis placing this as a separate basal lineage, while the maximum parsimony analysis placed it in a clade with northern Tanzanian haplotypes, supported by a bootstrap value of 78% (Fig. 2a). A second difference between the two trees was the placement of the *C. hottentotus mahali* haplotype from northern Pretoria (Patryshoek). With maximum likelihood analysis (Fig. 2b) this sequence was basal in a clade containing other *Cryptomys* species collected around Pretoria, that we refer to as *C. h. pretoriae* and *C. h. natalensis* haplotypes. A different topology was obtained with the maximum parsimony tree (Fig. 2a), where the Patryshoek haplotype formed a divergent, basal lineage in the *C. hottentotus* subclade of the genus *Cryptomys*. Although the latter subclade of *Cryptomys* is supported by a 100% bootstrap value, within the clade the relationships between the main taxa (*C. h. hottentotus*, *C. h. natalensis*, *C. h. nimrodi* and *C. h. pretoriae*) were less well supported (74, 78 and 89%), possibly explaining the differences obtained with the two tree-building methods. Finally, there was a small difference in the placement of the haplotypes from *C. whytei* (Thomas 1897), which were monophyletic in the likelihood analysis but paraphyletic on the parsimony tree (the haplotype from Mzuzu, Malawi falling outside the clade containing the geographically closely situated Mbala and Suma haplotypes). However, constraining monophyly for this clade did not increase the tree length and was thus equally parsimonious.

In addition to the overall phylogeographical trends evident within the family as a whole, some clear-cut patterns were also resolved within the major clades. In *Heterocephalus*, both trees support a north–south radiation into Kenya from ancestral lineages in Ethiopia, with all nodes supported by high bootstrap values (Fig. 2a). With maximum likelihood analysis, *Heliophobius* also shows a general phylogeographical pattern of ancestral populations being

more northerly in Kenya and northern Tanzania, with more derived populations occurring further south and west. The parsimony tree is less clear in this respect, dividing *Heliophobius* into two clades, one with Kenyan and northerly Tanzanian lineages, and the second with southerly Tanzanian and Malawian lineages. Rearrangement of this topology to that of the likelihood analysis resulted in an increase in tree length of just one step. The haplotypes from Malawi appear to represent a relatively early lineage, which is significant with respect to the influence of the Rift Valley as a geographical barrier to dispersal (see below and Discussion). The two species in the *Bathyergus* genus form two well-supported monophyletic groups that both reflect a north–south pattern of radiation. Of the *B. janetta* haplotypes, the more northerly Namibian one is basal, while of the *B. suillus* haplotypes, Rondawel (north) is basal with respect to the southern haplotypes (Stilbaai and Cape Town; see Fig. 1). In the *Cryptomys* genus, the *C. mechowi* subclade is characterized by species geographically distributed across central Africa, extending down at their most southerly range to northern South Africa. The basal radiation in the subclade contains *C. mechowi* and *C. bocagei*, both distributed across central Africa and in our data set represented by samples from Angola and Zambia. Haplotypes from *C. whytei* form the next lineage(s) in the clade, again from central African locations (western Tanzania, Zambia) and extending south into Malawi. The next lineages include haplotypes from Zimbabwe (*C. darlingi*) and Zambia (*C. anselli* and an unnamed *Cryptomys* species captured near Kalomo). Finally, the most derived taxon in the *C. mechowi* subclade in this phylogeny is the eusocial Damaraland mole-rat, *C. damarensis*. Within *C. damarensis*, although there was geographical structuring of haplotypes, there was no apparent clinal pattern to their distribution, possibly as a result of weak bootstrap support of nodes within the species clade and low levels of sequence divergence.

Within the *C. hottentotus* subclade, the currently accepted subspecies *C. h. hottentotus*, *C. h. nimrodi* and *C. h. natalensis* all form monophyletic clades, with all but one of the haplotypes from around Pretoria grouping with the latter (Krugersdorp and Hekpoort). The sample from Patryshoek (northern suburbs of Pretoria) that we refer to as *C. h. mahali* was distinct from nearby populations, and formed either a basal lineage within the *C. h. natalensis*/*C. h. pretoriae* clade (maximum likelihood, Fig. 2b) or the basal lineage in the *C. hottentotus* subclade (maximum parsimony, Fig. 2a). The phylogeographical pattern of radiation in the *C. hottentotus* subclade is less clear-cut due to these differences in the topology of the two trees. If the maximum likelihood tree is correct (Fig. 2b), then any clear pattern reflecting the spread into South Africa and parts of Zimbabwe from a (presumed) more northerly common ancestor of *Cryptomys* has been overshadowed and confused by subsequent movements. For example, in Fig. 2b the branching order, from the basal lineage, is *C. h. hottentotus* (South Africa), *C. h. nimrodi* (Zimbabwe) and *C. h. natalensis*/*Cryptomys* from Pretoria (South Africa). If the maximum parsimony tree is correct (Fig. 2a), then populations to the north of Pretoria form the basal lineage in the sample set, with *C. h. hottentotus* (South Africa)/*C. h. nimrodi* (Zimbabwe) and *C. h. natalensis*/*Cryptomys* from Pretoria, South Africa, forming reciprocally monophyletic clades. More work is required on these and other populations of *Cryptomys* in South Africa.

Phylogenetic analysis was also carried out on the 12s rRNA and cyt *b* sequences individually. Both parsimony and likelihood analysis of 12s rRNA resolved the main clades with the same topology seen in the combined analysis described above (e.g. Figure 3a). As a result of saturation effects (see below), cyt *b* was less suitable to resolve the deeper nodes in the phylogeny, and there was less congruence with 12s rRNA and the combined data set. The principal difference was a loss of monophyly of *Cryptomys* due to a monophyletic *C. mechowi* subclade and a group containing three monophyletic clades of the *C. hottentotus* subclade, *Bathyergus* and *Georychus*.

### *Intra- and interspecific sequence divergence*

For cyt *b*, plots of the number of transition substitutions against uncorrected *p*-distance were linear up to values of approximately 20% *p*-distance, but revealed that saturation of sites is occurring when genetic differences exceed this value. Transversions showed a linear increase up to the maximum *p*-distances of 25–30% ($r^2 = 0.79$; $P < 0.001$), and therefore these substitutions were used to calculate distances for all molecular clock estimates of divergence times for the combined 12s rRNA/cyt *b* data set. Similarly, Allard & Honeycutt (1992) found that saturation occurred in the 12s rRNA gene in bathyergids when pairwise divergence (*d*) exceeded 20%. As most of our values of *P* were below this, we used all substitutions to calculate divergence times from the 12s rRNA sequence data.

MODELTEST selected the HKY85 + G + I (HKY) model of sequence evolution for cyt *b* sequences. Both uncorrected *p* and HKY-corrected genetic distances within and between taxa for 128 cyt *b* sequences are displayed in Table 2. Uncorrected *p* distances between clearly defined species ranged from a minimum of 4.1 ± 0.19% between *B. suillus* and *B. janetta* to 26.4 ± 0.05% between *Heterocephalus glaber* and *Heliophobius argenteocinereus*. The disparity between the latter and the HKY distance (68.1%) illustrates clearly the effects of saturation at this locus for the more divergent taxa. High levels of sequence divergence occurred within some species (as currently recognized), with the greatest values among *H. argenteocinereus* populations: *p* distances between the Kenyan haplotype and the two samples from Malawi were 13.2 and 13.3%, respectively, and 12.6–13.3%

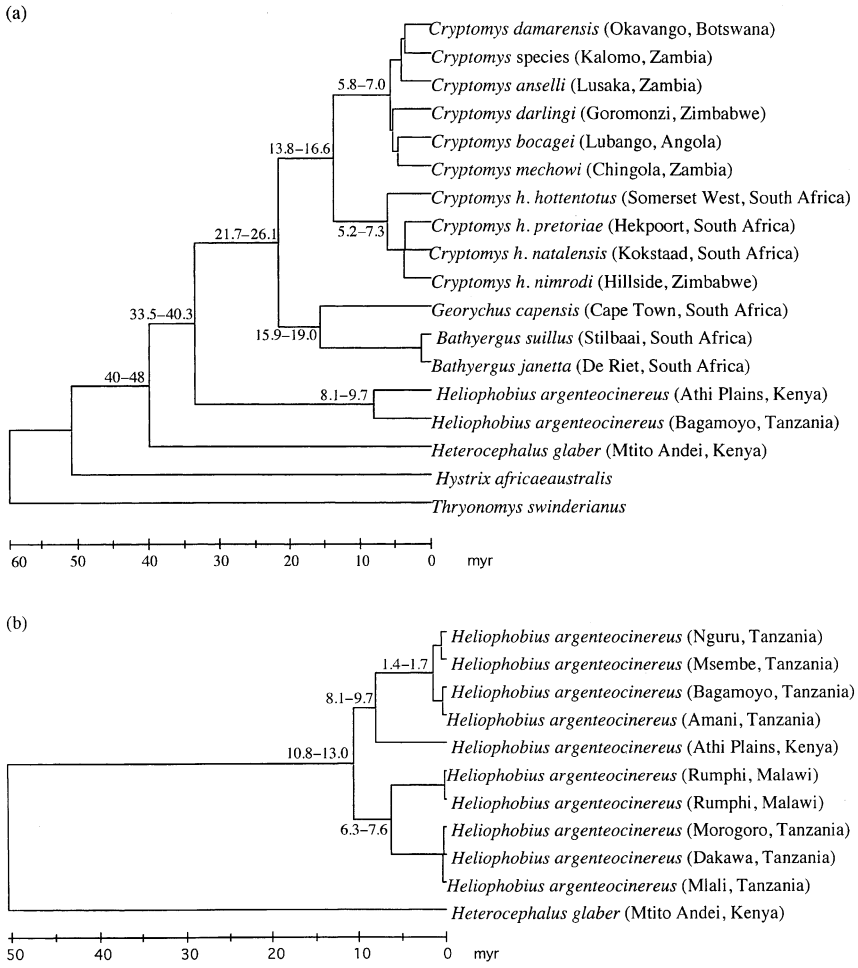


**Fig. 3** Linearized trees showing divergence times assuming a molecular clock calibration of 40 and 48 million years (Myr), respectively, for the basal node within the family. Scale bars are based on a divergence time of 40 Myr. Neighbour-joining trees were constructed from Tamura–Nei + gamma distances for (a) 12s rRNA sequences, with *H. africaeaustralis* and *T. swinderianus* as outgroups; (b) cyt *b*, with *H. glaber* as the outgroup.

between the Malawian and northern Tanzanian haplotypes. Among naked mole-rats, *p* distances were maximal between the extremes of the sampling range at 10.3 and 10.6% (Diredawa, Ethiopia vs. Dembalawachu, Ethiopia and Mtito Andei, Kenya, respectively). Some populations of *C. hottentotus* species from around Pretoria in South Africa were also highly divergent. In particular, the population at Patryshoek, 10 km to the north (*C. hottentotus mahali*), were on average 8.8% divergent from populations within the city. In contrast, *C. damarensis* ($n = 21$ sequences, 18 haplotypes) and *C. h. nimrodi* ($n = 7$ sequences, 6 haplotypes) had relatively low levels of genetic divergence, even over considerable geographical distances in the case of the former (mean $p \pm \text{SEM} = 1.4 \pm 0.11\%$, mean $p \pm \text{SEM} = 1.9 \pm 0.54\%$, respectively). The uncharacterized Zambian *Cryptomys* species from near Kalomo and *C. anselli* from Lusaka also had low levels of divergence at 2.9%, but it is probable that these constitute different species (see Discussion). Finally, the *G. capensis* haplotype from Cape Town, South Africa was highly divergent from the haplotype from further north at Virginia Farm, with a *p* distance of 13.7%.

### *Molecular clock estimates of divergence times*

Relative rate tests for variation in cyt *b* sequence evolution among lineages revealed no significant differences in the rates of transversion substitutions (the distance measure used to estimate divergence times). Nineteen tests were performed across the phylogeny, comparing successive pairs of lineages with an outgroup, as follows (abbreviations as Table 2): *C. dam*, *C. ans* vs. *C. dar*; *C. dam*, *C. dar* vs. *C. why*; *C. dam*, *C. dar* vs. *C. mec*; *C. dam*, *C. why* vs. *C. boc*; *C. dam*, *C. h. hot* vs. *B. sui* (Cape Town); *C. dam*, *C. h. hot* vs. *B. jan*; *C. dam*, *C. h. hot* vs. *G. cap*; *C. dam*, *B. sui* vs. *H. arg*; *C. dam*, *B. sui* vs. *H. gla*; *C. dam*, *H. arg* (Tanzania) vs. *H. gla* (Ethiopia); *C. dam*, *H. gla* vs. *Hystrix*; *C. dam*, *H. arg* vs. *Hystrix*; *C. h. nat*, *C. h. nim* vs. *C. h. hot*; *H. arg* (Kenya), *H. arg* (Tanzania) vs. *H. gla*; *B. jan*, *B. sui* vs. *G. cap*; *C. dam*, *C. h. hot* vs. *B. sui* (Stilbaai); *C. dam*, *H. arg* (Kenya) vs. *H. gla* (Kenya); *C. dam*, *H. arg* (Tanzania) vs. *H. gla* (Kenya); *H. gla* (Kenya), *H. gla* (Ethiopia) vs. *H. arg* (Tanzania); ($\chi^2 = 0.00–2.27$; $P = 0.06–1.00$).

Relative rate tests for variation in 12s rRNA sequence evolution among lineages also revealed no significant

**Table 2** Mean ± SEM cyt *b* genetic distances between sequences (%). Below diagonal HKY + G + I corrected distances, above diagonal uncorrected *p* distances, along diagonal, within clade uncorrected *p* distances; taxon abbreviations as follows: C. dam, *Cryptomys damarensis*; C. dar, *C. darlingi*; C. why, *C. whytei*; C. boc, *C. bocagei*; C. mec, *C. mechowi*; C. ans, *C. anselli*; C. kal, *Cryptomys* from Kalomo; C. h. hot, *Cryptomys hottentotus hottentotus*; C. h. nat, *C. h. natalensis*; C. h. prt, *Cryptomys h. pretoriae*; C. h. mah, *C. h. mahali*; C. h. nim, *C. h. nimrodi*; B. sui, *Bathyergus suillus*; B. jan, *B. janetta*; G. cap, *G. capensis*; H. arg, *Heliophobius argenteocinereus*; H. gla, *Heterocephalus glaber*; T. swi, *Thryonomys swinderianus* (outgroup); *n* = number of sequences within each taxon (no. haplotypes identified are shown in parentheses)

| | *C. dam* $n = 21(18)$ | *C. dar* $n = 1$ | *C. why* $n = 5(4)$ | *C. boc* $n = 1$ | *C. mec* $n = 7(7)$ | *C. ans* $n = 1$ | *C. kal* $n = 1$ | *C. h. nat* $n = 2(2)$ | *C. h. prt* $n = 12(12)$ | *C. h. mah* $n = 4(1)$ | *C. h. nim* $n = 7(6)$ | *C. h. hot* $n = 23(17)$ | *B. sui* $n = 4(4)$ | *B. jan* $n = 7(7)$ | *G. cap* $n = 2(2)$ | *H. arg* $n = 24(13)$ | *H. gla* $n = 6(6)$ | *T. swi* $n = 1$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. dam* | 1.4 ± 0.11 | 7.7 ± 0.4 | 7.6 ± 0.06 | 9.9 ± 0.06 | 10.8 ± 0.06 | 5.6 ± 0.06 | 7.1 ± 0.07 | 16.4 ± 0.04 | 17.6 ± 0.03 | 16.6 ± 0.03 | 17.6 ± 0.04 | 18.3 ± 0.03 | 17.2 ± 0.06 | 16.8 ± 0.06 | 19.3 ± 0.04 | 20.6 ± 0.03 | 24.0 ± 0.04 | 23.4 ± 0.05 |
| *C. dar* | 8.1 ± 0.05 | — | 7.6 ± 0.20 | 10.7 | 9.7 ± 0.33 | 6.7 | 7.7 | 18.1 ± 0.15 | 18.5 ± 0.07 | 17.6 ± 0.00 | 18.1 ± 0.15 | 19.6 ± 0.09 | 17.2 ± 0.30 | 16.9 ± 0.23 | 19.3 ± 0.06 | 21.0 ± 0.13 | 23.2 ± 0.25 | 23.5 |
| *C. why* | 9.8 ± 0.11 | 9.8 ± 0.35 | 3.7 ± 1.03 | 10.0 ± 0.33 | 10.2 ± 0.12 | 8.2 ± 0.36 | 9.3 ± 0.24 | 17.4 ± 0.10 | 18.1 ± 0.07 | 18.1 ± 0.09 | 17.8 ± 0.08 | 18.4 ± 0.05 | 16.7 ± 0.15 | 16.1 ± 0.11 | 18.7 ± 0.15 | 20.7 ± 0.07 | 22.9 ± 0.08 | 22.8 ± 0.35 |
| *C. boc* | 14.1 ± 0.12 | 15.5 | 14.0 ± 0.67 | — | 8.9 ± 0.25 | 9.9 | 11.3 | 16.9 ± 0.05 | 17.3 ± 0.10 | 16.9 ± 0.00 | 16.9 ± 0.2 | 17.3 ± 0.13 | 17.4 ± 0.29 | 17.1 ± 0.27 | 19.0 ± 0.41 | 20.9 ± 0.12 | 23.3 ± 0.18 | 23.8 |
| *C. mec* | 15.8 ± 0.13 | 13.7 ± 0.61 | 14.6 ± 0.22 | 11.9 ± 0.45 | 2.7 ± 0.36 | 10.8 ± 0.33 | 11.2 ± 0.40 | 18.0 ± 0.19 | 18.7 ± 0.08 | 18.1 ± 0.19 | 18.4 ± 0.11 | 18.8 ± 0.14 | 18.2 ± 0.13 | 18.1 ± 0.13 | 20.0 ± 0.39 | 20.5 ± 0.09 | 22.7 ± 0.17 | 23.1 ± 0.32 |
| *C. ans* | 6.7 ± 0.08 | 8.3 | 10.8 ± 0.66 | 13.8 | 16.2 ± 0.66 | — | 2.9 | 17.6 ± 0.22 | 18.2 ± 0.09 | 17.3 ± 0.00 | 18.7 ± 0.12 | 18.8 ± 0.06 | 17.4 ± 0.26 | 16.8 ± 0.38 | 19.4 ± 0.42 | 20.7 ± 0.12 | 23.9 ± 0.22 | 23.4 |
| *C. kal* | 8.8 ± 0.11 | 9.7 | 12.6 ± 0.47 | 16.5 | 16.7 ± 0.80 | 2.9 | — | 18.9 ± 0.20 | 18.9 ± 0.08 | 18.3 ± 0.00 | 19.6 ± 0.25 | 19.5 ± 0.06 | 18.9 ± 0.25 | 17.9 ± 0.46 | 20.6 ± 0.46 | 21.5 ± 0.14 | 25.0 ± 0.21 | 24.1 |
| *C. h. nat* | 29.9 ± 0.13 | 38.0 ± 1.10 | 34.6 ± 0.50 | 32.3 ± 0.16 | 37.3 ± 0.75 | 35.9 ± 1.16 | 40.6 ± 1.20 | 4.9 | 8.7 ± 0.08 | 9.6 ± 0.13 | 10.0 ± 0.18 | 11.8 ± 0.11 | 16.6 ± 0.16 | 16.9 ± 0.05 | 18.3 ± 0.45 | 21.4 ± 0.09 | 23.6 ± 0.18 | 23.3 ± 0.11 |
| *C. h. prt* | 34.5 ± 0.09 | 39.1 ± 0.26 | 37.1 ± 0.29 | 33.4 ± 0.36 | 40.0 ± 0.38 | 38.4 ± 0.35 | 40.5 ± 0.34 | 11.6 ± 0.15 | 2.0 ± 0.13 | 8.8 ± 0.04 | 9.1 ± 0.07 | 11.0 ± 0.05 | 18.3 ± 0.09 | 17.5 ± 0.06 | 18.2 ± 0.21 | 21.2 ± 0.04 | 23.1 ± 0.05 | 23.4 ± 0.09 |
| *C. h. mah* | 31.0 ± 0.11 | 35.5 ± 0.00 | 34.4 ± 0.39 | 32.3 | 37.9 ± 0.83 | 34.7 ± 0.00 | 38.1 ± 0.00 | 13.6 ± 0.24 | 12.0 ± 0.07 | — | 9.2 ± 0.08 | 9.8 ± 0.04 | 16.4 ± 0.14 | 16.7 ± 0.07 | 17.7 ± 0.23 | 19.7 ± 0.06 | 22.7 ± 0.11 | 23.3 ± 0.00 |
| *C. h. nim* | 34.8 ± 0.14 | 37.2 ± 0.58 | 36.0 ± 0.35 | 32.0 ± 0.72 | 38.8 ± 0.49 | 40.8 ± 0.53 | 44.4 ± 1.26 | 14.5 ± 0.38 | 12.4 ± 0.13 | 12.8 ± 0.15 | 1.9 ± 0.54 | 10.2 ± 0.07 | 17.8 ± 0.09 | 18.2 ± 0.10 | 18.2 ± 0.22 | 21.0 ± 0.07 | 23.8 ± 0.13 | 24.1 ± 0.14 |
| *C. h. hot* | 36.3 ± 0.09 | 43.3 ± 0.39 | 38.1 ± 0.21 | 32.6 ± 0.48 | 40.2 ± 0.5 | 40.4 ± 0.25 | 42.5 ± 0.28 | 18.0 ± 0.25 | 16.4 ± 0.11 | 13.9 ± 0.07 | 14.6 ± 0.14 | 2.1 ± 0.09 | 20.7 ± 0.06 | 19.0 ± 0.04 | 18.9 ± 0.10 | 23.0 ± 0.04 | 25.0 ± 0.10 | 24.9 ± 0.12 |
| *B. sui* | 31.5 ± 0.19 | 31.9 ± 0.97 | 30.0 ± 0.51 | 32.8 ± 1.09 | 35.5 ± 0.44 | 33.0 ± 0.75 | 38.4 ± 0.82 | 30.2 ± 0.49 | 36.0 ± 0.35 | 30.0 ± 0.42 | 35.3 ± 0.45 | 42.0 ± 0.31 | 2.1 ± 0.38 | 4.1 ± 0.19 | 17.2 ± 0.30 | 20.2 ± 0.06 | 22.8 ± 0.11 | 23.9 ± 0.12 |
| *B. jan* | 30.5 ± 0.18 | 30.9 ± 0.79 | 28.7 ± 0.34 | 32.2 ± 0.85 | 35.9 ± 0.52 | 30.9 ± 1.30 | 34.4 ± 1.75 | 31.4 ± 0.21 | 34.0 ± 0.24 | 31.4 ± 0.20 | 36.9 ± 0.37 | 41.1 ± 0.23 | 4.7 ± 0.19 | 0.9 ± 0.08 | 16.9 ± 0.21 | 19.6 ± 0.04 | 20.7 ± 0.17 | 22.8 ± 0.23 |
| *G. cap* | 38.6 ± 0.19 | 39.6 ± 0.13 | 36.8 ± 0.54 | 37.5 ± 2.11 | 42.8 ± 1.94 | 40.5 ± 2.75 | 45.7 ± 3.29 | 35.3 ± 1.46 | 34.5 ± 0.78 | 33.3 ± 0.67 | 35.5 ± 1.04 | 36.6 ± 0.31 | 31.5 ± 0.79 | 30.6 ± 0.61 | 13.7 | 21.5 ± 0.11 | 24.4 ± 0.46 | 23.2 ± 1.01 |
| *H. arg* | 40.7 ± 0.12 | 43.2 ± 0.64 | 42.1 ± 0.31 | 42.3 ± 0.50 | 41.3 ± 0.40 | 42.3 ± 0.52 | 45.4 ± 0.64 | 46.5 ± 0.46 | 45.7 ± 0.21 | 38.5 ± 0.24 | 45.3 ± 0.37 | 56.0 ± 0.26 | 39.8 ± 0.23 | 37.9 ± 0.14 | 45.2 ± 0.55 | 7.5 ± 0.32 | 26.4 ± 0.05 | 23.1 ± 0.12 |
| *H. gla* | 54.5 ± 0.23 | 50.6 ± 1.27 | 48.5 ± 0.37 | 50.7 ± 0.89 | 47.9 ± 0.81 | 54.6 ± 1.43 | 61.1 ± 1.63 | 52.4 ± 1.10 | 50.2 ± 0.29 | 47.9 ± 0.56 | 54.1 ± 0.78 | 61.8 ± 0.73 | 47.7 ± 0.56 | 39.2 ± 0.57 | 57.6 ± 2.62 | 68.1 ± 0.35 | 5.6 ± 0.96 | 23.4 ± 0.16 |
| *T. swi* | 50.3 ± 0.24 | 51.1 | 48.2 ± 1.83 | 52.6 | 49.9 ± 1.36 | 50.8 | 53.9 | 49.9 ± 0.64 | 49.7 ± 0.41 | 50.0 ± 0.00 | 54.3 ± 0.87 | 58.6 ± 0.78 | 52.9 ± 0.60 | 48.1 ± 0.77 | 50.8 ± 6.3 | 48.1 ± 0.57 | 49.0 ± 0.76 | — |

differences in the rates of substitutions (transitions and transversions). For this smaller data set, 12 tests were performed across the phylogeny, as follows: *C. dam*, *C. ans* vs. *C. dar*; *C. dam*, *C. dar* vs. *C. mec*; *C. dam*, *C. dar* vs. *C. boc*; *C. dam*, *C. mec* vs. *C. h. hot*; C. *dam*, *C. h. hot* vs. *B. sui* (Cape Town); *C. dam*, *C. h. hot* vs. *B. jan*; *C. dam*, *C. h. hot* vs. *G. cap*; C. *dam*, *B. sui* vs. *H. arg* (Kenya); *C. dam*, *B. sui* vs. *H. gla*; *C. dam*, *H. arg* (Kenya) vs. *H. gla*; *G. cap*, *B. sui* vs. *H. gla*; *C. h. prt*, *C. h. nat* vs. *C. h. hot*; ($\chi^2 = 0.00–2.86$; $P = 0.09–1.00$).

A likelihood ratio test on 12s rRNA tree scores obtained with and without a molecular clock enforced was not significant ($P > 0.01$), supporting the relative rate tests and confirming earlier studies that show the bathyergid 12s rRNA gene is evolving at a constant rate (Allard & Honeycutt 1992). However, in contradiction to the relative rate tests, a likelihood ratio test conducted on the combined 12S/cyt *b* data set showed a significant difference, suggesting rate heterogeneity among some lineages (likelihood ratio = 124.0; d.f. = 50; $P < 0.01$).

To time the divergence of the *Heliophobius* haplotypes not represented in the 12s rRNA data set in detail, a subset of cyt *b* sequences representing all 10 haplotypes, using *H. glaber* as an outgroup, were analysed separately. These lineages were also shown to be evolving in a clock-like manner following a likelihood ratio test (likelihood ratio = 17.6; d.f. = 9; $P > 0.01$).

Figure 3a summarizes the molecular clock-based divergence times of the main nodes within the phylogeny, estimated from 12s rRNA sequence data. At the generic level, the youngest of the internal nodes was dated to the Miocene, at 13.8–16.6 Myr, and represents the common ancestor of the two subclades forming the *Cryptomys* genus. This overlaps partially with the divergence of *Georychus*/*Bathyergus*, at 15.9–19 Myr, while the separation of the lineage leading to *Heliophobius* was estimated at 33.5–40.3 Myr. Despite the aforementioned ambiguity with rate heterogeneity tests, dates estimated from the combined 12s rRNA/cyt *b* data (not illustrated in Fig. 3) were in close agreement, with divergences estimated as follows: *Cryptomys* 12.3–14.8 Myr, *Bathyergus* 14.0–16.8 Myr, *Georychus* 20.1–24.1 Myr, *Heliophobius* 31.8–38.2 Myr.

For the *Heliophobius* haplotypes, the basal node in the genus was estimated to be 10.8–13.0 Myr (Fig. 3b), and 8.1–9.7 Myr for the split between Kenyan and Tanzanian lineages (Fig. 3a,b). Divergence times for the common ancestor of Malawian (Rumphi, west of the Rift Valley) and Tanzanian haplotypes (Mlali, Morogoro and Dakawa, east of the Rift) were estimated at 6.3–7.6 Myr. The timing of this node is especially important in establishing the role of the Rift Valley as a geographical barrier in the adaptive radiation and spread of the Bathyergidae (see Discussion).

## Discussion

This is the first extensive intra- and interspecific study of the Bathyergidae that applies molecular clock estimates of divergence times to examine their phylogeographical history. Previous molecular systematic studies of the Bathyergidae, based on both mitochondrial and nuclear loci but relatively small data sets, have all produced phylogenies that are congruent at the main nodes, giving a robust molecular phylogeny for the family (Allard & Honeycutt 1992; Faulkes *et al*. 1997a; Walton *et al*. 2000; Huchon & Douzery 2001). Morphologically based classification at the generic level is supported fully by molecular phylogenies, with each of the five genera forming monophyletic groups. Morphological traits also support the subdivision of the *Cryptomys* genus into the two distinct subclades resolved consistently by molecular phylogenies. Honeycutt *et al*. (1991) grouped *C. damarensis*, *C. mechowi*, *C. bocagei* (Figs 1 and 2) together with *C. zechi* (Ghana), *C. foxi* (Nigeria) and *C. ochraceocinereus* (found in Cameroon, Central African Republic and Uganda), which are not represented in our phylogeny. This grouping was based on the possession of small, thick-walled infraorbital foramina. In contrast, *C. hottentotus* species were found to have an elliptically shaped, thin-walled infraorbital foramen.

The placement of *Georychus* relative to *Cryptomys* and *Bathyergus* has been variable in previous phylogenies, with either a sister group relationship between *Georychus* and *Bathyergus*, or *Georychus* as a separate lineage between *Bathyergus* and *Cryptomys*. All of these previous studies have looked at a single *Georychus* sequence (e.g. Allard & Honeycutt 1992; Faulkes *et al*. 1997a) or two sequences from a single locality (Walton *et al*. 2000). As noted previously by Walton *et al*. (2000), subfamilial groupings within the Bathyergidae are not supported by molecular phylogenies. *Bathyergus (*subfamily Bathyerginae) forms either a clade within a paraphyletic Georychinae (*Heterocephalus*, *Heliophobius*, *Georychus* and *Cryptomys* or groups with *Georychus* in a monophyletic clade (Fig. 2a,b). Thus it would appear that the grooved upper incisors and clawed forefeet that are among the features defining the Bathyerginae are derived traits peculiar to *Bathyergus*, and perhaps an adaptation to digging the sandy soil characteristic of their particular habitat. All the recent studies have indicated that the eusocial naked mole-rat, *H. glaber*, is basal and divergent in the family, separated from the social *Cryptomys* genus by the three solitary genera, leading to the conclusion that either sociality or solitarity has been gained or lost more than once during their adaptive radiation thoughout sub-Saharan Africa (Allard & Honeycutt 1992; Faulkes *et al*. 1997a).

### *Phylogeography of the Bathyergidae*

Estimation of divergence times from DNA sequences suggests an ancient (Eocene), East African origin for the Bathyergidae. Allard & Honeycutt (1992), using sequence differences in the mitochondrial 12s rRNA gene and a variety of mammalian evolutionary rates as calibration points, estimated a divergence time from the common ancestor of the family at approximately 38 million years ago (Myr). A later study by Huchon & Douzery (2001) examined sequence differences at a nuclear locus, exon 28 of the von Willebrand factor gene. Using the appearance of the first South American hystricognath fossil in the Tinguiririan fauna of Chile to calibrate their molecular clock, they estimated a slightly earlier basal radiation at either 40 or 48 Myr, depending on the analysis chosen. These estimates predate, but are not inconsistent with the bathyergid fossil record, although the latter is rather limited and Africa is generally depauperate in Eocene/Oligocene strata (Lavocat 1978: 109–172). However, fossils from three genera have been found in Early Miocene fossil beds (24–14 Myr) in East Africa and Namibia (Lavocat 1973, 1978). The largest of these three fossil genera, *Bathyergoides*, is thought to be a sister group to the Bathyergidae. Another of the fossil genera, *Proheliophobius*, morphologically resembles some of the extant Bathyergidae (*Heterocephalus* and *Heliophobius*), while part of a skull of another fossil species, *Paracryptomys*, was found in the Early Miocene beds of the Namib desert in Namibia. In their review of the Early Miocene mammal fossils in East Africa, Van Couvering & Van Couvering (1976) compiled data from reports based on an expedition in 1909–19. They report the presence of fossil *Bathyergoidea* species at Rusinga and Songhor, near Lake Victoria, Kenya, in association with savanna habitat. More recently, Lavocat (1988) describes a new Miocene bathyergid from Fort Ternan, Kenya that he relates to modern *Heterocephalus*. The earliest (but unnamed) fossil resembling *Heterocephalus glaber* has also been recorded in Miocene deposits at Napak in Uganda, together with fossil *Bathyergoidea* (Bishop 1962). These strata have been dated at a minimum age of 17.8 Myr (Bishop *et al*. 1969). Later fossil *Heterocephalus* are known from the Pliocene of Tanzania (4.3 Myr Kakesio Beds, south of Laetoli; Denys 1989), and a further three named fossil species have also been described from the Pliocene and Pleistocene in East Africa: *H. quenstedti* (Laetoli, 3.7–3.5 Myr; Dietrich 1942), *H. atikoi* (Omo, Ethiopia, 2.5–1.8 Myr; Wesselman 1982) and *H. jaegeri* found from 1.7 to 0.8 Myr (Olduvai, Tanzania; Denys 1989). The coincident appearance of *Heterocephalus* fossils with extinct bathyergid ancestors also supports the early divergence of *Heterocephalus* within the family that is suggested by molecular data. While the aforementioned fossils are all in sub-Saharan Africa, interestingly, a review by Tchernov (1992) lists a fossil bathyergid (Gen. indet.) in Early Miocene deposits of the Levant (Negev), although in the primary source the identity of the fossil is unconfirmed (Tchernov *et al*. 1987). These fossils are not recorded in surveys of Pliocene and Pleistocene strata,

suggesting that if ancestral bathyergids had inhabited this region they may have become extinct in the later Miocene, and were replaced by *Spalax ehrenbergi*, a subterranean Myomorph rodent found from the Pliocene onwards.

While the earliest ancestral bathyergids may have been distributed more widely, the majority of fossils have so far been found in East/Central Africa, and basal lineages in the phylogenetic tree of extant taxa are also East African. Given the likelihood of an East African origin for the extant species within the family, it has been suggested (Honeycutt *et al*. 1991) that a possible route for the spread of the Bathyergidae from East Africa was via a corridor of fluctuating aridity linking east and southwest Africa (Van Zinderen Bakker 1967). This arid corridor has been implicated in the distribution of both modern and Early Miocene fossil faunas (Van Couvering & Van Couvering 1976). Thus arid- or mesic-adapted ancestral bathyergids could have exploited this corridor according to the prevailing conditions. Patterns of distribution of extant bathyergid taxa show clear trends with respect to the geography of the Rift Valley. With the exception of a small number of populations of *Heliophobius*, both the latter and *Heterocephalus* occur to the east of the Rift Valley. The genera *Bathyergus* and *Georychus* are restricted to South Africa with some populations of *B. janetta* just crossing into southern Namibia. *Cryptomys* occurs almost exclusively to the west of the Rift, the only exception so far being the population of *C. whytei* we identified at Suma, Tanzania (see Results). Apart from its possible role as a physical barrier, in the form of volcanic uplands and deep valleys (some forming the great lakes of Africa), it is also probable that the climate and vegetation changes that resulted indirectly from the rifting process have been of importance in the distribution and evolution of the Bathyergidae. The question of whether, or at what stage, the Rift Valley was influential, can now be examined in the context of estimated divergence times for the extant bathyergid taxa, and the limited fossil record.

Patterns of distribution of extant taxa suggest that an initial spread south from East Africa may have been constrained by the Rift Valley, effectively 'funnelling' the ancestral lineages down from the east into Southern Africa, from where the genus *Cryptomys* radiated north, eventually reaching as far as Central and West Africa (Fig. 4a–d), but again being constrained by the Rift Valley, this time with movement east being restricted. An alternative model is that early radiation of the family was independent of rifting and a general radiation from East Africa occurred, with rifting influencing the distribution of more recent lineages that have since replaced earlier populations. Estimation of the divergence times of the major nodes in the bathyergid phylogeny with respect to the chronology of rifting suggest that the latter scenario is more likely. Rift valley formation has occurred in a number of stages beginning as long ago as the Triassic Period (200 Myr) with the break-up of Gondwanaland and the formation of the Indian Ocean. Around 50 Myr, as the Somalian subplate began to separate from the African subplate to the west, the continent began to split. In East Africa, the Ethiopian highlands (a rifted upland volcanic region) already existed by the Late Oligocene (Baker *et al*. 1971), and major rifting then occurred in the Miocene, eventually producing two branches to the East Africa rift system, the Western rift and the Kenya rift, and the great African lakes and volcanos that characterize these regions. Kenya rift formation began first, from around 23 Myr, while in the Western rift volcanism commenced at about 12 Myr in the northern section and at around 7 Myr in the south (Van Couvering & Van Couvering 1976; Ebinger 1989). These processes continued through the Pliocene and Pleistocene. Thus, if our molecular datings are correct, divergence of the basal lineages in the Bathyergidae, *Heterocephalus* (40–48 Myr) and *Heliophobius* (32–40 Myr) occurred before the major rifting in the Miocene. Our estimates of the divergence of *Georychus*/*Bathyergus* from their common ancestor with *Cryptomys* (20–26 Myr) coincide with the beginning of volcanism in the Kenya rift, and possibly favoured the expansion into southern Africa rather than to the north and west. In addition, aridification of the Saharo–Arabian belt was beginning and would have isolated further the Bathyergidae to sub-Saharan Africa. If fossil evidence of the Bathyergidae or the sister taxon Bathyergoides is confirmed in the Negev of Israel, then this may represent either an early radiation north, or remnants of ancestral lineages that spread into Africa from Eurasia. The first fossil bathyergids are also found during this period (from the early Miocene) in East Africa and Namibia, confirming that southern Africa was also being colonized at this time.

During the later Early and Middle Miocene, we estimate that the *Cryptomys* genus diverged into its two subclades (12–17 Myr). Again, this is a critical period in Rift Valley formation as volcanism and rifting was progressing in the Kenya rift, and also just beginning in the Western rift. While the *C. hottentotus* subclade appears to have speciated almost exclusively in South Africa, the *C. mechowi* subclade underwent a more extensive radiation, particularly in Zambia and Central Africa, resulting a wide diversity of genetically divergent chromosomal forms (see below). Interestingly, this is coincident with the onset of volcanism in the adjacent Western rift, and presumably resulted in considerable environmental challenges as climate and vegetation changed. This period has been reported previously as a time of faunal turnover (Van Couvering & Van Couvering 1976). In the Early Miocene the Congo basin through to central Kenya is thought to have been heavily forested lowland with broadleaved woodland, which was gradually broken up as volcanos formed (Van Couvering & Van Couvering 1976; Axelrod & Raven 1978). This woodland occurred along an east–west axis from Senegal to Ethiopia, and along a north–south axis between Ethiopia,

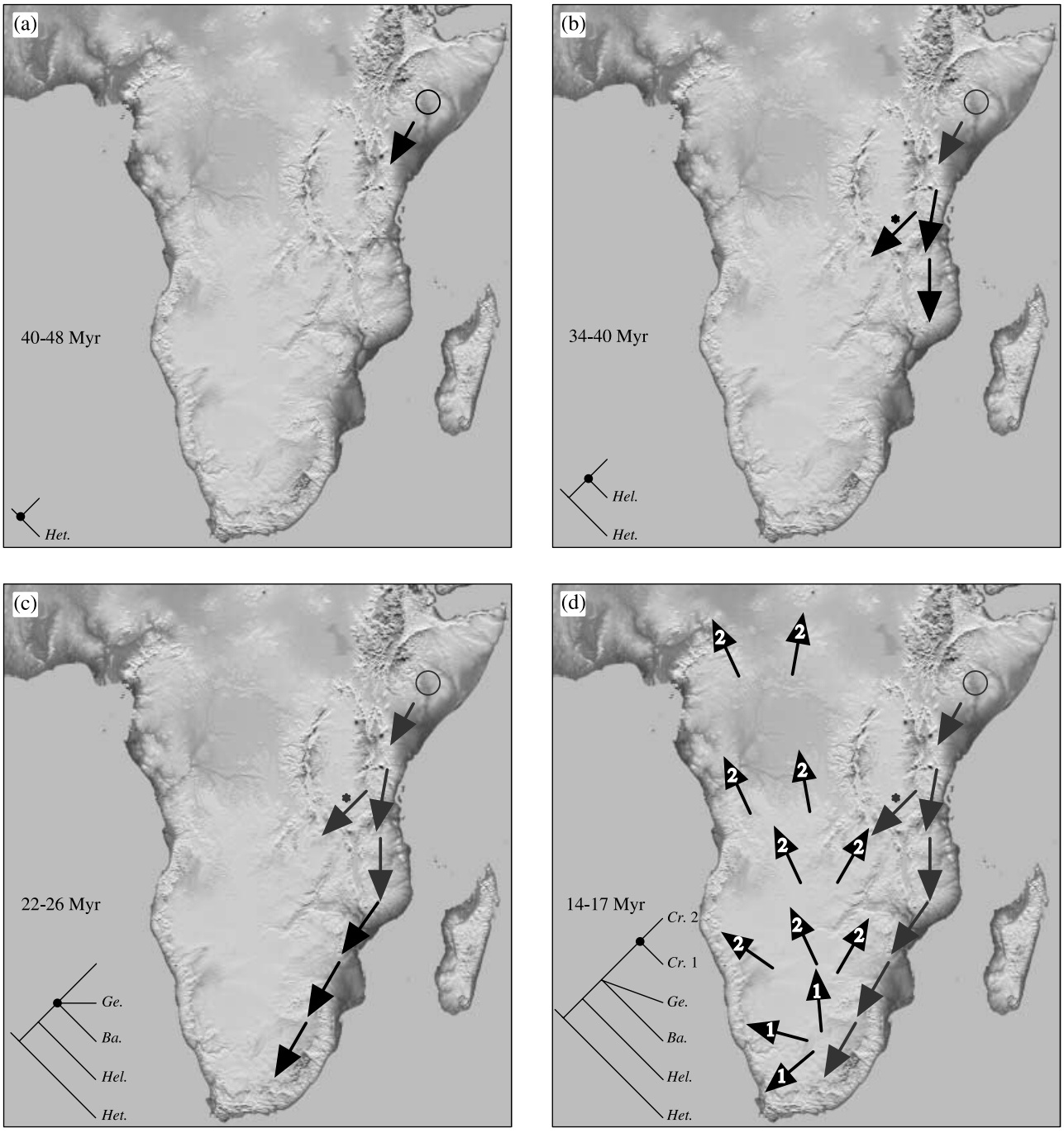


**Fig. 4** Phylogeographical trends in the Bathyergidae inferred from analysis of mitochondrial 12s rRNA sequence differences. (a) Initial divergence of the *Heterocephalus* (*Het.*) lineage from the common ancestor of the family in East Africa; (b) Radiation of *Heliophobius* (*Hel.*) and movement from East Africa into Southern Africa, with some populations crossing the Rift Valley (*); (c) *Bathyergus* (*Ba.*) and *Georychus* (*Ge.*) lineages diverge in South Africa; (d) *Cryptomys* (*Cr.*) diverges into two clades, one radiating predominantly in South Africa (1) and the other spreading north into Southern and Central Africa (2).

South Africa and Namibia). To the south and the west of this, the tropical lowland rain forest of the Congo Basin (and beyond) prevailed. According to our molecular clock estimates and the fossil evidence, it is likely that bathyergids already inhabited this broadleaved woodland vegetation zone. During the process of rifting in the Miocene, the climate difference between the zone occupied by the broadleaved forests and the rain forests increased, and the

broadleaved forests gradually became drier and turned into wooded savannas (e.g. in Tanzania, Kenya, Zambia, Zimbabwe, Namibia, South Africa) and where uplifting occurred into Afroalpine woodlands (Albertine Rift and the Ethiopian plateau). This suggests that the mole-rats must have survived in the wooded savannas and Afroalpine woodlands that experienced periodically extremely dry periods, particularly during the late Pleistocene. The increasing volcanism and formation of the East Africa rift during the Miocene appears to have isolated *Heterocephalus* and *Heliophobius* to the east and restricted *Cryptomys* to the west of the Rift. However, some recent 'leakage' across this boundary has occurred both ways among those taxa able to exploit the prevailing habitat. We have identified mole-rats caught in Suma, Tanzania as belonging to the *Cryptomys mechowi* subclade and assign this haplotype tentatively to *C. whytei*, as it groups with other sequences in the phylogeny from Mzuzu, Malawi and Mbala, Zambia. The type locality of *C. whytei* falls within this geographical range (Karonga, Malawi; Thomas 1897, cited in Ellerman 1940). The *Heliophobius* individuals from Malawi that we have examined appear to be relatively recent, rather than an early, relic population. We estimate that they shared a common ancestor with Tanzanian *Heliophobius* populations 6.3–7.6 Myr. It is possible that this divergence occurred before local rifting: the ages estimates for the Usangu Rift are less than 3 Myr, the Rukwa Rift less than 6 Myr and the Karonga basin rifting the northern part of Lake Malawi less than 5 Myr (Ebinger 1989).

### *Patterns of speciation and chromosomal evolution*

Historically, the taxonomy of the Bathyergidae has been problematic, although the application of DNA sequence analysis and karyotyping has helped to clarify some areas, and also to exemplify their taxonomic complexity. It is also apparent from the molecular phylogenetic data that there is no clear-cut relationship between social system, sequence divergence and chromosomal evolution. Deep divergences appear to be coupled with little or no chromosomal change while shallow divergences may be coupled with large changes in karyotype.

### *Basal clades* Heterocephalus *and* Heliophobius

Ellerman (1940), reviewing earlier studies, lists a total of four forms of *Heterocephalus*, which he considered as either synonyms or races of the type *H. glaber glaber*, based on variation in the number of molars and body size. However, the use of body size is particularly inappropriate for *Heterocephalus* because their social system gives rise to a high degree of intracolony polymorphism in this trait. Karyotypes of animals at the current extremes of the species range (Somalia and Kenya) show no variation in chromosome number ($2n = 60$; George 1979; Capanna & Merani 1980). Genetic structuring within and between geographical locations of *H. glaber* has been investigated previously in detail by examining sequence variation in the mitochondrial control region (Faulkes *et al*. 1997b). For parity with other species in the data set, this study has examined a more limited number of samples at the cyt *b* locus, but across a large geographical range. Despite genetic differences of up to 10.6% at cyt *b* between some populations, and a lifestyle that may be conducive for speciation (high reproductive skew, reduced effective population size and highly substructured populations), the available morphological and karyotypic data suggest that *Heterocephalus* is a monotypic genus. Furthermore, northern and southern Kenyan animals that differ by 3% at cyt *b* will hybridize readily in captivity (J. U. M. Jarvis & C. G. Faulkes, unpublished data).

For *Heliophobius*, Ellerman (1940) lists nine types, but concluded that with the exception of one (*Heliophobius spalax*), they should be viewed as races of *Heliophobius argenteocinereus*. More recently, Honeycutt *et al*. (1991) re-examined the holotype for *H. spalax* and concluded that the characters used to differentiate it from *H. argenteocinereus* were due to age variation. This study has revealed that while within populations mitochondrial sequence differences were low, between geographical areas genetic divergence was high (up to 13.3%). In addition, a recent study of Zambian populations of *Heliophobius* reports small chromosomal differences ($2n = 62$; Scharff *et al*. 2001) compared with the karyotype of Kenyan *Heliophobius* ($2n = 60$; George 1979). Unlike *Heterocephalus*, morphological differences have been reported between some types, including pelage colour, skull morphology and body size, and given the large genetic differences reported here and differences in karyotype noted by Scharff *et al*. (2001) this genus requires further study, in particular to establish whether nuclear genetic loci also show high rates of interpopulation divergence.

### *The South African solitary genera (*Georychus *and* Bathyergus*)*

Both these solitary genera show limited ranges compared with the rest of the family, and relatively few holotypes are described in the literature (five for *Bathyergus* and three for *Georychus*; Ellerman *et al*. 1953**)**. Currently three species are recognized, *G. capensis* ($2n = 54$), *B. suillus* ($2n = 56$) and *B. janetta* ($2n = 54$; Nevo *et al*. 1986). Although clearly different species, *B. suillus* and *B. janetta* show relatively low sequence divergence (up to 5.6%). A potential hybrid zone for *B. suillus* and *B. janetta* occurs at Rondawel, South Africa where these species are also sympatric with *C. h. hottentotus*. *Georychus* is considered to be a monotypic genus, although allozyme and mtDNA analysis of disjunct populations of

*Georychus* from Cape Town and southeastern KwaZulu-Natal has suggested that these may constitute separate species (Honeycutt *et al*. 1987, 1991; Nevo *et al*. 1987). Our cyt *b* sequence data also support high levels of genetic divergence (13.7%) between populations around Cape Town and those to the north.

### *Social mole-rats of the genus* Cryptomys

The *Cryptomys* genus is the most speciose and widely distributed of all the bathyergids, and their taxonomic status has historically been problematic. Ellerman (1940) lists 49 named forms of *Cryptomys*. Hayman (cited in Ellerman 1940) splits these into five groups, based primarily on the presence of a white head-spot, then colour and body size (head spot, colour and growth are now known to be unreliable characteristics and may vary considerably even within a species: Jarvis *et al*. 1991; Bennett & Faulkes 2000). Early descriptions of *Cryptomys* also failed to differentiate consistently between the two distinct groups within the genus identified by molecular phylogenies, despite reported morphological differences between these clades in the shape and wall thickness of the infraorbital foramen (Honeycutt *et al*. 1991). Hence, in the past, a number of taxa have been named as subspecies of *Cryptomys hottentotus* that molecular studies have shown to be genetically highly divergent and belong as separate species in the *Cryptomys mechowi* subclade. These include *C. darlingi* and *C. anselli* (the latter referred to previously as *C. amatus* by Faulkes *et al*. 1997a; Bennett & Faulkes 2000). Thus, although a review of earlier studies by Honeycutt *et al*. (1991) coupled with examination of unspecified museum voucher specimens proposed that just seven species of *Cryptomys* should be recognized, molecular phylogenies (Faulkes *et al*. 1997a; this study) suggest strongly that this is an underestimate, and despite the fact that many of the early descriptions of *Cryptomys* were based on highly variable morphological features, genetic analysis supports the species richness of the group. Within *Cryptomys*, the two subclades both contain robustly supported monophyletic groups, some of which attain high levels of sequence divergence. However, between them different patterns of chromosomal evolution are apparent, possibly giving rise to different patterns of speciation.

Taxa in the *C. mechowi* subclade show allozyme divergence (Filippucci *et al*. 1994, 1997) and considerable sequence and chromosomal diversity (reviewed in Bennett & Faulkes 2000). Recently, two new species from Zambia have been named by Burda *et al*. (1999): the aforementioned *C. anselli* and *C. kafuensis* (the latter not included in this phylogeny). Additional taxa not included in this study but thought to be distinct species are *C. zechi* from Ghana, *C. foxi* from Nigeria and Cameroon and *C. ochraceocinereus* from Cameroon, Central African Republic and Uganda (reviewed in Rosevear 1969). Both their geographical distribution and the morphology of their infraorbital foramen suggest that the latter three central African species should be grouped in the *C. mechowi* subclade. Limited sequence data from DNA extracted from museum samples (dried skin) of *C. zechi* also support this (C. G. Faulkes, unpublished data). One of these species (*C. foxi*) has also been karyotyped and shows the chromosomal divergence characteristic of the subclade ($2n = 66$; Williams *et al*. 1983).

Thus, from molecular (Faulkes *et al*. 1997a; this study) and cytological studies, we suggest that this subclade of *Cryptomys* may contain at least 11 (but probably many more) distinct phylogenetic species, some or all of which may also be biological species, as follows (citations refer to karyotype data): *C. damarensis* ($2n = 74$, 78, Nevo *et al*. 1986 and $2n = 80$, C. G. Faulkes *et al*. unpublished data), *C. darlingi* ($2n = 54$; Aguilar 1993), *C. whytei* ($2n = 46$; Chitaukali *et al*. 2001), *C. mechowi* ($2n = 40$; Macholán *et al*. 1993), *C. bocagei* ($2n = 58$; G.H. Aguilar, pers. comm.), *Cryptomys* collected near Kalomo, Zambia ($2n = 50$; G.H. Aguilar, pers. comm.), *C. anselli* ($2n = 68$) and *C. kafuensis* ($2n = 58$; Burda *et al*. 1999), *C. foxi* (Williams *et al*. 1983), *C. zechi* and *C. ochraceocinereus*. Other populations in Zambia are known to be karyotypically different and require further study (H. Burda, pers. comm.; Van Daele *et al*. submitted), and whether these populations are disjunct, sympatric or form hybrid zones is unknown. Vicariant populations of other mammals also occur in this region of south-central Africa, and it has been suggested that the patterns of drainage in large river systems and changes thereof may have had an important influence in driving speciation (Cotterill 2004; Van Daele *et al*. submitted).

Within the *C. hottentotus* subclade assignment of taxonomic status is more difficult, as although there is high sequence diversity between some populations, unlike the *C. mechowi* subclade, chromosomal evolution appears to be conserved. Our current sample set reveals three main groups, *C. h. hottentotus*, *C. h. natalensis* (both $2n = 54$; Nevo *et al*. 1986) and *C. h. nimrodi*. Both *C. h. nimrodi* and *C. h. hottentotus* form well-supported monophyletic clades (with 100% bootstrap values; Fig. 2b). The *C. h. natalensis* group includes divergent populations from around Pretoria, South Africa that we refer to as *C. h. pretoriae*. A further taxon that we refer to as *C. h. mahali*, despite their close proximity to *C. h. pretoriae* (30 km), appear to be highly divergent, and forms either a basal lineage in the *C. h. natalensis* group (Fig. 2a) or the basal lineage for the entire subclade (Fig. 2b). Both *C. h. pretoriae* and *C. h. mahali* share the same $2n$ of 54, characteristic of the *C. hottentotus* subclade (C. G. Faulkes *et al*. unpublished data). More work is now needed to extend the sampling range of all these to clarify further the phylogeographical patterns of radiation within this subclade, and possibly areas of sympatry and hybridization.

## Conclusions

In summary, an ancient origin of the Bathyergidae has given rise to a complex pattern of adaptive radiation and distribution of extant lineages. While early divergences within the family may have been largely independent of rifting, both molecular clock estimates and the fossil record suggest strongly that the patterns of distribution of later lineages were influenced directly by physical barriers imposed by rifting, and indirectly by accompanying climatic and vegetative changes. The different patterns of speciation observed both within the family and within the *Cryptomys* genus may have also been a result of these geological changes. It has been suggested that increased rates of genome evolution and diversity may be triggered by environmental stress (Nevo 2001). While the environment of South Africa was less affected by rifting, and the *Cryptomys* species distributed here appear to be karyotypically conserved, the increased rate of chromosomal evolution in the *C. mechowi* subclade of central African species correlates with the increased rifting during the Miocene that produced local climatic, vegetative and physical changes in the environment. Ultimately, this may explain the increased taxonomic diversity of the *Cryptomys* genus that has long puzzled taxonomists.

## Acknowledgements

We are greatly indebted to those who have assisted directly in providing samples, including Gonzalo Aguilar, Marna Herbst, Bill Stanley and the Field Museum of Natural History, Chicago, Glen Malherbe, Andrew Molteno, Peter Taylor, Conrad Lichtenstein, Dada Gottelli and Gus Mzumara. Also thanks to Tamsin Burland and Steve Le Comber for feedback on earlier versions of the manuscript, and the two referees for their detailed comments on the initial submission of this manuscript. Paul-Michael Agapow gave invaluable help with software. This work was funded by grants from the NERC (C. G. F.), National Research Foundation, South Africa (N. C. B. and J. U. M. J.), National Geographic Society (J. U. M. J.), University of Pretoria (N. C. B.), a DWTC project from the Belgian Federal Office for Scientific, Technical and Cultural Affairs and the Ecological Genetics Research Network of the Fund for Scientific Research, Flanders (E. V. and W. V.).

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This work is part of a long-term study of African mole-rat systematics by the authors C. G. F., N. C. B. and J. U. M. J., which began following a field trip across Southern Africa in 1992, and has been augmented over the years by ourselves and our coworkers. Understanding the phylogenetics of African mole-rats is integral to furthering our knowledge of the evolution and maintenance of highly social behaviour in these enigmatic animals.

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