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SYSTEMATICS AND BIOGEOGRAPHY OF THE MOZAMBIQUE THICKET RAT, *GRAMMOMYS COMETES*, IN EASTERN CAPE PROVINCE, SOUTH AFRICA

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Taxonomy of thicket rats (*Grammomys*) is highly provisional and the genus is in a critical need of a thorough revision. We compared *G. cometes* from Eastern Cape Province ($n = 150$) with *G. ibeanus*, *G. macmillani*, and the southern African *G. dolichurus*, applying analyses of a partial cytochrome-*b* (*Cytb*) sequence (375 base pairs), karyotypes, and cranial morphology. Genetically, *G. cometes* appeared to be very close to *G. dolichurus* (mean sequence divergence of 3.4% ± 0.8% *SE*), whereas *G. ibeanus* and *G. macmillani* were separated by a mean sequence divergence of 5.4% ± 1.2%. Nucleotide diversity among haplotypes was higher in *G. dolichurus* ($\pi = 0.0080 \pm 0.0010$ *SD*) than in *G. cometes* ($\pi = 0.0040 \pm 0.0009$). *G. cometes* and *G. dolichurus* showed the same diploid chromosome number ($2N = 52$) of mostly acrocentric autosomes. None of the karyotypes reported so far for various *Grammomys* species match the chromosomal sets we found in Eastern Cape Province. Discriminant function analysis on 5 cranial measurements that are not affected by age variation was successful in separating *G. cometes* and *G. ibeanus*, but *G. dolichurus* appeared very similar to the former. In spite of their close genetic and morphological proximity, *G. cometes* and *G. dolichurus* tend toward ecological segregation and behave as distinct biological species. *G. cometes* is endemic to the southern African subregion and the 4 Eastern Cape Province localities are possibly isolates. Specimens were caught in the Afromontane forest above 1,000 m elevation and the lowland riverine forests dominated by *Combretum caffra*.

Key words: chromosomes, cytochrome-*b* gene, *Grammomys*, morphology, systematics

Thicket rats of the genus *Grammomys* are endemic to sub-Saharan Africa (Musser and Carleton 2005) and in the past have frequently been reported under the generic name *Thamnomys* (de Graaff 1981; Delany 1975; Meester et al. 1964; Smithers and Lobão Tello 1976). Twelve species are currently recognized (Musser and Carleton 2005); this number is highly provisional and the genus is in a critical need of a thorough taxonomic revision (Bronner et al. 2003; Skinner and Chimimba 2005).

Two species have been reported uniformly for southern Africa (de Graaff 1981; Meester et al. 1986; Skinner and Chimimba 2005; Skinner and Smithers 1990): *G. dolichurus* and *G. cometes*. Until quite recently, both species were treated as being widely distributed in sub-Saharan Africa (Hutterer and Dieterlen 1984; Misonne 1974); the larger forms of the genus were frequently pooled under *G. cometes* and the smaller under *G. dolichurus* (Misonne 1974). Although *G. dolichurus* is still considered to range from the Cape region to southern Ethiopia, *G. cometes* is currently restricted to south of the Zambezi River and is endemic to the southern African subregion (Musser and Carleton 2005). Further north, *G. cometes* is replaced by *G. ibeanus*, a species that previously was assigned either a specific rank (Ellerman 1941; Musser and Carleton 1993) or was synonymized with *G. cometes* (Hutterer and Dieterlen 1984; Misonne 1974).

Although *G. dolichurus* is widespread along the eastern coast of southern Africa as far south as Port Elizabeth, *G. cometes* is mainly restricted to the northeastern portion of the subregion (de Graaff 1981; Musser and Carleton 2005; Skinner and Chimimba 2005; Skinner and Smithers 1990). The known occurrence of *G. cometes* in Eastern Cape Province is based on a single specimen from Pirie Forest near King William's Town (Musser and Carleton 1993), extending the previously known

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Table 1.—Specimens of *Grammomys* used in this study. Abbreviations: *Cytb*—cytochrome *b*; RSA—Republic of South Africa; GFRR—Great Fish River Reserve; m—male; f—female. For a complete list of specimens, see Appendix I.

Species	Country	Locality	*Cytb*	Karyotype	Morphology
G. ibeanus	Kenya	See Appendix I			19
	Tanzania	See Appendix I			25
	Malawi	Nyika Plateau	2		30
G. cometes	Mozambique	Inhambane			3
	RSA	Kosi Bay			1
	RSA	Pirie Forest			1
	RSA	Hobbiton	4	6 m/2 f	112
	RSA	Fort Fordyce	4	3 m/1 f	27
	RSA	GFRR	1	3 m/2 f	10
G. dolichurus	RSA	See Appendix I			3
	RSA	Port St. Johns	5	3 m/1 f	6

southern border of the geographic range in KwaZulu-Natal (Taylor 1998) approximately 500 km further southwestward. However, recent sources (Mills and Hes 1997; Skinner and Chimimba 2005; Stuart and Stuart 1997) missed this evidence of range extension.

Grammomys abounds with cryptic diversity and recent nominal species are based primarily on their karyotypes (Hutterer and Dieterlen 1984; Musser and Carleton 2005). Diagnostic characters separating the 2 southern African species also are vague, overlap, or even gradate in some areas (Meester et al. 1986; Taylor 1998). Information on chromosomal variation is limited and incomplete, but *G. dolichurus* is known to be polytypic (Dippenaar et al. 1983). Consequently, the identification of specimens is difficult. The morphological definition of *G. ibeanus* is unsatisfactory (Musser and Carleton 2005) and de Graaff (1981), in his detailed account on southern African rodents, provided no measurements for *G. cometes* because the paucity of material.

We report in this paper on morphological, chromosomal, and molecular properties of *G. cometes* from Eastern Cape Province. Next, we compare data sets on *G. cometes* with those on *G. dolichurus* from the subregion and on *G. ibeanus*. In doing so, we aim to define *G. cometes* as accurately and by as many data sets as possible, to reassess characters allowing its separation from the sympatric *G. dolichurus*, and to assess its taxonomic relations with *G. ibeanus*. Furthermore, we tested the monophyly of *Grammomys* and phylogenetic relationships of the genus with 8 other African murine genera using the partial sequence of the cytochrome-*b* (*Cytb*) gene. Our results are largely based on our own small mammal surveys conducted between 2002 and 2005 in various forest types in Eastern Cape Province. Additional information was derived from voucher specimens in museum collections.

Materials and Methods

Thicket rats were trapped between 2002 and 2005 in 5 localities: Hobbiton on Hogsback, 32°33′S, 26°57′E; Fort Fordyce Nature Reserve, 32°41′S, 26°28′E; Great Fish River Reserve, 33°04′–33°09′S, 26°37′–26°49′E; Silaka Nature Reserve near Port St. Johns, 31°34′S, 29°26′E; and the estuary of the Mzimvubu River near Port St. Johns, 31°40′S, 29°35′E. Trapping was performed using aluminum folding Sherman traps (23 × 8 × 9 cm; H. B. Sherman Traps, Inc., Tallahassee, Florida) and polyvinyl chloride live traps (Willan 1979) baited with rolled oats mixed with sunflower oil. Traps were set in lines with stations 20 m apart. Four traps were set at each station within a radius of <5 m. Two traps were placed on the ground and 2 >1 m above ground level and checked twice daily. The term ''trap night'' is used to describe a trap that was set for a 24-h period. All field procedures involving handling of animals in this study were in compliance with guidelines approved by the American Society of Mammalogists (Gannon et al. 2007).

Captured animals were sacrificed and the following external measurements were taken: W—body mass (g), HB—head and body length (mm from snout to anus), TL—length of tail (mm from anus to tail tip excluding terminal hair), HF—length of hind foot (mm without claws), E—ear length (mm). Voucher specimens (carded skins, skulls, postcranial skeletons, and alcoholic material) have been deposited in ZFMK and UPK (see Appendix I for collection acronyms).

Among numerous voucher specimens of *Grammomys* from throughout the range of the genus and examined in BMNH, SMF, and ZFMK, 85 were used for comparison with material collected in Eastern Cape Province (see Appendix I for list of localities and collections). External measurements, date of collection, and localities were recorded from specimen tags. In order to avoid taxonomic confusion regarding the scope of *G. dolichurus*, we considered only southern African material of this species.

Cytochrome-b sequence.—Sixteen *Grammomys* specimens (Table 1) were analyzed for variation in the mitochondrial *Cytb* gene. Three types of tissue were used: samples of dry skin, tissue samples from complete specimens in 70% ethanol, and tissue samples from muscles removed from freshly collected specimens and stored in 96% ethanol.

Total DNA from tissue preserved in ethanol was extracted using QiaAmp DNA Blood and the Tissue Mini purification kit (Qiagen, Valencia, California) and that from museum skins was extracted on a KingFisher apparatus using the genomic DNA purification kit for King Fisher (Thermo Fisher Scientific,

Waltham, Massachusetts). A 375-base pair (bp) *Cytb* fragment was amplified using ''universal'' primers L14771 and H15149 (Irwin et al. 1991). Because of the degraded nature of the DNA isolated from the museum skins and alcohol-preserved specimens, amplification of larger fragments of the *Cytb* gene using existing primers was unsuccessful in these samples.

Amplification of DNA fragments was performed on a BIORAD thermocycler (Bio-Rad Laboratories, Hercules, California) using a 50-μl reaction volume containing 2.5 mM $MgCl_2$, 0.5 μM forward and reverse primer, 0.25 mM deoxynucleoside triphosphates, and 1 unit of Bioline Taq (Bioline UK Ltd., London, United Kingdom) in the supplied ammonium buffer. Cycling conditions included an initial step of 95°C for 15 min, followed by 35 cycles of denaturation (1 min at 94°C), primer annealing (1 min at 55°C), and DNA extension (1 min at 72°C).

Polymerase chain reaction products and negative controls were checked on a 1.5% agarose gel. Double-stranded polymerase chain reaction products were purified with Wizard SV Gel and the PCR Clean-UP System (Promega, Madison, Wisconsin). Sequencing was performed on ABI PRISM 3130 Genetic Analyzer using BigDye Terminators chemistry (Applied Biosystems, Foster City, California).

The program CodonCode Aligner (Ewing et al. 1998) was used to align forward and reverse sequences. The resulting consensus sequences for each individual were aligned using Clustal W (Thompson et al. 1997) in combination with Bioedit (Hall 2004). Nucleotide and amino acid composition was analyzed using the program Mega 3.0 (Kumar et al. 2004). The total number of base frequencies in each position was estimated with the program DAMBE 4.2.13 (Xia 2000; Xia and Xie 2001). Nucleotide diversity (π) was estimated using DnaSP (Rozas and Rozas 1999).

We assessed phylogenetic relationships among *Grammomys* haplotypes (*Aethomys chrysophilus* as outgroup—Ducroz et al. 2001; Jansa et al. 2006) and among 8 African Murinae genera (*Otomys irroratus*, subfamily Otomyinae, as outgroup). Additional sequences were downloaded from GenBank (Appendix II). The hierarchical likelihood ratio test and the Akaike information criterion implemented from the program Modeltest 3.06 (Posada and Crandall 1998) were used to identify the most appropriate model of DNA substitution for the data. Under both algorithms the general time reversible model plus invariant sites and gamma distribution of variable sites (GTR+I+G—Rodríguez et al. 1990) were chosen to assess phylogenetic relations within the Murinae (gamma-distributed shape parameter [α] = 2.05, proportion of invariable sites [I] = 0.54), and the Tamura-Nei model (TrN+G) was chosen for assessing relations among *Grammomys* haplotypes (α = 0.26). These 2 models were implemented in the Bayesian analysis (Mau et al. 1999; Rannala and Yang 1996; Yang and Rannala 1997) to reconstruct evolutionary trees (program MrBayes—Huelsenbeck and Ronquist 2001), and to calculate maximum-likelihood pairwise genetic distances (PAUP 4.0b10—Swofford 2002). Phylogenetic trees were obtained using 4 Markov chain Monte Carlo chains running simultaneously for 200,000 generations (among haplotypes) and for 1 million generations (among murine genera) with the resulting trees sampled at every 10th generation.

Karyotypes.—Twenty-one specimens of 2 southern African species were karyotyped (Table 1) using the preparation of in vivo bone marrow chromosomes (Robbins and Baker 1978). The slides were stained conventionally by Giemsa and C-banded by the modified technique of Sumner (1972).

Morphology.—Only specimens of adults (molars at least moderately worn) were used in morphometric comparisons. Ansell (1974) did not find significant sexual dimorphism in Zambian *G. ibeanus*; consequently we ignored this factor. Twelve linear measurements were scored from each skull using a vernier caliper to the nearest 0.1 mm. Definitions and acronyms are as follows: CbL—condylobasal length, RoL—length of rostrum, MxT—maxillary toothrow length (on crowns), DiL—length of diastema, FiL—length of incisive foramen, ZyB—zygomatic breadth, BcB—braincase breadth, IoC—interorbital constriction, RoB—breadth of rostrum across molars, BcH1—braincase height, BcH2—braincase height across bullae, BuL—length of bullae. Discriminant function analysis was used to assess overall phenetic similarity among a priori defined groups. Statistical tests were run in Statistica 5.5 (StatSoft, Tulsa, Oklahoma).

Results and Discussion

Cytochrome-b sequence.—A total of 8 haplotypes was identified among the 16 *Grammomys* partial *Cytb* sequences (375 bp) and an additional haplotype was downloaded from GeneBank (Appendix II). Altogether, 68 sites (18.1%) were polymorphic, with a total of 69 mutations within which 60 sites (16.0%) were parsimony informative. No stop-codon insertions or deletions were present in the alignment. As expected under neutral evolution (Martin and Palumbi 1993) the majority of polymorphic sites were at 3rd positions (49 variable sites, 72.0% of all variable sites), followed by 1st positions (14 variable sites, 20.6% of all variable sites), and 2nd positions (5 variable sites, 7.4% of all variable sites).

The Bayesian analysis on *Grammomys* haplotypes reached stationarity at around 200,000 generations (20,000 saved trees), so that the last 18,000 trees were used to compute a 50% majority-rule consensus tree. The 4 independent analyses converged on similar log-likelihood values, and the mean lnL score for the posterior distribution of these trees was 1,046.05. The majority consensus tree rooted with *A. chrysophilus* (Ducroz et al. 2001; Jansa et al. 2006) was unresolved at the basal branching (Fig. 1). *Grammomys* haplotypes fell into 2 major clades separated by a mean sequence divergence of 17.0% ± 2.3% *SE* (TrN genetic distance).

The 1st clade included southern African samples and the 2nd comprised a sample from Malawi and a specimen from Tanzania. The southern African clade was further divided into 2 lineages separated by a mean sequence divergence of 3.4% ± 0.8%. These 2 clades, with a high bootstrap support (100%), are interpreted as representing 2 distinct species, *G. cometes* and *G. dolichurus*, respectively. Two geographic samples from north of the Zambezi River were represented by 3 haplotypes

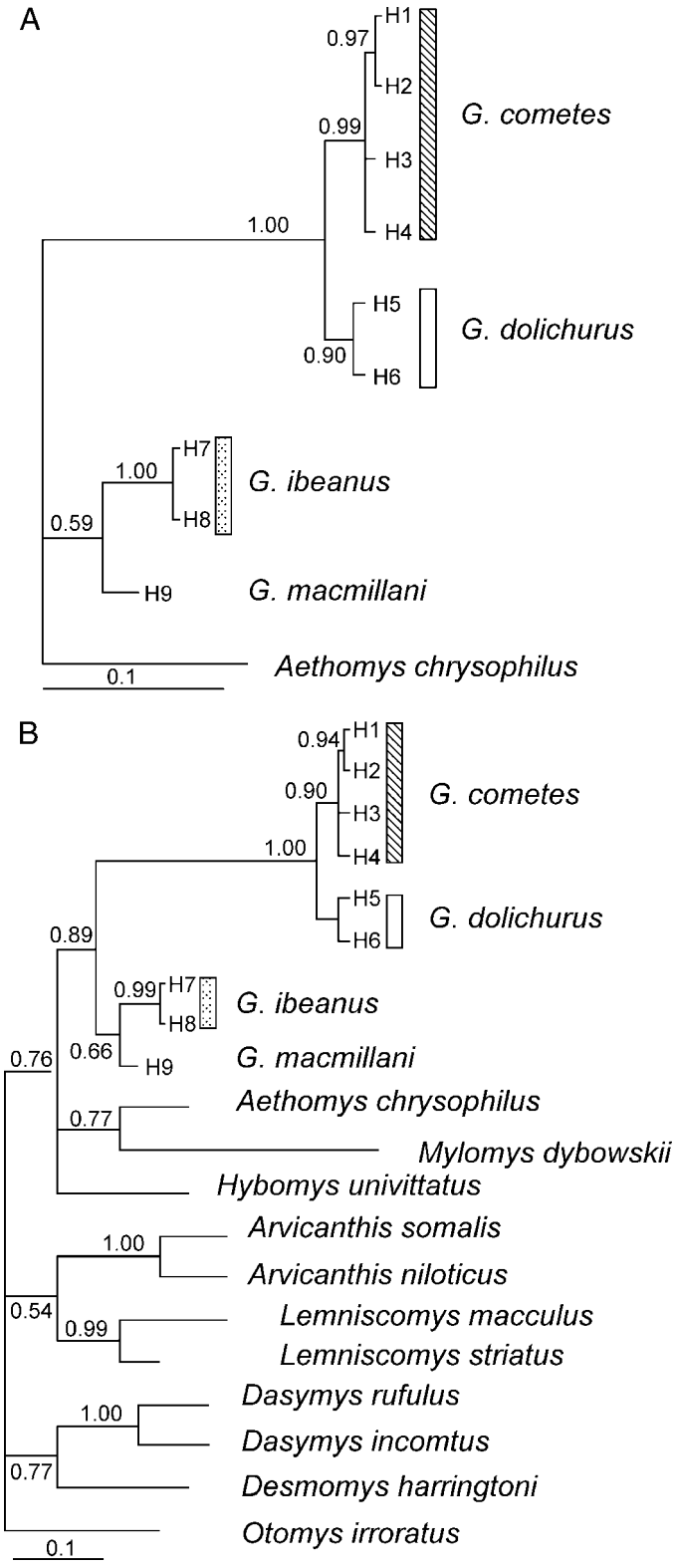

Fig. 1.—Fifty percent majority-rule consensus trees. Above is phylogenetic tree of 18,000 trees from a Bayesian analysis of 9 cytochrome-*b* (*Cytb*) haplotypes of *Grammomys* rooted with *Aethomys chrysophilus*. The bottom consensus tree of 80,000 trees from a Bayesian analysis of 9 *Cytb* haplotypes of *Grammomys* and 10 other species from the subfamily Murinae is rooted with *Otomys irroratus*. Numbers above branches represent posterior probability values (>0.50).

that fell into 2 clusters. These 2 groups were separated by a mean sequence divergence of 5.4% ± 1.2%, but support for branching was low (59%). Two closely related haplotypes from Malawi are ascribed to *G. ibeanus*, whereas Jansa et al. (2006) identified the Tanzanian haplotype as *G. macmillani*. Earlier allocations of the latter to *G. dolichurus* (Castiglia et al. 2003; Ducroz et al. 2001) must be erroneous, considering that the type locality of this species is in South Africa.

Nucleotide diversity was highest between 2 haplotypes of *G. dolichurus* ($\pi = 0.0080 \pm 0.0010$ *SD*), lowest between haplotypes of *G. ibeanus* ($\pi = 0.0026 \pm 0.0006$), and intermediate among haplotypes of *G. cometes* ($\pi = 0.0040 \pm 0.0009$). Contrary to this, haplotype diversity was higher in *G. cometes* ($hd = 0.750 \pm 0.122$ *SD*) than in *G. dolichurus* ($hd = 0.400 \pm 0.237$). Although such discrepancy might be indicative of a rapid population growth from a small effective population (Avise 2000), samples are by far too small for sound conclusions.

To test the monophyly of *Grammomys*, we ran Bayesian analysis on 9 haplotypes of *Grammomys* and 10 species of African Murinae, representing 7 genera and 4 divisions (sensu Musser and Carleton 2005). *O. irroratus* (subfamily Otomyinae) was used as an outgroup. Bayesian analysis became stationary at around 1,000,000 generations (equaling 100,000 trees) so that the last 80,000 trees were used to compute a majority-rule consensus tree. The 4 independent analyses converged on similar log-likelihood values, and the mean lnL score for the posterior distribution of trees was 2,232.33. Hence, the 50% majority consensus tree provided strong support (89%) for the monophyly of *Grammomys* (Fig. 1).

The topology of the clade for *Grammomys* retained the same clustering pattern of haplotypes as in the above analysis (Fig. 1). Three divisions of Murinae (*Aethomys*, *Arvicanthis*, and *Hybomys*) emerged as a possible sister group to the division for *Grammomys* and support for the monophyly of this clade was modest (76%). Of note was that the genera in Murinae did not form a monophyletic cluster and that the division for *Arvicanthis* emerged as paraphyletic because of the sister position of *Desmomys* to *Dasymys* (Fig. 1).

Karyotypes.—All 4 geographic samples karyotyped from Eastern Cape Province (3 of *G. cometes* and 1 of *G. dolichurus*) showed the same diploid number of chromosomes 2N = 52 (Fig. 2). The autosomal complement consisted of 25 pairs of gradually diminishing size. Most of the autosomes were acrocentric. Two pairs were metacentric or submetacentric (numbers 18 and 21), and 2 other pairs were submetacentric or subtelocentric (numbers 6 and 9). The X chromosome was submetacentric and extraordinarily large. Its size comprised approximately 20% of the length of the haploid complement of females. The Y chromosome probably was 1 of the acrocentric or subtelocentric chromosomes. The centromeric regions and the short arms of certain autosomes stained positively in C-banded preparations. The Y chromosome as well as the long arm and the centromeric area of the X chromosome also were C-positive (Fig. 2). We have not found any consistent differences in the karyotype between the geographical populations or species studied. Differences were indicated in the proportion of

acrocentric and subtelocentric autosomes and in the size and centromere position in the Y chromosome. It seems that this variation only represents polymorphism between individuals within populations.

Various diploid numbers were reported from different regions and for various species of *Grammomys*, ranging between 27 and 76 (Table 2). Part of this variation is due to the presence of supernumerary chromosomes (Civitelli et al. 1989). None of these karyotypes match the chromosomal sets we found in Eastern Cape Province. Within southern Africa, Dippenaar et al. (1983) report 2 diploid numbers for *G. dolichurus*: 2N = 52 from Ngoye Forest and 2N = 44 from Woodbush. The diploid number of *G. ibeanus* from Nyika Plateau, 2N = 44–48 (Chitaukali et al. 2000), is distinct from the one we found in *G. cometes*.

The diploid number of 52 chromosomes is the most frequently reported and is seemingly widespread across the range of *Grammomys*. However, the fundamental number of arms may vary. Matthey (1971) recorded dimorphism in the X chromosome that may be related to its large size and presumably high content of heterochromatin.

Morphology.—Although a large proportion of specimens of adult *G. cometes* showed reproductive activity (scrotal testes, embryos, or presence of placental scars), this group was not homogeneous. Further division of the sample of adults from Hobbiton on Hogsback into 4 age groups based on abrasion of the 1st lower molar revealed 7 characters not affected by age: HF, E, MxT, IoC, BcH1, BcH2, and BuL (1-way analysis of variance: $F < 2.7$, $P > 0.05$). Details on nongeographic variability will be provided elsewhere. Overall phenetic similarity among samples of *Grammomys* was thus assessed by discriminant function analysis on 5 cranial variables that were not subjected to age variation. Seven operational taxonomic units were defined at this early stage of analysis: 1—*G. ibeanus* (Kenya and northern Tanzania), 2—*G. ibeanus* (southern Tanzania and Malawi), 3—*G. dolichurus* (Republic of South Africa), 4—*G. cometes* (Mozambique and KwaZulu-Natal), 5—*G. cometes* (Great Fish River Reserve), 6—*G. cometes* (Hobbiton on Hogsback), and 7—*G. cometes* (Fort Fordyce). Specimens were classified on the basis of characters by Hutterer and Dieterlen (1984) and the above evidence derived from partial *Cytb* sequences. Because multivariate statistics require complete data sets and because missing data were not substituted by estimates, 208 of a total 238 specimens were included in subsequent analyses.

Fig. 2.—C-banded karyotype of a male *Grammomys cometes* from Hobbiton on Hogsback.

Table 2.—Summary of conventional chromosomal sets reported in the genus *Grammomys*, except for the *G. rutilans* (= *poensis*) group, arranged according to descending numbers. Species name is the same as originally reported. Note that *G. gazellae* is currently considered to be a junior synonym of *G. macmillani*; *G. surdaster* is synonymized with *G. dolichurus* (Hutterer and Dieterlen 1984; Musser and Carleton 2005). Abbreviations: RSA—Republic of South Africa; ECP—Eastern Cape Province; 2N—diploid number; FN—fundamental number of chromosomal arms; FNa—fundamental number of automosomal arms.

Species	Locality	2N	FN/FNa	Source
G. gazellae	Central African Republic	68–76	82/	Petter and Tranier 1975
G. gazellae	Central African Republic	56–71		Civitelli et al. 1989
Grammomys	Somalia	56–61	/70–75	Roche et al. 1984
G. caniceps	Kenya	56	78/	Hutterer and Dieterlen 1984
G. dolichurus	Central Africa	52	66/	Matthey 1971
G. surdaster	Katanga	52	66/	Petter and Tranier 1975
G. buntingi	Ivory Coast	52	66/	Petter and Tranier 1975
G. cometes	RSA, Eastern Cape Province	52	62/58	This study
G. dolichurus	RSA, Port St. Johns	52	62/58	This study
G. dolichurus	RSA, Ngoye Forest	52		Dippenaar et al. 1983
G. ibeanus	Malawi, Nyika Plateau	44–48		Chitaukali et al. 2000
G. dolichurus	RSA, Woodbush	44		Dippenaar et al. 1983
G. minnae	Ethiopia	32	64/	Hutterer and Dieterlen 1984
Grammomys	Tanzania	27	/39	Fadda et al. 2001

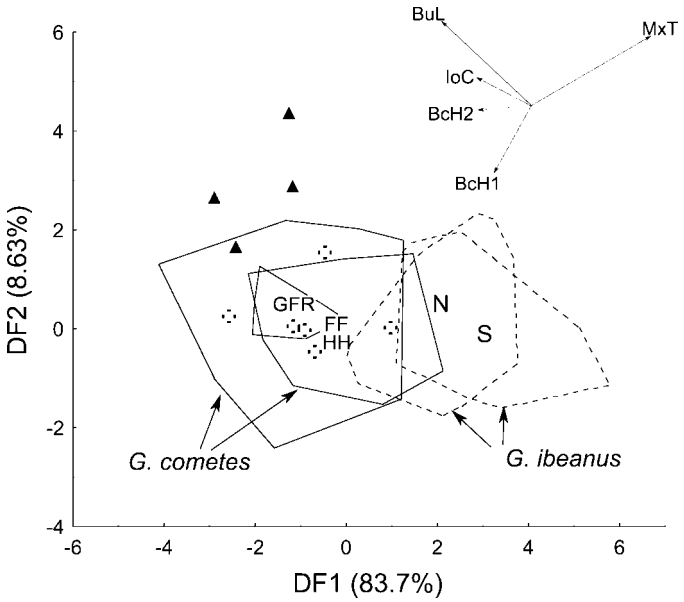

FIG. 3.—Projection of specimens onto the first 2 discriminant functions derived from a discriminant function analysis on 5 $\log_{10}$-transformed cranial variables for 7 a priori defined groups. The percentage of variance explained by a variate is in parentheses. Polygons enclose all the specimens within a group and sample acronyms are plotted on group centroids. *Grammomys cometes* from Eastern Cape Province (bold line): GFR—Great Fish River Reserve; HH—Hobbiton on Hogsback; FF—Fort Fordyce. *Grammomys ibeanus* (dotted line): N—northern group; S—southern group. Triangles—*G. cometes* from Mozambique and KwaZulu-Natal; crosses—*G. dolichurus*. Insert shows character vectors. See text for character designations and for further definition of groups.

Discriminant function analysis (Wilks' $\lambda = 0.218$, $P < 0.0001$) classified 67.3% of specimens into their proper group. The majority of misclassified specimens (52 of 58 misclassified) were placed within geographic samples but from the same species. The proportion of misclassified specimens was highest in *G. dolichurus* (4 of 6). A projection of discriminant function scores onto the first 2 discriminant functions (DFs), which explained 92.3% of variance in the original data set, separated samples of *G. cometes* and *G. ibeanus* along DF1 (Fig. 3). The 2 species primarily differed in molar length (high loadings for *G. ibeanus*) and in length of the bullae (high loadings for *G. cometes*). The importance of bullar size in distinguishing these 2 species already has been revealed by Musser and Carleton (2005). All 4 specimens of *G. cometes* from Mozambique and KwaZulu-Natal matched the Eastern Cape Province conspecifics along DF1 axis but attained higher loadings for DF2. Surprisingly, the entire sample of *G. dolichurus* appeared morphologically very close to *G. cometes* from Eastern Cape Province.

To improve discrimination between species, we pooled the 2 samples of *G. ibeanus* and 3 samples of *G. cometes* from Eastern Cape Province. Discriminant function analysis on 4 operational taxonomic units classified 96.7% of specimens into their proper group (Wilks' $\lambda = 0.275$, $P < 0.0001$) and 16 misclassified specimens (of 17 total) were allocated to a wrong species group. Results were nearly identical to the previous discriminant function analysis on 7 operational taxonomic units (not shown). Another discriminant function analysis on 3 operational taxonomic units (2 pooled samples of *G. cometes* and *G. ibeanus*, and 1 of *G. dolichurus*) did not improve classification results (92.3% of specimens classified properly). Again, *G. dolichurus* phenetically resembled *G. cometes* (not shown).

The 2 species of *Grammomys* from the southern African subregion appeared similar, both cranially and dentally. Characters reported in the literature for distinguishing *G. dolichurus* from *G. cometes* involve size and color (de Graaff 1981; Skinner and Chimimba 2005; Skinner and Smithers 1990; Smithers and Lobão Tello 1976; Taylor 1998). *G. cometes* is on average larger but measurements overlap, which makes size (either HB or CbL) of little taxonomic help. This overlap is partly due to heterogeneity in the adult age group as defined in this study. Thus, *G. dolichurus* does not attain the maximum measurements of *G. cometes*, and very old specimens of *G. cometes* with heavily worn molar cusps were outside the range for *G. dolichurus*: CbL $>$ 27.5 mm, ZyB $>$ 15.0 mm. In addition, *G. cometes* had longer ears than *G. dolichurus*, with the cut-off point at approximately 18 mm (Table 3). There were several outliers among the studied *G. cometes* regarding ear length, but remeasuring this trait on dry skins showed that values $<$ 18 mm were invariably underestimates and thus obviously erroneous. We thus suggest that ear length is of help in distinguishing the 2 species of *Grammomys* in southern Africa.

The fur color ("less gray" in *G. dolichurus*—cf. de Graaff 1981; Taylor 1998) appeared prone to individual variation in our material. The samples of *G. cometes* from Hobbiton on Hogsback and from Fort Fordyce were of both extremes (paler–darker) and the coloration of specimens of *G. dolichurus* from Port St. Johns was fairly dark (wood brown). A few specimens of *G. cometes* from Great Fish River Reserve were clearly paler than *G. dolichurus*, showing bright russet tints on the back, particularly the posterior. Consequently, we do not suggest coloration be used for morphological identification.

Grammomys cometes is reported to "often but not always" have a subaricular tuft of white hairs, whereas *G. dolichurus* lacks it entirely (Skinner and Chimimba 2005; Skinner and Smithers 1990). We could not score this trait in the majority of BMNH specimens because the tuft is hidden under the dried ears of the prepared skins. Thus, we considered only material collected and processed by ourselves, where the ear was laid forward on 1 side of voucher skins. Presence or absence of the white subauricular tuft was subject to much geographic variation in *G. cometes*. The white tuft was present in all specimens from Hobbiton on Hogsback ($n = 108$) but was absent in 37.0% of animals from Fort Fordyce ($n = 27$). In agreement with published data (Skinner and Smithers 1990), none of 5 *G. dolichurus* from the Port St. Johns area had such a tuft of white hairs. Thus, this character is only of possible auxiliary value in taxonomic identification and can be misleading because of interpopulation variation, at least in *G.*

TABLE 3.—Summary statistics for external and cranial traits of *Grammomys* used in this study. Given are samples size (in parentheses), mean ± *SD* (upper row), and range (lower row). See Appendix I for further details on geographic origin of samples and text for character acronyms. Note that *G. cometes* includes material from Eastern Cape Province. Probability level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, $^{n.s.}$ $P > 0.05$. The largest homogeneous sets (in parentheses) were derived from Scheffe's test.

Character	1, *G. ibeanus*	2, *G. cometes*	3, *G. dolichurus*	*F*-value Homogeneous sets
HB	(43) 112.4 ± 6.83	(143) 121.8 ± 6.56	(7) 114.1 ± 4.34	37.38****
	100–126	103–143	110–122	(1, 3) (2)
TL	(41) 174.5 ± 14.66	(122) 173.7 ± 9.25	(5) 171.2 ± 3.42	$0.14^{n.s.}$
	127–203	147–203	167–176	(1, 2, 3)
HF	(45) 23.7 ± 1.07	(143) 24.8 ± 0.80	(7) 24.5 ± 1.47	25.44****
	21.0–26.2	22.9–27.3	22.0–27.0	(1, 3) (2, 3)
E	(40) 18.5 ± 2.01	(141) 19.9 ± 0.97	(7) 17.2 ± 0.35	32.50****
	16.0–22.0	17.9–22.0	16.9–17.7	(1, 3) (2)
W	(33) 43.8 ± 6.69	(143) 40.7 ± 6.23	(4) 33.7 ± 2.50	6.21**
	35–58	27–65	31–36.5	(1) (2, 3)
CbL	(61) 28.21 ± 0.92	(142) 28.11 ± 0.92	(7) 26.67 ± 0.66	8.62***
	26.3–30.1	26.2–30.1	25.6–27.2	(1, 2) (3)
RoL	(60) 14.64 ± 0.48	(144) 14.51 ± 0.48	(7) 13.84 ± 0.46	8.87***
	13.4–15.6	13.1–15.7	13.2–14.4	(1, 2)
MxT	(65) 4.72 ± 0.16	(144) 4.42 ± 0.11	(7) 4.39 ± 0.11	120.61****
	4.4–5.1	4.2–4.8	4.2–4.5	(1) (2, 3)
DiL	(60) 7.72 ± 0.37	(144) 7.66 ± 0.37	(7) 7.18 ± 0.33	6.61**
	6.8–8.8	6.5–8.6	6.7–7.7	(1, 2) (3)
FiL	(60) 7.13 ± 0.32	(144) 7.18 ± 0.37	(7) 6.63 ± 0.27	8.06***
	6.4–8.1	5.8–8.0	6.3–7.1	(1, 2) (3)
ZyB	(59) 15.05 ± 0.52	(142) 15.05 ± 0.42	(6) 14.22 ± 0.35	9.99****
	14.0–16.1	14.0–16.4	13.8–14.8	(1, 2) (3)
BcB	(55) 12.71 ± 0.39	(144) 12.70 ± 0.32	(7) 12.24 ± 0.23	6.37**
	12.0–13.6	11.9–13.4	11.8–12.5	(1, 2) (3)
IoC	(59) 4.59 ± 0.19	(144) 4.76 ± 0.19	(7) 4.61 ± 0.31	17.70****
	4.2–5.0	4.0–5.2	4.1–5.0	(1, 3) (2, 3)
RoB	(59) 5.76 ± 0.23	(144) 5.63 ± 0.27	(6) 5.42 ± 0.10	15.69****
	5.4–6.3	5.3–6.1	5.3–5.6	(1) (2) (3)
BcH1	(56) 8.96 ± 0.29	(144) 9.20 ± 0.28	(6) 8.87 ± 0.28	16.95****
	8.4–9.7	8.3–9.8	8.4–9.2	(1, 3) (2)
BcH2	(56) 10.74 ± 0.33	(144) 11.07 ± 0.32	(6) 10.83 ± 0.27	21.62****
	9.9–11.8	10.2–11.9	10.3–11.1	(1, 3) (2)
BuL	(57) 5.31 ± 0.24	(142) 5.63 ± 0.20	(7) 5.61 ± 0.18	45.78****
	4.7–6.1	5.2–6.1	5.3–5.8	(1) (2, 3)

cometes. The taxonomic value of a subauricular white spot has already been questioned in earlier studies (e.g., Ansell 1974).

Different counts have been reported for mammary formula in *G. cometes* (de Graaff 1981). Roberts (1951) gave the number as 6 nipples (2 inguinal pairs in addition to 1 pectoral), Meester et al. (1964) reported 4 (inguinal only), whereas Ellerman (1941) stated that it can be either. Twenty-two females from Fort Fordyce and Hobbitton on Hogsback invariably showed 3 pairs of mammae (1 pectoral and 2 inguinal).

Distribution.—Musser and Carleton (2005) report *G. cometes* endemic to the southern African subregion in Mozambique (Smithers and Lobão Tello 1976), eastern Zimbabwe (Melseter and Umtali districts—Musser and Carleton 2005), southeastern Transvaal (Mpumalanga—Skinner and Smithers 1990), and KwaZulu-Natal (Taylor 1998). No records are available from Lesotho (Lynch 1994) and from Swaziland (Monadjem 1998). Musser and Carleton (2005) report it for Free State but Lynch (1975, 1985) and de Graaff (1981) provide no records. The northern border of the geographic range of *G. cometes* is on the Zambezi River in Mozambique (Smithers and Lobão Tello 1976), whereas *G. ibeanus* occurs further north in Malawi (Chitaukali et al. 2002) and in northeastern Zambia (Ansell 1978), but not in Mozambique (Skinner and Smithers 1990; Smithers and Lobão Tello 1976). Until the record from Pirie Forest near King William's Town (Eastern Cape Province) was published (Musser and Carleton 1993), the southernmost localities were from KwaZulu-Natal (Ngoye Forest and the Royal Natal National Park); the report for the Mpumalunga Province was dubious (Taylor 1998). Our records are from the Amathole forest complex in the Drakensberg Range close to Pirie Forest (Fig. 4). To bridge the gap of approximately 500 km between records in Amathole and KwaZulu-Natal, we sampled in an Afromontane forest fragment located 14 km north and 4.5 km west of Ugie (Witteberg forest complex; elevation 1,500–1,600 m) in February 2005 but failed to catch any thicket rats (1,400 trap nights). Regarding the hitherto reported distribution, and considering possible mistakes in identification of animals, it is not possible at this point to conclude to what extent the Amathole population is an isolate. Clearly, more fieldwork remains to be done in various

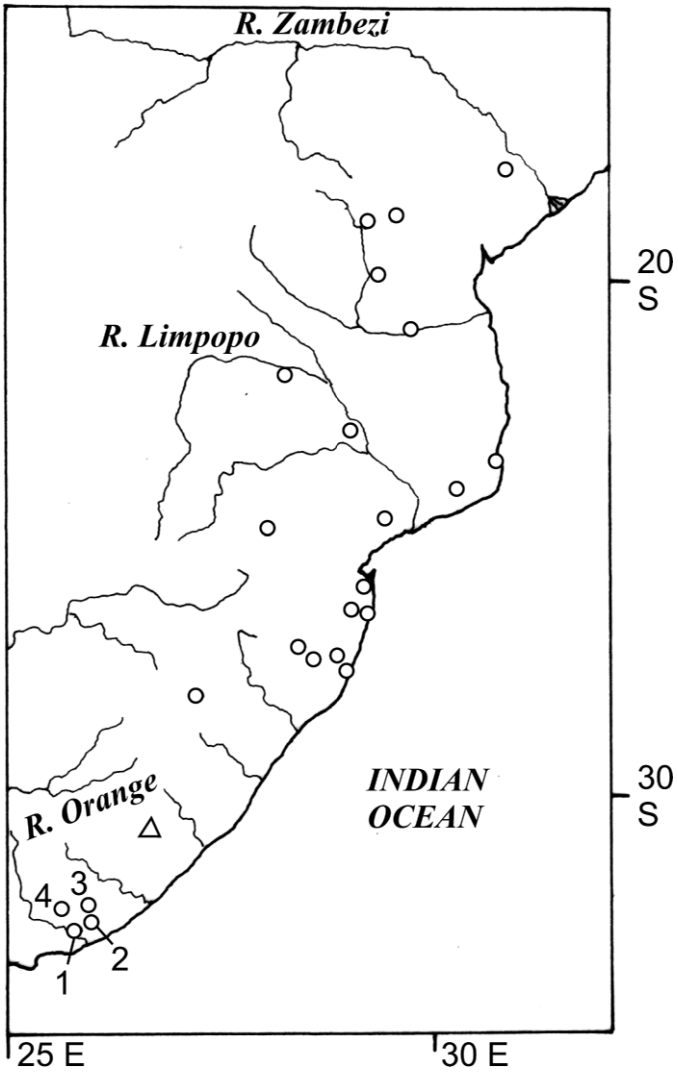

Fig. 4.—Map of the eastern part of the southern African subregion showing localities of *Grammomys cometes* (dots), based on de Graaff (1981), Smithers and Lobão Tello (1976), and Taylor (1998). Absence of *G. cometes* in the Transkei forest complex 14 km north and 4.5 km west of Ugie is indicated by a triangle. Eastern Cape Province records: 1—Pirie Forest; 2—Great Fish River Reserve; 3—Hobbiton on Hogsback; 4—Fort Fordyce. References: Musser and Carleton (2005): 1; records of BK, WH, and RMB: 2–4.

forest fragments along the Great Escarpment mountain ridge, which de Graaff (1981) saw as the western border of the species range (Fig. 4). *G. cometes* is rare in South Africa and Mugo et al. (1995) report merely 6 localities from the entire country.

In Eastern Cape Province we collected *G. cometes* in 2 different woodland types: the *Podocarpus*-dominated Afromontane forest above 1,000 m above sea level (Hobbiton on Hogsback and Fort Fordyce) and the lowland riverine forest at 95 m above sea level dominated by *Combretum caffra* (Great Fish River Reserve). All specimens ($n = 156$) were trapped at night, which supports earlier statements that *G. cometes* is nocturnal (Skinner and Chimimba 2005). The majority of rats were collected in traps set on trees: 75% in Hobbiton and 82% in Fort Fordyce; deviation from random was significant in both cases ($\chi^2 > 14$, $P < 0.001$). *G. cometes* appeared to be strictly tied to a closed canopy forest. Pine plantations in Amathole are not a suitable habitat for this species (R. M. Baxter, in litt.). In Great Fish River Reserve we also failed to catch *G. cometes* in the arid and shrubby Valley Thicket vegetation during 8,406 trap nights that yielded 457 other small mammals.

The only report of *G. cometes* for Eastern Cape Province published so far (Pirie Forest) is based on a single specimen deposited in the American Museum of Natural History (Musser and Carleton 1993). Another voucher specimen from the same locality that was collected in September 1897 (BMNH) has the following note on its tag: ''Very rare in this part.'' During our summer trapping sessions (December–February 2002 and 2003) in the Amathole forest region, we found *G. cometes* to be one of the core small mammals in the forest ecosystem, along with *Myosorex cafer* and *Graphiurus murinus*. The abundance of *G. cometes* in our samples of small mammals varied from 17.9% in Fort Fordyce and 18.3% in Hobbiton to 23.4% in Great Fish River Reserve. In spite of this, population densities were fairly low. In Hobbiton on Hogsback we collected 118 specimens of *G. cometes* in 2,324 trap nights (i.e., 5.1 specimens per 100 trap nights), in Fort Fordyce 27 specimens in 3,228 trap nights (0.8 per 100 trap nights), and in Great Fish River Reserve 11 in 496 trap nights (2.2 per 100 trap nights).

Systematics and phylogeny.—In the genus *Grammomys*, species are loosely defined (Hutterer and Dieterlen 1984; Taylor 1998), thus one can assume a possible bias in the taxonomic interpretation of our results. However, we feel confident that the taxonomic designation of our material is consistent with the current use of the specific names. *G. dolichurus* is the only thicket rat reported thus far from the Port St. Johns district (de Graaff 1981) and *G. cometes* was confirmed recently from Pirie Forest near King William's Town (Musser and Carleton 1993), a forest fragment that is linked directly to Hogsback as part of the Amathole forest (see Fig. 4). Identification of specimens from Mt. Nganda as *G. ibeanus* accords with the opinion by Chitaukali et al. (2000, 2002). *G. macmillani*, which was recently reported for the southern African subregion in Zimbabwe (Bronner et al. 2003; Musser and Carleton 1993, 2005), is karyologically very distinct from our material (cf. Table 2). Our nomenclature is thus consistent with that applied by earlier authors and accords also with Musser and Carleton (2005) and Skinner and Chimimba (2005).

The 2 southern African thicket rats, *G. cometes* and *G. dolichurus*, appeared very similar morphologically and genetically, and also were identical in their karyotypes. Sequence divergence between them (3.4%) is so low that it hardly supports their separation into 2 distinct biological species. Genetic distance values between 2% and 11% are indicative of conspecific populations or valid species (Bradley and Baker 2001) and the lowest reported sequence-divergence value between rodent sister species is 1.2% (Baker and Bradley 2006).

In spite of their close genetic and morphological proximity, *G. cometes* and *G. dolichurus* behave as distinct biological

species. Their ranges partially overlap in southern Africa (Skinner and Smithers 1990). *G. cometes* usually occurs sympatrically with *G. dolichurus* (de Graaff 1981) and the 2 species also were found syntopically (Taylor 1998). In addition, they tend toward ecological segregation: *G. cometes* prefers denser and more developed forest than *G. dolichurus*, which, in turn, also occurs in open woodland and in shrubland (de Graaff 1981; Taylor 1998). Thus, *G. cometes* seems restricted to the forest biotic zone, whereas *G. dolichurus* also occurs in the southern savannah woodland (Rautenbach 1978). Therefore, it is plausible to continue regarding *G. cometes* and *G. dolichurus* as 2 separate species.

The gene tree, derived from a partial *Cytb* sequence, represents a minuscule fraction of the genetic history of thicket rats in South Africa. Besides, the history of a gene is not necessarily equal to the history of a species (Avise 2000) and a phylogenetic approach to taxonomy based on single gene loci can be misleading (Avise and Ball 1990).

Our results show that in all 3 data sets (molecular, chromosomal, and morphological), *G. cometes* appears more divergent from *G. ibeanus* than from *G. dolichurus*. On the other hand, Tanzanian *G. macmillani* is genetically linked to *G. ibeanus* and not to southern African *G. dolichurus*. Note that Misonne (1974) in his oversimplified taxonomic division of the genus into 2 broadly sympatric and polytypic species (putting aside a very distinct *G. rutilans*—cf. Hutterer and Dieterlen 1984), the larger *G. cometes* and the smaller *G. dolichurus*, synonymized *G. ibeanus* with the former and *G. macmillani* with the latter. These 2 size-based species presumably differentiate regionally at the pan-African scale. Our results suggest that the actual evolutionary history of *Grammomys* might be just the opposite, namely an ancient regionally based divergence followed by a relatively recent speciation that also involved size displacement in young sympatric species pairs.

Nucleotide diversity was highest in *G. dolichurus* and lowest in *G. cometes*. This difference suggests that *G. dolichurus* is evolutionarily the older species, which would concur with its putative broad pan-African occurrence (Musser and Carleton 2005). Presuming this, *G. cometes* likely evolved from *G. dolichurus* in the southern African subregion. Such a scenario is tenuous because molecular evidence can be interpreted in other ways; for example, by different demographic histories of the 2 species.

The degree to which localities where *G. cometes* was captured in the Amathole forest complex are isolates is not known. Samples from Mozambique and KwaZulu-Natal morphologically differ from those from Eastern Cape Province. Lacking chromosomal and molecular evidence, the possible taxonomic significance of these differences remain unknown and is thus an area for further research.

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APPENDIX I

List of specimens used in this study. Collection acronyms: BMNH—Natural History Museum London; SMF—Senckenberg Forschungsinstitut und Museum, Frankfurt; UPK—University of Primorska, Koper; ZFMK—Zoologiches Forschungsinstitut und Museum A. Koenig, Bonn.

Grammomys cometes (n = 154).—Mozambique: Boguno, Inhambane (BMNH, $n = 3$; includes type of *G. cometes*). Republic of South Africa: Kosi Bay, KwaZulu-Natal (SMF, $n = 1$); Pirie Forest, King William's Town (BMNH, $n = 1$). Great Fish River Reserve near Grahamstown, 95 m aove sea level (ZFMK, $n = 10$); Hobbiton on Hogsback, the Amathole Mts., 1,200 m above sea level (ZFMK, $n = 112$); Fort Fordyce, the Katberg Mts., 1,040 m above sea level (UPK, $n = 27$).

Grammomys ibeanus (n = 74).—Kenya: Mt. Nyiru (BMNH, $n = 3$; includes type of *G. lutosus*); Mt. Elgon, 2,740 m (BMNH, $n = 4$); Uraguess (= Gargues) Mts. (BMNH, $n = 3$); Nanyuki (BMNH, $n = 1$); Molo (BMNH, $n = 1$; type of *G. ibeanus*); Aberdare National Park (BMNH, $n = 7$). Tanzania: Ngorongoro (crater), 1,700 m, 2,300 m (BMNH, $n = 15$); Momela Lakes, 30 km NE Arusha (BMNH, $n = 1$); Lushoto, near Wilhelmastal (BMNH, $n = 1$); Mazumbai Estate, Lushoto District, Tanga Region, 1,400 m above sea level (BMNH, $n = 1$); Kambai Forest Reserve, Usambara Mts., Muheza District, Tanga Region, 335 m (SMF, $n = 1$); Mbeya, Poroto Mts. (BMNH, $n = 3$); Irambo Mission, Poroto Mts., Tukuyu District, Mbeya Region, 2,050 m (SMF, $n = 3$). Malawi: Nyika Plateua (BMNH, $n = 13$); Mt. Nganda, Nyika National Park, 1,531–2,255 m (SMF, $n = 17$).

Grammomys dolichurus (n = 10).—Republic of South Africa: Ngoye Forest Reserve (BMNH, $n = 1$); Ngqeleni, 800 m (BMNH, $n = 2$); KwaZulu-Natal, no locality (BMNH, $n = 1$); Port St. Johns, 150 m (BMNH, $n = 1$); Port St. Johns: the estuary of the Mzimvuba River, near sea level (UPK, $n = 1$); Port St. Johns, Silaca Nature Reserve, below 100 m above sea level (UPK, $n = 4$).

APPENDIX II

List of species of Muridae whose cytochrome-*b* sequences were used in this study. GenBank accession numbers are in parentheses. Taxonomy and nomenclature follow Musser and Carleton (2005).

Murinae.—Grammomys division: *Grammomys macmillani* (AF14121). *Grammomys cometes* (all from Republic of South Africa): Fort Fordyce, haplotypes H1 and H2 (EU275248, EU275249); Great Fish River Reserve, haplotype H3 (EU275250); Hobbiton on Hogsback, haplotype H4 (EU275251). *Grammomys dolichurus*: Port St. Johns, haplotype H5 (EU275252); Silaca Nature Reserve, haplotype H6 (EU275253). *Grammomys ibeanus*: Malawi, Mt. Nganda, Nyika National Park, haplotypes H7 and H8 (EU275254, EU275255). *Arvicanthis* division: *Arvicanthis somalicus* (*neumanni*) (AF004574), *Arvicanthis niloticus* (AF004572), *Mylomys dybowskii* (AF141212), *Lemniscomys macculus* (AF141208), *Lemniscomys striatus* (AF141211), *Desmomys harringtoni* (AF 141206). *Dasymys* division: *Dasymys incomtus (AF141217), Dasymys rufulus (AF141216). Hybomys division: Hybomys univittatus (AF141219). Aethomys division: Aethomys chrysophilus (AF004587).*

Otomyinae.—Otomys irroratus (AF141222).