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Phylogeny and Divergence-Date Estimates of Rapid Radiations in Muroid Rodents Based on Multiple Nuclear Genes

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Abstract.—The muroid rodents are the largest superfamily of mammals, containing nearly one third of all mammal species. We report on a phylogenetic study comprising 53 genera sequenced for four nuclear genes, GHR, BRCA1, RAG1, and c-myc, totaling up to 6400 nucleotides. Most relationships among the subfamilies are resolved. All four genes yield nearly identical phylogenies, differing only in five key regions, four of which may represent particularly rapid radiations. Support is very strong for a fundamental division of the mole rats of the subfamilies Spalacinae and Rhizomyinae from all other muroids. Among the other "core" muroids, a rapid radiation led to at least four distinct lineages: Asian *Calomyscus*, an African clade of at least four endemic subfamilies, including the diverse Nesomyinae of Madagascar, a hamster clade with maximum diversity in the New World, and an Old World clade including gerbils and the diverse Old World mice and rats (Murinae). The Deomyinae, recently removed from the Murinae, is well supported as the sister group to the gerbils (Gerbillinae). Four key regions appear to represent rapid radiations and, despite a large amount of sequence data, remain poorly resolved: the base of the "core" muroids, among the five cricetid (hamster) subfamilies, within a large clade of Sigmodontinae endemic to South America, and among major geographic lineages of Old World Murinae. Because of the detailed taxon sampling within the Murinae, we are able to refine the fossil calibration of a rate-smoothed molecular clock and apply this clock to date key events in muroid evolution. We calculate rate differences among the gene regions and relate those differences to relative contribution of each gene to the support for various nodes. The among-gene variance in support is greatest for the shortest branches. We present a revised classification for this largest but most unsettled mammalian superfamily. [Adaptive radiation; calibration; classification; molecular clock; Murinae; Sigmodontinae.]

The Mammalia comprise at least 146 families in 27 orders, yet a single family, the Muridae (the sole member of the superfamily Muroidea; Musser and Carleton, 1993), represents nearly one third of its total species diversity (Musser and Carleton, 1993). Its species are distributed on every major landmass in the world except Antarctica and New Zealand and include many of the most ecologically abundant and taxonomically diverse mammals. Phylogenetic knowledge of the group is critical to many nonevolutionary studies, particular physiology, genomics, immunology, and oncology, because most biomedical research uses model organisms belonging to the Muroidea. Increasingly, researchers are expanding studies beyond the mouse (*Mus*) and rat (*Rattus*), for the purpose of tracing the evolution of key traits. A robust phylogeny is crucial to interpreting current synthetic studies as well as identifying species and clades for future studies. Additionally, wild muroid species are the natural hosts or vectors of many human pathogens, including hantaviruses, arenaviruses, and the plague, and some evidence points to cospeciation between virus and rodent lineages (Mills et al., 1997; Hughes and Friedman, 2000). Because of their taxonomic diversity and broad geographic distribution, murids are involved in many biogeographic debates, including those over the Great American Interchange and the timing of their entry into South America (Hershkovitz, 1972; Savage, 1974; Simpson, 1980; Baskin, 1986), Holarctic interchanges (Conroy and Cook, 2000), and African connections (Jansa et al., 1999). Furthermore, there is intrinsic interest in understanding why this group is so much more diverse than any comparable mammalian clade, and more broadly, has sustained one of the highest net speciation rates among land vertebrates.

The phylogeny of muroids has been one of the most intractable problems in mammalogy, perhaps because their presumably rapid radiation left little opportunity for the evolution of unique synapomorphies and because morphological systematists had to rely largely on dental characters, which are particularly prone to adaptive convergence among rodents. Systematists have generally agreed on the composition of the subfamilies and to a certain extent on the number of the subfamilies, between 16 and 20. Although many of these subfamilies have been elevated to family status by some authors, the key taxa composing them have remained largely the same, regardless of taxonomic rank within the Muroidea.

The taxonomic, ecological, physiological, and morphological diversity of the Muroidea is apparent even from a brief overview of the group. Body sizes span over two orders of magnitude, from less than 10 g (*Baiomys*) to more than 2500 g (giant African pouched rat, *Cricetomys;* the maned rat *Lophiomys* and muskrat *Ondatra* are nearly as large), exceeding the range seen in any other mammalian family. The Old World mice and rats (Murinae) are the largest mammalian subfamily, comprising over 500 species (Musser and Carleton, 1993). Recent debate has focused on whether or not the spiny mice, *Acomys* and its relatives (that share with Murinae the derived molar pattern of three rows of cusps), are members of the Murinae. Possibly related to the Murinae are the Gerbillinae, the gerbils and jirds, primarily

bipedal, hopping species that live in African and Asian deserts.

The second-largest mammalian subfamily is the Sigmodontinae. Conventionally it includes all the New World mice, about 450 species, but some authors divide the Sigmodontinae s.l. into two or three subfamilies, most importantly distinguishing the Neotropical (and predominantly South American) Sigmodontinae s.s. (>300 species) from the almost exclusively North American Neotominae. These groups are morphologically and ecologically diverse, and display a wide array of diets, including fish and crustaceans. Familiar species include the deer mice (*Peromyscus*), wood rat (*Neotoma*), cotton rat (*Sigmodon*), and leaf-eared mouse (*Phyllotis*). They are often grouped with the Old World hamsters (Cricetinae) and sometimes the voles, lemmings, and muskrat (Arvicolinae). The Arvicolinae are a diverse Holarctic group (>125 species), many of whose members have been the subjects of physiological, ecological, and behavioral studies, particularly in relation to population cycles (Elton, 1942).

The remaining subfamilies are considerably less speciose but include many of the most specialized forms. The Dendromurinae (climbing mice) of sub-Saharan Africa are the largest of the remaining subfamilies, comprising about 20 species. Other African forms include the generally large-bodied pouched rats (Cricetomyinae), the diurnal herbivores in the Otomyinae (e.g., the whistling rat, *Parotomys*), and the rock rats (Petromyscinae). Of particular interest are a group of eight genera endemic to the island of Madagascar, the Nesomyinae, whose morphologies are so diverse that each has been separated into its own subfamily in some classifications. Monophyly of the Nesomyinae has been challenged on morphological and molecular grounds (Jansa et al., 1999).

Some of the most extreme morphologies can be found in the fossorial mole rats and bamboo rats in the Rhizomyinae, a group including both African and Indian species. Some debate has arisen over whether they are related to the blind mole rats in *Spalax* (Spalacinae), a western Asian lineage whose eyes, completely covered by fur, have undergone fascinating patterns of molecular evolution in lens-crystallin and visual-pigment genes (Hendriks et al., 1987).

No comprehensive morphological cladistic study of the Muroidea has included a broad sampling of all the constituent subfamilies. Morphological cladistic studies have been infrequent within subfamilies or among closely related groups and number only four (Carleton, 1980; Denys and Michaux, 1992; Denys et al., 1995; Steppan, 1995). The core of muroid systematics has been developed by paleontologists who have tackled broad-scale treatments more frequently than have neontologists. Neontologists have been dissuaded by the enormous number of taxa and by the high frequency of morphological convergence combined with a relatively low number of unique synapomorphies (Musser and Carleton, 1993). Because of the reliance on paleontological studies, murid systematics is largely based on dental characters (few extinct murid species are represented by anything else). In his influential classification, Simpson (1945) grouped the Muroidea into four families; the two fossorial groups Spalacidae and Rhizomyidae (which he did not think were closely related) included relatively few species, and the bulk of the diversity was split between the Cricetidae and the Muridae. Many paleontologists have considered the Cricetidae to be ancestral to the Muridae. Carleton and Musser (1984) highlighted the ground breaking comprehensive treatment by Chaline et al. (1977), who divided Muroidea into eight families (Table 1).

TABLE 1. Past systematic treatments for subfamilies now considered part of the Muroidea (Musser and Carleton, 1993).

Simpson, 1945	Chaline et al., 1977	Lavocat, 1978
Cricetidae	Cricetidae	Cricetodontidae
Sigmodontinae s.l.	Sigmodontinae s.l.	Afrocricetodontinae
Cricetinae	Cricetinae/Calomyscinae	Nesomyidae
Arvicolinae	Spalacinae	Nesomyinae
Lophiomyinae	Myospalacinae	Lophiomyinae
Nesomyinae	Lophiomyinae	Mystromyinae
Myospalcainae	Platacanthomyinae	Tachyoryctinae
Muridae	Nesomyidae	Gerbillinae
Murinae (incl. Cricetomyinae)	Nesomyinae	Otomyinae
Dendromurinae (incl. Petromyscinae)	Otomyinae	Muridae
Otomyinae	*Mystromys*?	Murinae
Spalacidae	Rhizomyidae	Dendromurinae
Spalcinae	Gerbillidae	
Rhizomyidae	Arvicolidae	
Rhizomyinae	Dendromuridae	
Platacanthomyidae (related to Myoxidae)	Dendromurinae	
	Petromyscinae	
	Cricetomyidae	
	Muridae	
	Murinae	
	Hydromyinae	

Note: Lavocat (1978) included only African groups.

Molecular studies followed, but slowly. Hänni et al. (1995) concluded from the mitochondrial 12S gene not only that *Acomys* and related genera were not murines but that the Cricetinae were closer to the Murinae and that the Gerbillinae were the outgroup to the Murinae + Cricetinae + "acomyines." This conclusion conflicted with immunological distance data (Sarich, 1985) and DNA-DNA hybridization (Chevret et al., 1993a), which indicated that "acomyines" (= deomyines, see below) were more closely related to gerbillines than to murines. Michaux and Catzeflis (2000) used the nuclear gene LCAT to provide the first phylogenetic analysis of the Muroidea based on broad sampling. They found that the mole-rat subfamilies (Spalacinae and Rhizomyinae) were the sister group to all other muroids, but they were unable to resolve the branching pattern among most of the remaining subfamilies and speculated that robust resolution may never be achieved. In the following year, the addition of vWF exon 28 data resolved four clades: Calomyscinae, an African radiation (Nesomyinae, Mystromyinae, Dendromurinae, Cricetomyinae), a cricetine group (Cricetinae, Arvicolinae, Myospalacinae, and Sigmodontinae s.l. as represented by Neotominae,), and a murine group (Murinae, Otomyinae, Gerbillinae, Deomyinae) (Michaux et al., 2001). Sampling within subfamilies was limited, however.

Objectives

Here we present the most comprehensive phylogenetic study of the Muroidea to date in terms of both the number of taxa and the number of sampled nucleotides per species. We sample representatives of 16 out of the possible 20 subfamily-level taxa (13 out of 17 under Musser and Carleton's [1993] treatment). The only missing subfamilies are the rare or depauperate Lophiomyinae (maned rat), Platacanthomyinae (Malabar spiny mouse and blind tree mouse), Myospalacinae (fossorial zokors), and Mystromyinae (mouse-like hamster). Our 53 genera are distributed such that we have multiple representatives from 12 subfamilies (5 muroid subfamilies are monotypic). The large size of Muroidea and historical difficulties in defining relationships indicate that a large amount of data will be required for robust resolution. We sequenced four large unlinked nuclear exons, growth hormone receptor (GHR), breast cancer gene 1 (BRCA1), recombination activating gene 1 (RAG1), and the proto-oncogene c-myc, for a total of approximately 6400 aligned sites. In light of the difficulties researchers have encountered in defining robust clades with mitochondrial sequences (Hänni et al., 1995; Engel et al., 1998; Jansa et al., 1999; Smith and Patton, 1999) and the high rates of molecular evolution (Wu and Li, 1985; Adkins et al., 2001) within this group, we chose slowly evolving genes to minimize homoplasy. Indeed, the high evolutionary rates in muroids make alignment of introns across subfamilies unreliable or impossible.

Our taxonomic and character sampling allow us to address for the first time four key issues: (1) test for monophyly of the polytypic subfamilies, (2) resolve relationships among subfamilies, (3) identify rapid radiations, and (4) revise the fossil calibrated dating scheme. We concentrated our sampling in the two most speciose groups, the Murinae and Sigmodontinae, so for the first time, we can explore the evolution of these major radiations within the broader phylogenetic context. In particular, using the finer-scale sampling within the Murinae, we reevaluate the phylogenetic placement of the most commonly cited muroid fossil calibration (Jacobs and Downs, 1994) and apply molecular approaches to date key events in muroid evolution and biogeographic history.

Phylogenetic Hypotheses Tested

In addition to estimating optimal phylogenies, we adopted a hypothesis testing framework (Huelsenbeck and Rannala, 1997; Steppan and Sullivan, 2000) and tested a suite of a priori hypotheses that have been proposed in the past. These hypotheses are summarized in Table 3 and include monophyly of each polytypic subfamily (hypotheses 2 to 13), paleontological higher taxa (14 to 29, 33), and molecular-based hypotheses (20–32, 34).

Here, we follow the taxonomy of Musser and Carleton (1993) with the following exceptions. *Acomys* and its relatives are removed from the Murinae into the related "acomyine group" (Denys and Michaux, 1992; Hänni et al., 1995), a clade that also includes *Deomys*, a genus historically associated with the Dendromurinae. Several authors identifying this group as monophyletic have used the name Acomyinae (Dubois et al., 1999; Michaux and Catzeflis, 2000; Michaux et al., 2001) without diagnosing it, making it a nomina nuda (Musser and Carleton, in press). The oldest available name for this group is Deomyinae (Musser and Carleton, in press). We also follow the recommendations of Reig (1980, 1984), Slaughter and Ubelaker (1984), Steppan (1995), and D'Elía (2000) in separating the Neotominae and Tylomyinae from the Sigmodontinae.

Materials and Methods

Specimens and Genes Sequenced

We included 53 genera representing 13 subfamilies according to Musser and Carleton (1993). Specimen identification and locality information are listed in Appendix 1, GenBank accession numbers in Appendix 2. Debate continues over whether a well-resolved phylogeny that is both accurate and strongly supported is best arrived at through an increase in the number of nucleotides sampled in a limited subset of species (more sequence) or through sampling of a smaller number of nucleotides in a larger representation of species (more species, Hillis et al., 2003; Rosenberg and Kumar, 2003). The sequencing strategy we used struck a balance between full gene and species representation and the complete exclusion of some species. GHR was chosen to have the most complete representation among species for several reasons: intermediate rate of substitution (see results), ease of

amplification and sequencing across species with a common set of primers, and proven usefulness for rodent systematics (Adkins et al., 2001, 2003). The remaining genes have also proven useful for rodent systematics (Adkins et al., 2001, 2003; DeBry, 2001; Steppan et al., 2004) but were sequenced in a smaller set of species. The reduced set of species sequenced for RAG1, BRCA1, and c-myc was selected with the intention to represent exemplars from each of the major subclades.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from liver or muscle by PCI (phenol/cholorform/isopropanol)/CI (chloroform/isopropanol) "hot" extraction (Sambrook et al., 1989). Exon 10 of GHR, exon 11 of BRCA1, the single exon of RAG1, intron 2/exon 3 of c-myc (3′ region of intron 2, and entire translated region/partial untranslated region 3′ UTR) were amplified with primers and under reaction conditions described previously (Adkins et al., 2001; Steppan et al., 2004). Amplification and sequencing strategy for c-myc and RAG1 followed Steppan et al. (2004), except that the 3′ 800 bp were amplified and sequenced in addition to the 2200 bp sequenced previously for RAG1. Primers S91 and S92 were used to amplify c-myc intron2-exon 3. RAG1 was amplified in three overlapping fragments with primer pairs S70/S73 and S77/S71 (Steppan et al., 2004) and S78/S69. This latter fragment was sequenced with the amplification primers S78 (GAGACCCTTACTGCTATTCTAA), S69 (TTCCATTGAATCTTGGCTTTCCA), and the internal primers S121 (ATGAGGATGAATGGCAACTT) and S123 (TGCDTCTACAGTCTCTTGGGTCA).

Reagent concentrations varied with primer sets for each gene region in amount of $MgCl_2$ (2 mM for c-myc and RAG1; 4 mM for GHR and BRCA1). Cycling conditions were also different for different genes; annealing temp was 60°C for c-myc, 51°C for RAG1, 45–50°C for GHR, and 50–55°C for BRCA1. DNA from nesomyine species was extracted from small skin biopsies (3 to 4 mm^3) according to the same protocols as for tissues. Because of the small amount of template DNA for nesomyines, 40 cycles of PCR rather than 30 were performed with a low annealing temperature (45°C).

Negative (no DNA) controls were included with every reaction to reveal instances of DNA contamination of reagents. PCR products were visualized on an agarose gel with ethidium bromide, and successful amplifications were isolated from a low-melting-point gel with Wizard PCR prep reagents (Promega) or precipitated directly with polyethylene glycol (PEG). Both strands of each PCR product were completely sequenced with PCR primers and an arrangement of internal primers (available from the authors upon request) that varied depending on the species by automated DNA sequencing on an ABI 377 or ABI 3100 machine using big-dye terminator chemistry (Applied Biosystems).

Results of individual sequencing runs for each species were combined into contiguous sequences with AssemblyLign (Oxford Molecular) or Sequencher (GeneCodes), and regions of ambiguity or disagreement resolved through manual inspection of sequence traces. Initial multiple alignments of sequences across species were performed with ClustalX (Thompson et al., 1997); manual refinement consolidated indels and brought indels into the coding frame. c-myc intron 2 and 3′ UTR sequences (approximately 200 bp each) could not be aligned reliably across muroid subfamilies and were excluded from the current analyses. Maximum aligned sequence lengths were 940 bp (GHR), 1811 bp (BRCA1), 3077 bp (RAG1), and 570 bp (c-myc). Parsimony-informative indels were coded as presence/absence characters regardless of length and appended to the data sets. Sequences for the genes were concatenated for each taxon.

Phylogenetic Analyses

Heterogeneity of nucleotide composition among informative sites was determined using PAUP* version 4.0b10 (Swofford, 2002) for all taxa, Muroidea and Dipodoidea only, and Muroidea only. Phylogenetic analyses were conducted for each gene separately under maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian approaches with the programs PAUP* version 4.0b10 (Swofford, 2002) and MrBayes V3.0 (Huelsenbeck and Ronquist, 2003). All MP analyses used heuristic searches with tree bisection-reconnection (TBR) branch swapping and 30 random-addition replicates. All transformations were weighted equally, including indels. A sequential optimization approach (Swofford et al., 1996; Fratti et al., 1997) was used to estimate the ML phylogeny. Initial trees were generated under MP. ML parameter values were estimated under a nested array of substitution models for the MP trees as implemented in Modeltest 3.04 (Posada and Crandall, 1998), with parameters for nucleotide substitution rates and among-site rate variation; a portion of the sites were assumed to be invariable (I), and rates among all sites were assumed to vary according to a gamma distribution (Γ; Yang, 1994). Likelihood-ratio tests were used to identify the simplest models of sequence evolution that adequately fit the data and phylogeny (Yang et al., 1995). Models and parameters are summarized in Table 2. A ML search was then conducted under the preferred model with parameters fixed to the values estimated on the MP tree. Heuristic searches were conducted with 10 (total data) to 30 (individual genes) random-addition replicates and TBR branch swapping. Model parameters were reestimated from the initial ML tree and the process repeated until the topology remained constant. The optimal phylogeny was always found on the first search.

Nonparametric bootstrapping (Felsenstein, 1985) was performed on all data partitions (200 replicates for ML total data set, 100 replicates for ML individual genes, 500 replicates MP). Bootstrap analyses using likelihood were limited to 4,000 rearrangements. Restricting the number of rearrangements reduces the chances that the optimal tree will be found for each replicate but is a conservative procedure more likely to reduce bootstrap values

TABLE 2. Comparison of estimated transformational properties for the four sequenced genes. Ti/tv; transition ratio. Transition rates estimated with likelihood under a General Time Reversible model. I; estimated proportion of invariant sites. Γ; shape parameter for among-site rate variation under a gamma distribution with four rate categories, estimated with and without invariant sites in the model. Preferred model from nested likelihood-ratio tests and, where different, Akaike Information Criterion.

	Ti/tv	A-C	A-G	A-T	C-G	C-T	G-T	I	Γ (I)	Γ (NO I)	Preferred model (AIC)
GHR	2.34	1.01	4.74	0.72	1.31	4.74	1	0.12	1	0.73	HKY+Γ (TVM+I+Γ)
RAG1	3.12	1.52	5.11	0.6	1.03	8.11	1	0.4	1.15	0.37	GTR+I+Γ
BRCA1	1.97	1.33	3.5	0.7	0.95	4.49	1	0.02	2.56	2.26	GTR+Γ
c-myc	2.89	0.81	4.17	0.78	0.57	4.73	1	0.48	0.83	0.23	HKY+I+Γ

than to inflate them (Steppan et al., 2004). The ML bootstrapping was performed with PAUP* on a 36-processor cluster.

Analyses were performed on individual genes and on a concatenation. A partition-homogeneity test (200 replicates) on the set of taxa represented by all four genes indicated slight, but nonsignificant ($P = 0.07$), heterogeneity in signal among genes. Each gene contained parsimony-informative indels, all corresponding to whole codons. The recoded presence/absence characters for indels were excluded from the ML and Bayesian analyses.

Bayesian analysis of the total data set used the GTR+I+Γ model as in the likelihood analyses with the addition of partitioning by codon position. Parameters were estimated for each position separately ("unlinked"). Five independent analyses of four chains were run for 4, 4, 4, 7, and 10 million generations, respectively; trees and parameters were recorded every 100 generations. Most runs used the default heating and swap parameters, but one of the 4 million generation runs was "hot" to explore parameter space more fully: temp = 0.5, five chains, swapfreq = 2. Partition frequencies were examined in 200,000 generation bins from the 7 million generation run. All but a few nodes were very stable. The only nodes that varied significantly were those within a poorly resolved region of the sigmodontines, and in particular involving the only pair of sigmodontines, *Oryzomys* and *Irenomys*, that had no characters in common. Averaged over several million generations, all Bayesian analyses including the "hot" run yielded very similar results. Although stable partition frequencies and overall likelihood were achieved by generation 200,000 (with the sigmodontine exception), we excluded the first 5 million generations as the "burn-in" period.

Hypothesis Testing

A priori hypotheses were tested with parsimony- and likelihood-based approaches. Tree searches were conducted with constraints enforced to match predicted topologies for each hypothesis. Differences in tree scores between all equally optimal trees from constrained searches and the optimal trees overall were subjected to the Templeton's (Templeton, 1987) and Kishino-Hasegawa (Kishino and Hasegawa, 1989) tests under parsimony and a one-tailed Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa, 1999) test with restricted likelihood as implemented in PAUP* 4.0b10 (Swofford, 2002). Bonferroni's correction for multiple tests was applied to parsimony analyses because unlike the SH test they have no correction for multiple comparisons.

Divergence Date Estimation

The maximum-likelihood phylogeny estimated from the full set of sequences was pruned of those taxa represented by fewer than three gene regions, and this topology was used as the basis for divergence-date estimation. The adherence of this data set and topology to a global molecular clock was determined by a likelihood-ratio test comparing the likelihoods (GTR+I+Γ) assuming a molecular clock and assuming no constraints upon rates of evolution.

Estimation of divergence dates was restricted to those species represented by three or four gene regions for two reasons. First, we use bootstrap resampling to measure how consistently the data support the estimated date at each node. Use of the full set of taxa would cause some taxa to be represented primarily by missing sites in some of the resampled data sets, and those branches would have misleadingly variable lengths that could artificially inflate the variance in estimated divergence dates. Second, individual genes may exhibit taxon-specific rate variation that could distort branch lengths or result in systematic errors. For comparison and to allow estimation of divergence dates for nodes not represented by three or more gene regions, divergence dates were also estimated for all muroid taxa represented by GHR sequences.

Divergence dates were estimated with the program r8s (version 1.5) (Sanderson, 2002) by three different methods. (1) A uniform rate of DNA substitution (option LF) was assumed across the entire phylogeny. (2) The method of nonparametric rate smoothing (option NPRS) permits a different rate of substitution on each branch, while minimizing the sum of squared differences in estimates of substitution rates across adjacent branches. (3) Penalized likelihood (option PL) is an intermediate model between LF and NPRS that maximizes the log likelihood of a model with different rates on each branch minus a roughness penalty that costs the model more as the magnitude of the changes in rates increases. The relative contribution of the two components (log likelihood and roughness penalty) is determined by a smoothing parameter. The model becomes more clocklike as the smoothing parameter increases. The optimal smoothing parameter

was chosen via a cross-validation method (Sanderson, 2002). Initially smoothing values ranging from 10^0 to 10^3, with the exponent incremented by 0.25 were evaluated. This was followed by a cross validation search with 0.1 increments of the exponent around the optimal value of the first search to determine the smoothing parameter used in the estimation of divergence dates.

Under the same fixed topology and values of the GTR+I+Γ maximum-likelihood model, sites were bootstrap resampled 100 times and branch lengths recalculated. The outgroups (*Graphiurus*, *Aplodontia*, *Sciurus*, and *Glaucomys*) were pruned from each tree and divergence dates estimated with the clock calibrated by the first occurrence of a murine with modern dentition at 12 Mya (Jacobs and Downs, 1994) and the origin of Myodonta constrained between 50 and 70 Mya (Flynn et al., 1985). We assigned the 12-Mya date (Jacobs and Downs, 1994) to the most recent common ancestor (MRCA) of all murines, not to the MRCA of *Mus* and *Rattus*. (See Discussion for more on the justification and consequences of this interpretation.) Additional calibration points were considered, but only this one has a clear series of transitional fossils bracketing the evolution of a defining apomorphy, allowing it to be assigned much more precisely to a single node. The minimum age of the myodont clade can be assigned with some confidence. Although the maximum age cannot be definitively assigned, the age used here exceeds the age of the oldest known member of the Muroidea or Dipodoidea by at least 15 My and probably includes the true age. The mean and standard deviation of the age estimate at each node were calculated by the r8s program.

RESULTS

Properties of Genes

All four genes exhibit similar patterns of nucleotide transformation rates (Table 2). When a HKY model of evolution is applied, the transition/transversion ratio ranges from approximately 2 in BRCA1 to approximately 3 in RAG1. The most complex rate model is preferred for RAG1 and BRCA1, whereas simpler models (HKY and TVM) appear appropriate for GHR and c-myc. The patterns of among-site rate variation are similar in GHR, c-myc, and RAG1; 12% to 48% of sites are invariant (c-myc and RAG1 show the strongest constraint) and the alpha shape parameter is approximately 1 for the variable sites. BRCA1 stands apart from the others in its much more uniform pattern of among-site rate variation. Only 2% of sites are estimated to be invariant (when estimated in addition to gamma-distributed rates), and the shape parameter is more uniform, whether estimated with invariant sites or not. This pattern appears to reflect greatly relaxed constraint on the protein structure, an observation that is also reflected in the higher rates of insertions and deletions than in the other genes (see Indels section). Alignments for each gene are available from TreeBase accession number SN1719.

There was no significant heterogeneity in nucleotide composition within Muroidea ($P > 0.5$). Although there was significant heterogeneity when outgroups were included ($P = 0.012$ Myodonta, $P < 0.0003$ all taxa), most of that heterogeneity was attributable to two taxa, *Allactaga* and *Pedetes* and restricted to BRCA1. Because those taxa are well removed from the ingroup, no further corrections for nucleotide bias were made.

Phylogenetic Analyses

The single ML tree is shown in Figure 1. The sister group to the Muroidea appears to be the Dipodoidea (the largely bipedal or saltatorial jumping mice and jerboas), forming the Myodonta. Within the muroids, every subfamily with multiple representatives is monophyletic except for the Murinae and possibly the Cricetomyinae, as discussed below. A deep division separates the two major clades within the Muroidea: the mole rats (Spalacinae and Rhizomyinae) and all remaining subfamilies ("Muroidea s.s."). The Rhizomyinae are monophyletic, including both Asian (*Rhizomys*) and African (*Tachyoryctes*) genera. The mole rat subfamilies appear to have diverged well after their split from the Muroidea s.s. Likewise, the modern subfamilies of the Muroidea s.s. did not start diversifying until well after their split from the mole rats.

Phylogenetic analyses of individual genes produced congruent phylogenies with the exception of rearrangements around five key regions of the tree. These five key regions are (as labeled on Fig. 1) (I) the basal nodes among Muroidea s.s. (excluding Rhizomyinae and Spalacinae); (II) relationships among core "cricetid" clades (Cricetinae/Arvicolinae/Sigmodontinae); (III) relationships among major lineages of Sigmodontinae s.s., excluding *Sigmodon*; (IV) relationships among major lineages of Murinae; and (V) relationships among the three genera of Deomyinae. Each pairwise comparison of gene trees exhibits conflict in three to four of these key regions except for RAG1 and c-myc, which differ only in one minor aspect within the Murinae. No conflicts are apparent elsewhere among the trees. Notably, the regions of conflict do not involve well-supported nodes in otherwise robust topologies. Only one of the 20 regions of pairwise conflict (an average of three regions of conflict for each of the six pairwise gene comparisons) is moderately well supported by parsimony bootstraps in excess of 70% for both genes. This one region involves the branching pattern among the major lineages of Murinae (excluding *Batomys*). GHR places the *Mus-Praomys* clade basal to all other core murines with bootstrap support for intervening nodes of 72% and 42%, whereas c-myc places an African clade (*Arvicanthis-Parotomys*) basally with support for intervening nodes at 68% and 71% MP bootstrap (only 66% and 58% ML). In only one other case do both conflicting sets of nodes have MP bootstrap percentages even above 50%. Therefore, no meaningful conflict appears among the four genes because all topological conflict is limited to nodes that are poorly supported in either one or both genes.

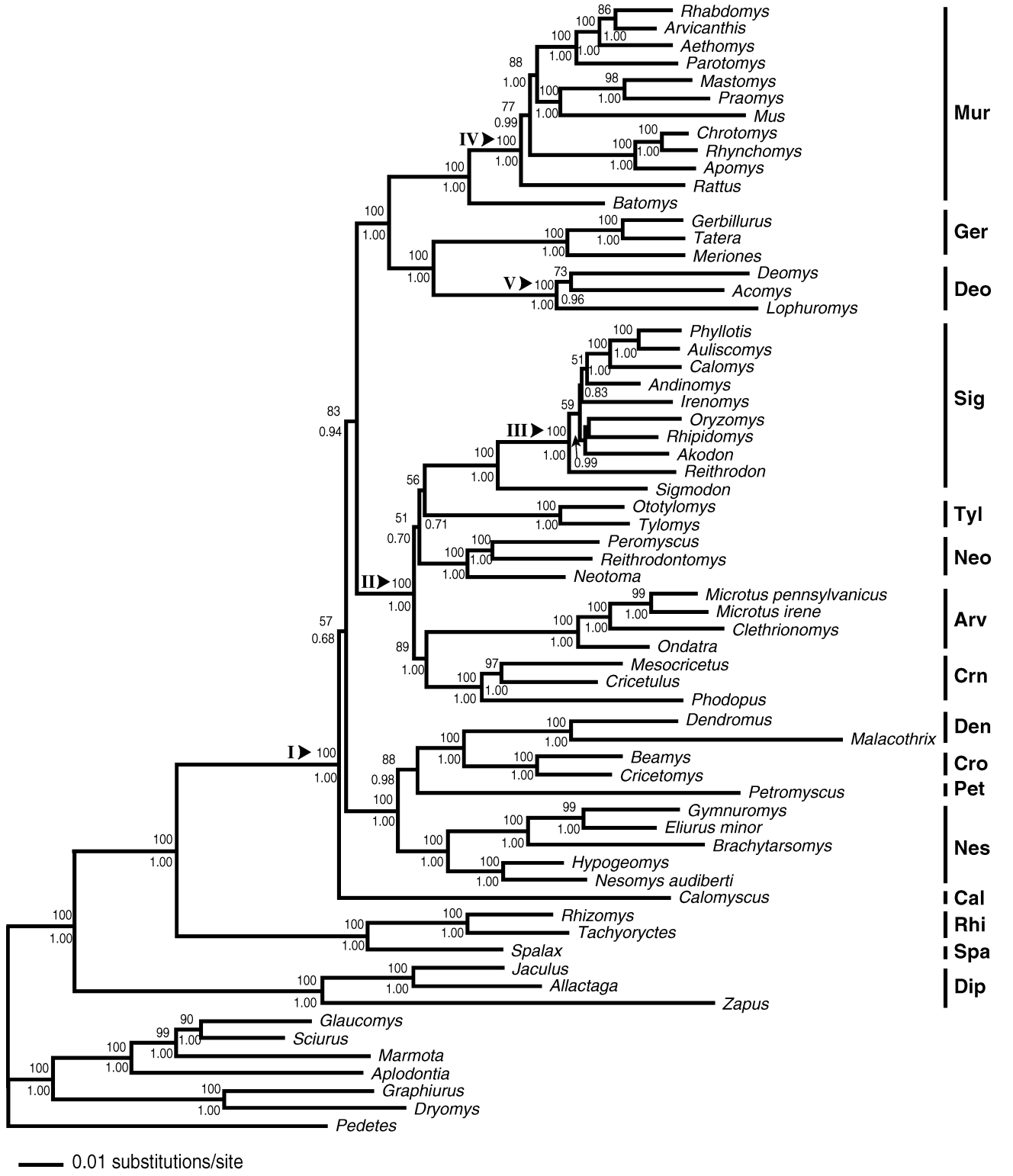

FIGURE 1. Maximum-likelihood phylogram for the Muroidea under the GTR+I+Γ model. Numbers above branches refer to ML bootstrap percentages. Numbers below branches refer to Bayesian posterior probabilities (greater than 0.50). Clade names are abbreviations from the first three letters of the subfamily or family names (except Crn = Cricetinae; Cro = Cricetomyinae). Dipodidae and unlabeled taxa are outgroups. Note the longer branch lengths among the Myodonta. Arrows indicate regions of conflict and poor resolution discussed in the text.

Within the Muroidea s.s. there appear to be four major regions of the total-data phylogeny where a rapid radiation led to short branch lengths and the absence of robust resolution of the branching order. These are the same regions that exhibit conflict among the individual gene trees. The first of these poorly resolved regions is the basal region of the Muroidea s.s., including four major lineages (node I). These are the Calomyscinae, an African clade (Nesomyinae, Petromyscinae, Dendromurinae, Cricetomyinae), a "cricetid group" (Cricetinae, Arvicolinae, Sigmodontinae, Neotominae, Tylomyinae), and a "murid group" (Murinae, Deomyinae, Gerbillinae). The Calomyscinae are sister to the other three clades, but support for this relationship is weak (57% ML bootstrap, 0.68 posterior probability "pp"). Within this larger clade, three of the four individual gene trees (not BRCA1) support the grouping of the "cricetid group" with the "murid group" to the exclusion of the African clade, despite very short internal branches. The grouping of the two former clades is moderately to well supported; individual-gene ML bootstrap percentages range from 56% (GHR) to 94% (RAG1), and the total data set shows 83% (ML) to 84% (MP) bootstrap and 1.00 pp.

We refer to the first of the three major clades as the African group because all included taxa are endemic to Africa or Madagascar. Monophyly of this clade is well supported; individual gene bootstraps range from 69% (c-myc) to 99/100% (GHR/BRCA1). Support for monophyly is strong from the total data set (100% MP and ML bootstrap, 1.00 pp). The Malagasy endemic Nesomyinae are monophyletic (100% bootstrap, 1.00 pp) and the sister group to the remaining subfamilies (89% ML bootstrap, 0.98 pp).

The second major clade is the cricetid group. The cricetid group is well supported as a clade; individual gene ML bootstraps range from 55% to 99%, the total-data bootstrap is 100%, and 1.00 pp. Branching sequence among the five constituent subfamilies (Cricetinae, Arvicolinae, Neotominae, Sigmodontinae, Tylomyinae) is poorly resolved and represents the second region of conflict among the individual gene trees (node II). All four genes do agree on the unrooted network within this clade and differ only in how it is rooted with respect to other muroids. The total-data analysis yields an endemic New World clade (Neotominae, Tylomyinae, Sigmodontinae) and a hamster-vole clade (Cricetinae, Arvicolinae) but with weak support (44% MP, 51% ML bootstrap, 0.82 pp). Among the three New World groups, no clear resolution emerges.

Within Sigmodontinae are two major lineages, the cotton rat *Sigmodon* (widespread in North and South America) and all other groups, most of which are endemic to South America or have their greatest diversity there. Support for this fundamental division is strong: 74% to 100% bootstrap within genes and 100% bootstrap, 1.00 pp among genes. However, relationships among the seven sampled tribes or "unique lineages" (sensu Smith and Patton, 1999) is effectively unresolved as this is the least resolved region of the tree with the shortest branches (node III) and only one node has greater than 50% ML bootstrap support. Bayesian posterior probabilities appeared to cycle between tree islands involving *Oryzomys* and *Irenomys* (that shared no characters in common) with 2 to 3 million generation periodicity. This provides evidence that in some cases, short Bayesian runs of less than a few million generations may not properly characterize parameter space. The nodes that varied in Bayesian analysis also had very low bootstrap frequencies and by either method appear poorly supported.

The third major lineage includes the largest subfamily, the Murinae (Old World mice and rats); the historically associated Deomyinae (spiny mice); and the Gerbillinae (gerbils). All four genes robustly support the sister-group relationship of the Deomyinae with the Gerbillinae. Relationships within these three subfamilies are also well supported with two exceptions, which are the third and fourth regions of conflict among gene trees. The first of these is the base of the Deomyinae (node V), where all three possible resolutions are represented among the individual genes. The resolution from the total data is predictably weak; *Lophuromys* is the outgroup and only 58% MP and 73% ML bootstrap (but 0.96 pp).

Monophyly of the Murinae is strongly supported by all genes. Every gene also agrees on the placement of the Philippine endemic rat *Batomys* as the sister group to all other murines, including the other Philippine genera (*Apomys, Chrotomys, Rhynchomys*). This latter clade represents the fourth region of conflict (node IV) where internal branches are very short. Four well-supported clades (76% to 100% bootstrap, depending on clade and gene, 1.00 pp) can be detected within the core murine clade given our taxonomic sampling; the Asian *Rattus*, the Philippine endemic clade, a clade that includes the Asian *Mus* and African *Mastomys* and *Praomys*, and an African clade that includes the subfamily Otomyinae as represented by *Parotomys*.

Indels.—Twenty-four indels that were parsimony informative regarding muroid relationships were found among the three genes, as described above. Seventeen of those indels were perfectly congruent with the optimal tree (mapped onto Fig. 2) and seven were homoplasious. All homoplasious indels involved variation in the number of repeated amino acids or repeated pairs of amino acids. Indels support several clades that have been debated recently including the murid group (Murinae-Gerbillinae-Deomyinae; R3), Gerbillinae-Deomyinae (G3, B15), the Murinae (B7, B12, B16), the Deomyinae (G5, B11), the cricetid group (G4, G5), the Sigmodontinae s.s. (G2, B2), and the Sigmodontinae less *Sigmodon* (R2). Most of the indels are single amino acids (67%).

Three of the four indels longer than three residues deserve particular attention because three support major clades. The grouping of the predominantly South American Sigmodontinae with the North American Neotominae is supported by a four-residue duplication (R1) near the beginning of RAG1 (RAG1 data

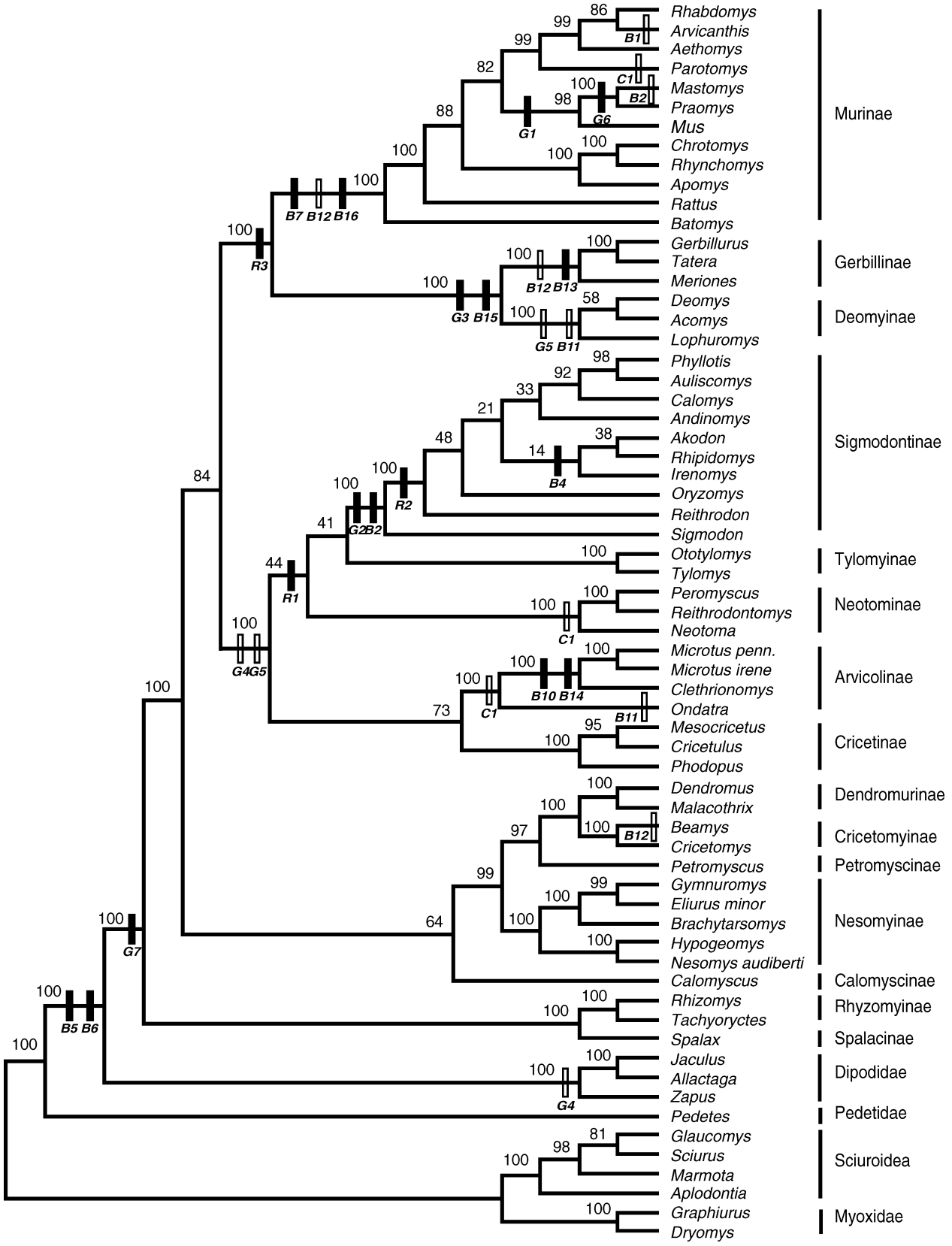

FIGURE 2. Maximum-parsimony phylogeny for the Muroidea with the insertions and deletions mapped. Closed bars indicate unique indels; open bars indicate homoplasious indels. Indels are numbered by relative sequence on each gene (G = GHR; B = BRCA1; R = RAG1; C = c-myc), e.g., G1 = first parsimony-informative indel in GHR. Numbers above branches are bootstrap percentages.

not available for Tylomyinae). A six-residue deletion in BRCA1 (B2) is shared by all sigmodontines. A five-residue deletion in BRCA1 (B15) is shared by the Gerbillinae and Deomyinae.

Hypotheses Tested

We tested 34 a priori hypotheses derived from the literature, summarized in Table 3. Monophyly of all subfamilies, as defined for this study, was supported. In all but four subfamilies, alternate topologies wherein they were not monophyletic could be rejected under either one or both optimality criteria at the 0.05 level (after Bonferroni correction to 0.0015 for Kishino-Hasegawa and Templeton's test). These four subfamilies without strong statistical support are the Rhizomyinae (MP $P = 0.04$, ML $P = 0.64$), Dendromurinae (MP $P = 0.0075$, ML $P = 0.549$), Tylomyinae (MP and ML $P > 0.5$), and Neotominae (MP and ML $P > 0.5$).

Every paleontological higher taxon can be rejected on the basis of these data. In many cases, highly significant *P*-values result from "misplacement" of only one

TABLE 3. Topological tests of a priori hypotheses. The optimal tree had a parsimony score of 9659 steps and log likelihood of −58,008.333. Δ Steps and Δ Likelihood are differences between the optimal and constrained phylogenies. When multiple equally parsimonious trees exist, the largest *P*-value is reported. Names and differences in optimality criteria in parentheses indicate hypotheses that were present in the optimal trees and for which the inverse constraint (clade not present in constrained search) was employed. Asterisk indicates significance at 0.05 level for likelihood or with Bonferonni correction to 0.0014 for parsimony. The hypotheses are: (1) monophyly of the Muridae; (2–13) monophyly of the 11 out of 16 polytypic subfamilies for which we have multiple lineages represented; (14) monophyly of Simpson's (1945) Cricetidae modified to exclude the Otomyinae (e.g., Michaux et al., 2001); (15) monophyly of Simpson's (1945) Cricetidae; (16) monophyly of Chaline et al.'s (1977) Cricetinae, which include *Calomyscus*; (17) monophyly of Chaline et al.'s (1977) Cricetidae, which also include the Sigmodontinae s.l., Lophiomyinae, Spalacinae, Myospalacinae, and Platacanthomyinae; (18) monophyly of Chaline et al.'s (1977) Dendromuridae; (19) monophyly of Lavocat's (1978) Nesomyidae; (20) exclusion of the Otomyinae from the Murinae (hypothesis 13 includes otomyines within the Murinae; Michaux et al., 2001); (21) sister-group status of Deomyinae+Murinae; (22) Deomyinae, Gerbillinae, and Murinae are a clade (Michaux and Catzeflis, 2000); (23) Petromyscinae+Nesomyinae are a clade; (24) Dendromurinae+Cricetomyinae are a clade (Michaux et al., 2001); (25) Dendromurinae+Rhizomyinae are a clade (Jansa et al., 1999); (26) Sigmodontinae+Neotominae are a clade (Sigmodontinae s.l.; Musser and Carleton, 1993); (27) Neotominae+Arvicolinae are a clade (Catzeflis et al., 1993; Engel et al., 1998); (28) Sigmodontinae+Cricetinae are a clade (Engel et al., 1998); (29) Arvicolinae, Cricetinae, and New World cricetids are a clade (Michaux et al., 2001); (30) status of *Sigmodon* as sister to all other sigmodontines (Dickerman, 1992; Engel et al., 1998; but cf. Smith and Patton, 1999); (31) Nesomyinae, Dendromurinae s.s., and Cricetomyinae as an African clade (Michaux et al., 2001) that includes other African subfamilies, e.g., Petromyscinae; (32) Rhizomyinae+Spalacinae as a clade (Michaux and Catzeflis, 2000); (33) membership of Spalacinae, but not Rhizomyinae, in the Muroidea (Hartenberger, 1998); (34) Murinae are more closely related to Cricetinae than to Deomyinae or Gerbillinae (Hänni et al., 1995).

	ΔSteps		ΔLikelihood	
Hypothesis	Score	*P*-value	Score	*P*-value
1. (Muroidea)	(17)	$P < 0.002$	(34.8)	$P = 0.073$
2. (Rhizomyinae)	(11)	$P < 0.04$	(35.0)	$P = 0.64$
3. (Nesomyinae)	(7)	$P < 0.0001^*$	(19.8)	$P = 0.041^*$
4. (Cricetomyinae)	(26)	$P < 0.0001^*$	(48.4)	$P = 0.266$
5. (Dendromurinae)	(16)	$P < 0.0075$	(28.4)	$P = 0.549$
6. (Cricetinae)	(25)	$P < 0.004$	(80.1)	$P < 0.001^*$
7. (Arvicolinae)	(39)	$P < 0.0001^*$	(147.7)	$P = 0.001^*$
8. (Tylomyinae)	(2)	$P > 0.5$	(22.1)	$P = 0.728$
9. (Neotominae)	(2)	$P > 0.5$	(20.3)	$P = 0.758$
10. (Sigmodontinae)	(37)	$P < 0.0001^*$	(102.9)	$P = 0.019^*$
11. (Deomyinae)	(90)	$P < 0.0001^*$	(85.9)	$P = 0.043^*$
12. (Gerbillinae)	(61)	$P < 0.0001^*$	(91.4)	$P = 0.032^*$
13. (Murinae (inc. Otomyinae))	(20)	$P = 0.0002^*$	(50.4)	$P = 0.218$
14. Simpson Cricetidae 1	70	$P < 0.0001^*$	142.4	$P < 0.001^*$
15. Simpson Cricetidae 2	158	$P < 0.0001^*$	408.0	$P < 0.001^*$
16. Chaline et al. Cricetinae	17	$P < 0.005$	27.5	$P = 0.387$
17. Chaline et al. Cricetidae	70	$P < 0.0001^*$	188.0	$P < 0.001^*$
18. Chaline et al. Dendromurinae	21	$P = 0.003$	30.2	$P = 0.536$
19. Lavocat Nesomyidae	211	$P < 0.0001^*$	601.6	$P < 0.001^*$
20. Murinae s.s.	48	$P < 0.0001^*$	1221.8	$P < 0.001^*$
21. Deomyinae+Murinae	35	$P < 0.0001^*$	53.1	$P = 0.040^*$
22. (Deomyinae+Murinae+Gerbillinae)	(10)	$P = 0.02$	(16.6)	$P = 0.657$
23. Petromyscinae+Nesomyinae	8	$P = 0.059$	4.2	$P = 0.98$
24. (Dendromurinae+Cricetomyinae)	(13)	$P = 0.02$	(28.0)	$P = 0.341$
25. Dendromurinae+Rhizomyinae	95	$P < 0.0001^*$	229.1	$P < 0.001^*$
26. (Sigmodontinae s.l.)	(2)	$P = 0.70$	(1.0)	$P = 0.99$
27. Neotominae+Arvicolinae	9	$P = 0.18$	11.6	$P = 0.452$
28. Sigmodontinae+Cricetinae	9	$P = 0.18$	12.6	$P = 0.749$
29. (Cricetid group)	(12)	$P = 0.014$	(27.0)	$P = 0.367$
30. (*Sigmodon* vs. core sigmodontines)	(25)	$P < 0.0005^*$	(47.3)	$P = 0.091$
31. (African clade)	(11)	$P < 0.025$	(90.3)	$P = 0.003^*$
32. (Spalacinae+Rhizomyinae)	(18)	$P < 0.001^*$	(81.4)	$P = 0.023^*$
33. Rhizomyinae not in Muroidea	18	$P < 0.001^*$	81.4	$P = 0.023^*$
34. Murinae+Cricetinae	61	$P < 0.0001^*$	128.6	$P < 0.001^*$

or a few taxa. In other cases, such as Lavocat's concept of the Nesomyidae, the proposed family seems little different from a random assortment of muroid subfamilies. No morphological cladistic data sets exist, however, that could be used to conduct the reverse analyses, that is, reveal whether the morphological data can reject the molecular hypothesis. Therefore we cannot say whether the data sets conflict significantly or whether the morphological data (predominantly molar characters) are simply uninformative or weakly informative at these deeper levels of muroids.

We also tested several a priori hypotheses from molecular studies. Those arising from nuclear data were consistent with the optimal trees from our study: 22, 24, and 29 to 32 (Table 3). Alternative trees were significantly worse in only three of them (30 to 32), however. In contrast, the two hypotheses tested from mitochondrial data—(25) Dendromurinae+Rhizomyinae (Jansa et al., 1999) and (34) Murinae+Cricetinae (Hänni et al., 1995)—were much worse than our optimal trees and rejected at very high significance, even though we employed less restrictive backbone constraints. This result suggests that mitochondrial genes can yield unreliable results at the deeper nodes among muroids, possibly as a result of saturation. Finally, in a treatment consistent with traditional classifications, removing the Otomyinae from the Murinae is in strong disagreement with the data; the optimal trees conforming to this constraint are 48 steps and 1221 log-likelihood units worse.

Divergence-Date Estimation

The results of divergence-date estimation are shown in Table 4 and Figure 3. The likelihood of the phylogeny is significantly worse under the assumption of a molecular clock ($-\ln\text{likelihood} = 47513 - 47621$, $df = 31$, $P = 1 \times 10^{-29}$). The same was also true when only the muroids were considered. The poor fit to a molecular clock is supported by the fact that the optimal smoothing factor for the PL model for the concatenated data set was 1.0, which permits considerable change in substitution rates across branches. Therefore, the dates estimated without the assumption of a molecular clock may be more reliable. In general the standard deviation of the dates estimated on the basis of bootstrap-resampled data sets are less than 10% of the mean, probably because of the large number of nucleotides (mean = 5220, SD = 816) sampled in each species. Although the divergence dates were estimated under very divergent assumptions (global clock versus a unique rate for each branch), the range of divergence dates at each node averaged only about 12% of the mean age estimate. This result agrees with the appearance of the phylogram in Figure 1, where lineages clearly do vary in rate but there is no branch that is extremely long or short. Given that the degree of rate heterogeneity appears rather mild in Figure 3, our ability to detect it with such high statistical

TABLE 4. Estimated dates of divergence (Mya) for nodes in Figure 3 based on a concatenation of gene regions by three methods and based on the GHR gene alone.

	Concatenation of all gene regions						GHR
	Global clock		Rate smoothing		Penalized likelihood		Penalized likelihood
	Date	SD	Date	SD	Date	SD	Date
A	50	0.00	50.0	0.01	50.0	0.00	50.1
B	25.9	0.75	24.5	1.21	25.5	0.86	22.9
C	15.0	0.73	14.5	0.92	15.3	0.79	13.5
D	7.1	0.66	7.6	0.84	7.7	0.77	8.4
E	24.7	1.1	23.3	1.36	24.2	1.05	22.0
F	22.5	0.65	20.6	1.18	21.4	0.79	19.9
G	12	—	12	—	12	—	12
H	10.3	0.20	8.8	0.35	9.6	0.27	8.6
I	10.0	0.39	8.3	0.45	9.2	0.42	8.0
J	9.7	0.37	7.9	0.43	8.7	0.40	8.0
K	6.6	0.53	5.4	0.49	5.8	0.50	5.6
L	8.8	0.30	6.9	0.39	7.8	0.33	7.2
M	20.0	0.64	17.6	1.13	18.7	0.78	16.4
N	9.3	0.55	8.2	0.76	8.6	0.61	7.6
O	13.1	0.51	10.6	0.75	11.7	0.55	10.3
P	12.2	0.55	9.8	0.74	10.8	0.57	9.9
Q	19.2	0.63	18.7	1.06	19.6	0.79	16.8
R	18.5	0.65	17.9	1.05	18.8	0.80	16.3
S	8.2	0.46	7.3	0.61	8.0	0.53	7.7
T	6.5	0.38	5.5	0.46	6.2	0.42	5.4
U	13.8	0.69	13.5	0.96	14.1	0.80	12.4
V	17.6	2.17	17.3	2.35	18.1	2.25	16.8
W	9.8	0.60	11.0	0.81	10.9	0.74	7.7
X	12.8	0.58	12.3	0.85	13.1	0.69	12.0
Y	7.7	0.45	7.2	0.63	7.8	0.52	9.4
Z	6.8	0.73	6.4	0.84	6.9	0.76	8.2
ZZ	5.0	0.41	4.5	0.53	5.0	0.46	6.4

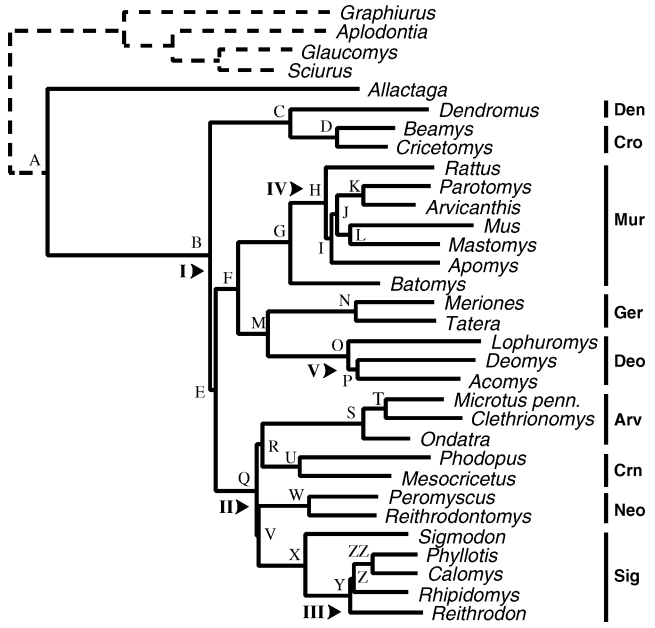

FIGURE 3. Estimated divergence dates are shown at each node. Letters on nodes refer to those in Table 4. Myodonta in solid lines, more distant outgroups indicated by dashed lines.

significance might be attributable to the unusually large amount of sequence data for each taxon.

Using the calibration of 12 Mya for the origin of modern murines (*Batomys* versus other murines), we were able to estimate dates for a series of key phylogenetic events. The basal radiation of the Muroidea s.s. ("B") occurred between 24.5 and 25.9 Mya (22.1 to 27.4, the 95% confidence limits around the greater and lesser estimates for the node in question). Between 23.3 and 24.7 Mya (95% CI 20.6 to 26.9 Mya) the murid group split from the cricetid group ("E"). The divergence between the Murinae and the Deomyinae-Gerbillinae was estimated at 20.6 to 22.5 Mya (CI 18.3 to 23.8 Mya; "F"), and the Deomyinae and Gerbillinae split at 17.6 to 20.0 Mya (CI 15.4 to 21.3 Mya; "M"). Much smaller intervals were estimated for the successive divergence events among the Cricetinae, Arvicolinae, and Sigmodontinae. At 18.7 to 19.6 Mya (CI 16.6 to 21.1 Mya) the Arvicolinae-Cricetinae split from the Sigmodontinae-Neotominae ("Q"), and comparatively soon afterward the Arvicolinae diverged from the Cricetinae at 17.9 to 18.8 Mya (CI 15.8 to 20.4 Mya; "R"). After the basal cricetid split, an even shorter interval preceded the divergence of the Neotominae and Sigmodontinae at 17.3 to 18.1 Mya (CI 12.7 to 22.5 Mya; "V"). The Sigmodontinae originated at least 12.3 to 13.1 Mya (CI 10.6 to 14.5 Mya; "X").

For comparison to the estimates of divergence dates based on the concatenated genes and to examine nodes not represented by three or more gene regions, we also estimated divergence dates based only on GHR (optimal smoothing factor = 1.5) for all of the myodont taxa represented by that gene. Generally divergence date estimates from GHR fell well within the confidence interval for dates estimated based on the full concatenation. Some of the nodes of particular interest that are not represented in Figure 3 are Muroidea s.s. + Spalacine/Rhizomyines at 39 Mya; Spalacinae versus Rhizomyinae at 19.8 Mya; nesomyines versus "Afrocricetodontines" at 18.6 Mya; base of the Nesomyinae at 14.8 Mya; *Petromyscus* versus dendromurine/cricetomyines at 16.7 Mya; and Tylomyinae versus Sigmodontinae at 16.2 Mya.

DISCUSSION

Features of Gene Evolution

The four genes used in the present study are functionally independent, unlinked, and exhibit unique evolutionary patterns. The rate of substitution for GHR, c-myc, and BRCA1 is relatively homogeneous across the length of their sequences, but RAG1 has a much higher rate of substitution among the 5′ ~1000 bp than among the remaining ~2000 bp. This difference is illustrated in Figure 4, where pairwise distances calculated independently for GHR, BRCA1, c-myc, and the two regions of RAG1 are plotted against pairwise distances calculated from the concatenated data set for those taxa represented by all four genes. Visually, the gene regions fall into three groups: c-myc and the ~2000 3′ bp of RAG1, which have very similar low rates; GHR and the 5′ end of RAG1, which have an intermediate rate; and BRCA1, which has the highest rate. The pattern of divergence appears to be linear across the range of pairwise divergences for GHR, RAG1, and c-myc, but the curve for BRCA1 does appear to flatten at high divergence, suggesting saturation of substitutions, although this trend is complicated by considerable dispersion of points. Consistent with the possibility of saturation, a polynomial regression fits the plot for BRCA1 significantly better than a simple linear regression ($F = 83.3$; $df = 1273$; $P \approx 1.6 \times 10^{-17}$). The relative rates of nucleotide substitution are also reflected in the incidence of insertions and deletions per 1000 bp of sequence: 0 in the conservative two thirds of RAG1, 5.3 in c-myc, 13 in the variable domain of RAG1, 18.1 in GHR, and 23.8 in BRCA1.

Perhaps as a partial consequence of different rates and patterns of substitution, the four genes contribute unevenly to support of individual nodes across the phylogeny (Fig. 5 and Table 5). If the bootstrap support for a node drops markedly when a particular gene is removed from the concatenation, that gene provides the majority of the support for that node. As would be expected, branch length and strength of bootstrap support are generally correlated, although each gene exhibits at least one exception to this pattern. Removal of GHR or c-myc causes the smallest effect on bootstrap values, probably because those two genes contain only 22.5% (429 sites) of the parsimony-informative sites, but their absence does have considerable impact on the support of three nodes. When GHR is removed, the bootstrap support for node "E" decreases by 14 points, and when

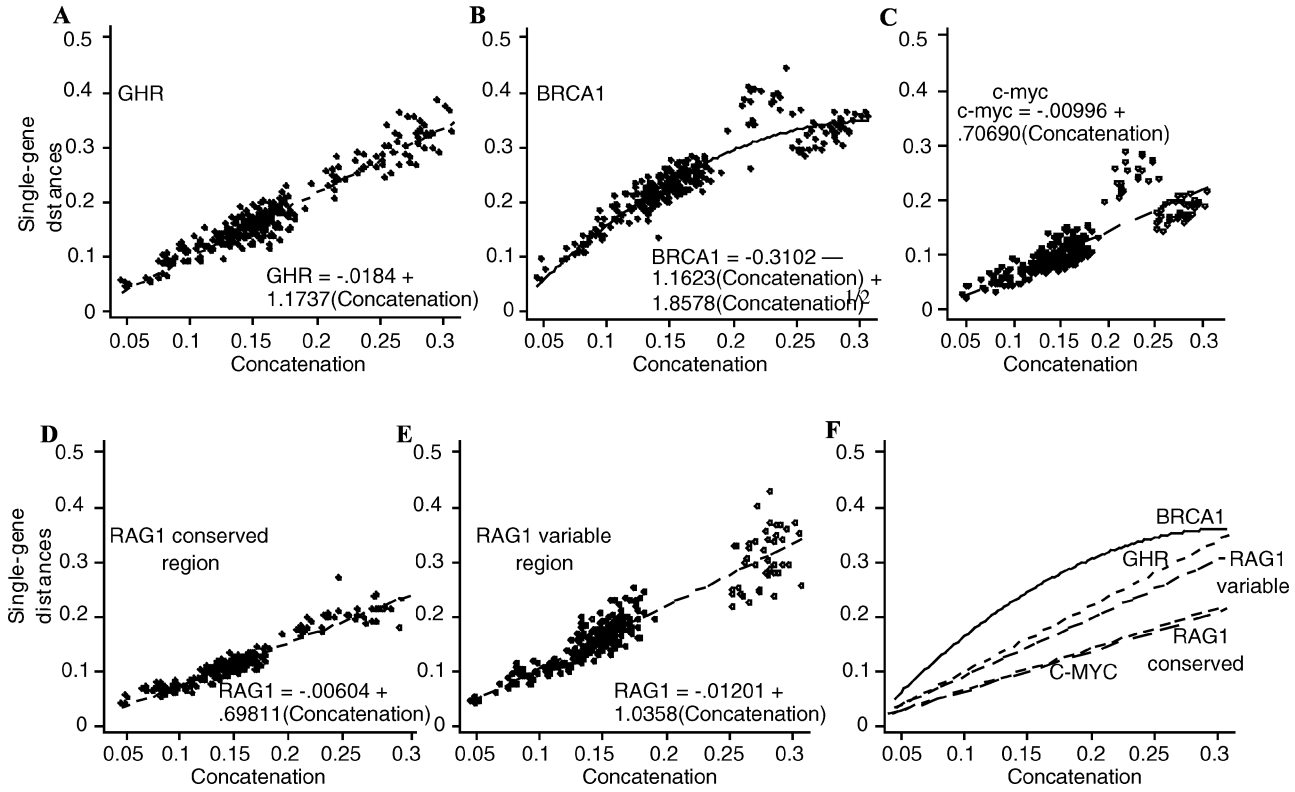

FIGURE 4. Plot of pairwise distances calculated independently for GHR (A), BRCA1 (B), c-myc (C), the conservative 3′ 2077 bp of RAG1 (D), and the more variable 5′ 1000 bp of RAG1 (E) versus pairwise distances calculated from the concatenation of all four genes. The graphs are restricted to those taxa represented by all four genes. Maximum-likelihood distances were calculated from the GTR+Γ+I model. Model parameters were estimated for each gene, and the concatenation based on the maximum-likelihood phylogeny derived from the concatenated data set (Fig. 1). The predicted-distances values derived from the equations shown in parts A–E are plotted together (F) to illustrate relative rates of divergence among the gene regions.

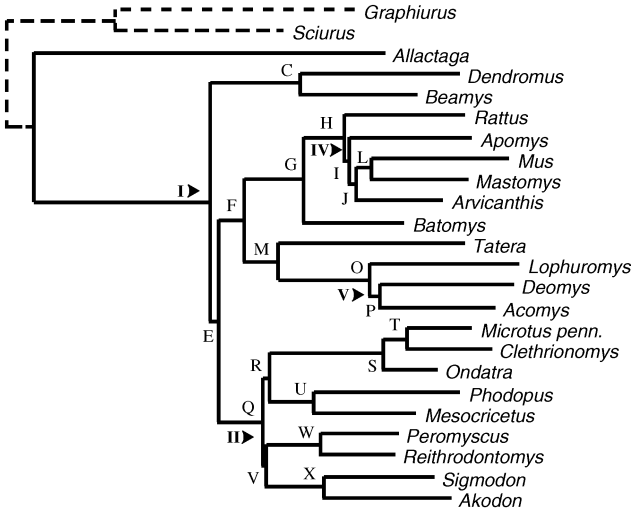

FIGURE 5. Maximum-likelihood phylogeny for the taxa represented by all four gene regions in this study. Relative contribution of each gene to the bootstrap support listed in Table 5. Letters on nodes refer to those used in Tables 4 and 5 and Figure 3. Myodonta in solid lines, more distant outgroups indicated by dashed lines. Only those nodes from Figure 4 that are represented by taxa with all four genes are shown here.

c-myc is removed the support for node "I" decreases by 17 points and that for "J" by 11. The branches leading to nodes "I" and "J" are two of the shortest on the tree, so the result for c-myc is somewhat surprising because it is the most conservative gene and might be expected to provide few synapomorphies for short branches. This point illustrates the difficulty of predicting where on a tree the phylogenetic signal from a molecule will reside or at what level of divergence a gene will perform optimally based solely on that gene's rate of substitution. Removal of BRCA1 (42.3% of informative sites) or RAG1 (35.2%) has a greater effect on bootstrap values, as might be expected from the greater number of parsimony-informative sites involved. Removal of BRCA1 disproportionately affects support for shorter branches (nodes I −52, J −59, P −31, R −67, and V −50) and in these cases is consistent with expectations based on its comparatively high rate of substitution. Support for node "E" appears to reside primarily in RAG1 because its support decreases by 55 when RAG1 is removed. In terms of phylogenetic accuracy, the removal of GHR and c-myc individually or in combination results in the same topology as Figure 5, but no combination of three or two (data not shown) genes resulted in bootstrap support values across all nodes as high as those based on all four genes combined. Both the

TABLE 5. Bootstrap proportions for nodes in Figure 5 based on the full concatenated data set, single genes, and the full concatenation with single genes removed.

			Change in bootstrap proportion relative to full concatenation							
			Single gene bootstrap proportion				BP after single gene removal			
Node	Length	Full data bootstrap	GHR	BRCA1	RAG1	c-myc	GHR removed	BRCA1 removed	RAG1 removed	c-myc removed
C	0.0289	100	0	0	−1	−50	0	0	0	0
E	0.0028	85	−7	−79	+11	−67	−14	+13	−55	+2
F	0.0078	100	−10	−2	−7	−40	0	−1	0	0
G	0.0192	100	−1	0	0	−5	0	0	0	0
H	0.0128	100	−2	0	−1	−14	0	0	0	0
I	0.0017	72	−67	+14	−58	+5	0	−52	+20	−17
J	0.0022	78	−75	+3	−56	−53	+5	−59	+13	−11
L	0.0048	96	−48	−38	−25	−11	−2	+1	−5	−5
M	0.0108	100	−5	−1	−1	−10	0	0	0	0
O	0.0298	100	0	0	0	−12	0	0	0	0
P	0.0032	66	−54	−1	−12	−24	+7	−31	−1	−3
Q	0.0138	100	−3	0	0	−58	0	0	0	0
R	0.0025	87	−72	+9	−87	−79	+3	−67	+5	−3
S	0.0365	100	0	0	0	−21	0	0	0	0
T	0.0075	100	0	0	−16	−58	0	0	0	0
U	0.0137	100	0	0	−1	−63	0	0	0	0
V	0.0012	59	−45	+24	−39	−58	+1	−50	+7	+3
W	0.0173	100	0	0	0	−17	0	0	0	0
X	0.0182	100	−4	0	0	−15	0	0	0	0

accuracy and strength of phylogenetic hypotheses are therefore improved by inclusion of all four genes, even when taxonomic sampling is incomplete.

Differences in relative support among genes for the five key regions of phylogenetic conflict may also be due to differences in history of lineage sorting, such that the gene histories may actually be different from each other. The branch lengths in these five regions are consistent with periods between speciation events on the order of a million years or less where lineage sorting may be a possibility for nuclear genes.

Phylogenetic Relationships

The data yield a highly resolved, robust phylogeny, as evidenced by the high bootstrap percentages, high posterior probabilities, and significant differences between the optimal and constrained topologies. The major results include the deep division between the mole-rat subfamilies (Spalacinae and Rhizomyinae) and the remaining core members of the Muroidea. Within this core group are four distinct lineages that diverged from each other over a relatively short time: the monotypic Calomyscinae, a diverse African clade, a "cricetid" clade with maximum diversity in the New World, and an Old World murine clade. The results confirm the recent removal of *Deomys* from the Dendromurinae and its placement in an elevated clade best referred to the Deomyinae, the sister-group relationship between the Deomyinae and Gerbillinae, as well as the placement of the Otomyinae within the Murinae rather than as a separate subfamily (Chevret et al., 1993b). In contrast to Jansa et al. (1999), the Malagasy endemic Nesomyinae are monophyletic and appear to be the sister group to other members of the African clade. Within the cricetid group, the Neotominae, Tylomyinae, and Sigmodontinae do appear to form a clade, weakly supported, consistent with common usage of Sigmodontinae s.l.

Detailed sampling within several subfamilies in addition to the broad sampling across subfamilies yielded additional insights. Within the Sigmodontinae, a relatively long period of time followed the separation of *Sigmodon* before the rapid radiation that characterized the mostly endemic South America radiation. Within the Murinae, distinct geographic lineages are apparent, including two in the Philippine islands (one old, *Batomys*, one recent, the *Apomys* clade), two Asian clades, and an African clade that includes the Otomyinae. Additional sampling is underway in this group.

Several comprehensive classifications of Muroidea have been done previously, all by paleontologists. Notably, our results show that none of the polytypic families in those classifications represent monophyletic groups or even paraphyletic ones (Table 1). For example, Simpson's (1945) Muridae and Cricetidae both represent polyphyletic groups. Chaline et al. (1977) proposed a unified classification of Muroidea that divided it into eight families. Their Rhizomyidae correctly grouped the African *Tachyoryctes* and the Asian *Rhizomys*, but most of their proposed groupings above the subfamily are incongruent with our findings. Their Cricetidae in particular are polyphyletic, including not just the core members we recognize (Cricetinae, Sigmodontinae, Neotominae) but also taxa that are members of distantly related clades: the Spalacinae and Calomyscinae (which they place within the Cricetinae). Their Nesomyidae include not just the paraphyletic subgroup of our African clade but also the Otomyinae, which are clearly just derived murines. Their Dendromuridae are paraphyletic in excluding the Cricetomyinae (and Nesomyinae). Lavocat's (1973, 1978)

higher taxa conflict similarly with our DNA data. It appears that morphological data, particularly dental, have been at least as prone to convergence as many morphologists have suspected. For example, the Murinae and Dendromurinae of Simpson's (1945) Muridae share similar, but not identical, triserial cusp arrangements of the molars. Our results validate the reluctance of Musser and Carleton (1993) to accept the morphological hypotheses.

Systematics

The findings presented here in conjunction with concordant findings from other recent studies using different genes (Michaux et al., 2001) are sufficiently strong to warrant systematic changes. We present our conclusions here partially to simplify the terminology for the remainder of the paper. Nomenclaturally we follow the ICZN (1999) but where options exist have made decisions to facilitate a conversion to Phylocode. In support of separating sigmodontines, neotomines, and tylomyines into distinct subfamilies (Reig, 1980; Reig, 1984; Steppan, 1995; reviewed in D'Elía, 2000), we note that the estimated divergence dates within and among these groups are as old as or older than divergences in other subfamilies. We do not suggest that rank be determined directly by time or divergence level, but in the absence of significant shared history or biologically important synapomorphies, there is no basis for uniting these three taxa in the Sigmodontinae. The "African" clade so strongly supported by these data is similar to several taxa used previously, particularly the Afrocricetodontinae (Lavocat, 1978) and Nesomyidae (Chaline et al., 1977). Unfortunately, all of the available names refer to groups that include subfamilies clearly unrelated to the clade revealed by nuclear genes. The nomenclatural code nevertheless requires that the oldest family-level name be applied, which in this case is Nesomyidae.

Revised Classification of Muroidea

Muroidea
- Spalacidae
  - Spalacinae
  - Rhizomyinae
- Eumuroida, new taxon
  - Calomyscidae, new rank
  - Nesomyidae
    - Nesomyinae
    - Petromyscinae
    - Mystromyinae
    - Dendromurinae
    - Cricetomyinae
  - Cricetidae
    - Cricetinae
    - Arvicolinae
    - Neotominae
    - Tylomyinae
    - Sigmodontinae
      - Oryzomyalia, new taxon
  - Muridae
    - Murinae
    - Deomyinae
    - Gerbillinae
- Lophiomyinae, Muroidea incertae sedis
- Platacanthomyinae, Muroidea incertae sedis
- Myospalacinae, Muroidea incertae sedis

The Eumuroida are defined as the most recent common ancestor of the Murinae, Cricetinae, Nesomyinae, and Calomyscinae and all of its descendants. It contains the core, or "true," muroids and excludes the mole rat subfamilies Spalacinae and Rhizomyinae. The Nesomyidae ("African mice") are defined as the most recent common ancestor of the Nesomyinae, Dendromurinae, Petromyscinae, and Mystromyinae and all of its descendants. Good evidence from vWf exon 28 and LCAT genes indicates that the Mystromyinae are members of this clade (Michaux et al., 2001). The Deomyinae are defined as the most recent common ancestor of *Deomys*, *Acomys*, *Lophuromys*, and *Uromys* (several data sets support the inclusion of *Uromys* in this clade) and all its descendants. We revise the definition of the Cricetidae to be the most recent common ancestor of the Cricetinae, Arvicolinae, Sigmodontinae, Neotominae, and Tylomyinae and all of its descendants. The Oryzomyalia are a clade within the subfamily Sigmodontinae defined as the most recent common ancestor of the Akodontini, Oryzomyini, Phyllotini, Thomasomyini, and Reithrodontini and all of its descendents, excluding the Sigmdontini. The revised definition of the Muridae is the most recent common ancestor of the Murinae, Deomyinae, and Gerbillinae and all of its descendants. Without new sequences, data are still insufficient to place the Lophiomyinae, Platacanthomyinae, and Myospalacinae.

Divergence Dates and Biogeography

Divergence dates among murid rodents are very controversial. In general, dates estimated on the basis of molecular data and a nonmurid calibration of a molecular clock have generated dates much earlier than paleontological dates (i.e., 27 to 43 Mya for *Mus* versus *Rattus*; Kumar and Hedges, 1998; Cao et al., 2000; Huchon et al., 2000). This discrepancy is largely due to the application of a uniform molecular clock across all taxa, despite the fact murids have a higher rate of sequence evolution than most mammals (Wu and Li, 1985; Adkins et al., 2001). Recently, the application of a relaxed molecular clock has been shown to give a much better fit to paleontological dates (Adkins et al., 2003).

Our results indicate that attributing the origin of *Progonomys* at 12 Mya to the MRCA of *Mus* and *Rattus* is incorrect and would result in an overestimation of divergence dates when extrapolated from this calibration. Most studies that cite Jacobs and Downs (1994)—or related studies from that group—include only two murines, *Mus* and *Rattus*. Thus the only node that could be associated with *Progonomys* would be the MRCA of those two genera, which is incorrect. Jacobs and Downs

(1994) viewed *Antemus* (13.75 to 12.5 Mya) as part of a lineage leading to murines with the synapomorphic triserial cusp arrangement of modern murines first seen in *Progonomys* at 11.8 to 12.1 Mya. If the detailed and well-calibrated fossil record from the Siwalik Group of deposits in Pakistan is an accurate reflection of murine history, then *Progonomys* is either the MRCA of extant murines or a predecessor. Some researchers (e.g., Huchon et al., 2002) cite Jacobs and Downs (1994) and assign 14 Mya to the *Mus-Rattus* split, perhaps because that is the approximate transition from the "cricetid" *Potwarmus* (14.3 Mya), through a transitional form at 14.1 Mya, to the first murine with triserial cusps, *Antemus*, at 13.75 Mya. However, *Antemus* does not have the modern murine configuration and probably predates the MRCA of extant murines. We estimate the *Mus-Rattus* split at 8.8 to 10.3 Mya, significantly less than the 12 to 14 Mya (Robinson et al., 1997; Ruedas and Kirsch, 1997; Ducroz et al., 1998; Verneau et al., 1998; Dubois et al., 1999; Barome et al., 2000; Huchon et al., 2000, 2001, 2002; Michaux et al., 2001; Salazar-Bravo et al., 2001) cited in many studies but compatible with the date of 10 Mya used in some studies (She et al., 1990; Smith and Patton, 1999). See Ruedas and Kirsch (1997) for further discussion.

Michaux et al.'s (2001) survey of the LCAT and vWf genes across muroid diversity parallels our study in many respects, and one of their two calibration points is also used in ours, although we assign 12 Mya to the divergence of *Batomys*, rather than *Mus* from *Rattus*. Significantly, the dates presented here overlap with or barely exceed the dates estimated by Michaux et al. (2001). This concordance is noteworthy because two very different approaches were used. Michaux et al. (2001) applied a global molecular clock to a linearized tree, whereas we applied a relaxed clock to a phylogeny exhibiting heterogeneous rates of substitution. In both studies the dates estimated from molecules are usually older than those estimated from the fossil record.

Murinae.—Our estimate of the divergence of otomyines from other murines at 5.4 to 6.6 Mya, supports the 4.5 to 6.0 Mya date of Senegas and Avery (1998). It is noteworthy that within one million years of the *Mus-Rattus* split clades (nodes H, I, and J) diverged that are now confined to Africa (otomyine-arvicanthine group), the Philippines, and Eurasia, suggesting rapid geographic expansion or simultaneous subdivision during this period. That the sister group (*Batomys*) to the main murine radiation is isolated in the Philippines means either that the center of origin for murines was in Southeast Asia or that the Philippines is a refuge for the earliest murine lineage.

Sigmodontinae–Neotominae.—The date at which muroids entered South America is quite controversial (reviewed in D'Elía, 2000; Pardiñas et al., 2002). From before the origin of muroids to the formation of the Panamanian land bridge about 4 Mya (Iturralde-Vinent and MacPhee, 1999), South America was isolated from the other continents. Therefore, if muroids entered South America directly via a land route, they had to have entered no earlier than 4 Mya (Baskin, 1978, 1986; Patterson and Pasqual, 1972; Simpson, 1950). Debate has centered on two points, whether the entry occurred before the land bridge (early arrival) or after (late arrival) and whether the major diversification took place in North/Central America or South America. Four major sets of models have been proposed: South American diversification and late arrival (Simpson, 1950), which would make the sigmodontines an explosive radiation; Central American diversification followed by late arrival of either many lineages (>20; Patterson and Pasqual, 1972) or a moderate number (6 to 8; Baskin, 1986); South American diversification after an early arrival more than 20 Mya (Hershkovitz, 1972; Savage, 1974); or a mostly South American diversification after the arrival of a small number of lineages around 5 to 7 Mya during a period of low sea level (Marshall, 1979). Therefore the various models differ in the number of lineages to invade South America and the location of the early radiation: many lineages (20 to 40, Patterson and Pasqual, 1972), several (≈2 to 5, Marshall, 1979; 6 to 12, Baskin, 1986), one or a few (late, Simpson, 1950; early, Hershkovitz, 1972).

We estimate that *Sigmodon* diverged from other Sigmodontinae 12.3 to 13.1 Mya. This is incompatible with Simpson (1950) at one extreme and Hershkovitz (1972) and Savage (1974) at the other. The dates are several million years earlier than those favored by Baskin (10 My; 1986) and Marshall (7 to 10 My; 1979). Fossils that can clearly be ascribed to the Sigmodontinae only appear around 4 Mya, and until this gap is filled, the biogeography of these groups cannot be definitively resolved. However, the divergence between *Reithrodon* and other Oryzomyalia (node III) is estimated at 6.0 to 8.8 My (including estimation error) and divergence among the remaining Oryzomyalia tribes during the next million years or so (Fig. 3). Additional sampling with mitochondrial and nuclear DNA data indicate that, given our topology, all the remaining major lineages except for the fish-eating rats Ichthyomyini must have diverged during the same brief period (Engel et al., 1998; Smith and Patton, 1999; Weksler, 2003). We suggest that the most plausible explanation for such a burst of speciation and morphological divergence is dispersal of a single lineage into South America followed by an adaptive radiation. This interpretation suggests that the ancestor to the Oryzomyalia had reached South America by 6 Mya, before a continuous land bridge formed, in keeping with Marshall's (1979) model of lower sea level. If the rapid radiation was a consequence of expansion into South America, then our molecular data provide the first clear evidence regarding the number of lineages involved in the colonization and the timing of the event. In this view, three major lineages are likely to have migrated (ancestor of Oryzomyalia, some Sigmodontini, and some of the semiaquatic tribe Ichthyomyini (Dickerman, 1992; Weksler, 2003)), perhaps at different times, rather than the large numbers of species required by most other hypotheses (Baskin, 1978, 1986; Marshall, 1979; Simpson, 1980; Jacobs and Lindsay, 1984; Czaplewski, 1987), a

complex scenario criticized by Steppan (1996). It seems improbable that large numbers of sigmodontine lineages would have crossed the land bridge even though none of the contemporaneous and ecologically diverse neotomyines were able to. We argue against a North American center of diversification and support an autochthonous model for the major South American radiation.

Arvicolinae.—Michaux and Catzeflis (2000) and Michaux et al. (2001) estimated the divergence of *Microtus*, *Clethrionomys*, and *Dicrostonyx* at 7.4 to 9.3 Mya, whereas Chaline and Graf (1988) suggested 3 to 5 Mya. Our estimate of 5.5 to 6.6 Mya for the divergence of *Microtus* from *Clethrionomys* and 7.3 to 8.2 Mya for that of *Ondatra* is most compatible with Conroy and Cook (1999; ~5.7 Mya for all genera) and reaffirms the impression from molecular data that the genera of arvicolines began to diverge earlier than the fossil record would suggest. Conroy and Cook's (1999) estimate of 9.8 Mya for the divergence of the Arvicolinae and Murinae is much younger than our estimate of >22 Mya and may indicate saturation of the third-codon-position transversions in mtDNA.

Eumuroida.—In general, the dates estimated in our study are slightly older than those derived from fossils (Carleton and Musser, 1984) or molecules (Engel et al., 1998; Michaux and Catzeflis, 2000; Michaux et al., 2001), but given the branch lengths observed and the rates of substitution, the dates are consistent in some ways with paleontological work. Our estimate of approximately 24.5 to 26 Mya for the eumuroid radiation places the origin of the modern muroids (Eumuroida) near the border between the Miocene and Oligocene (24 to 26 Mya), a little earlier than the Miocene date derived from most paleontological studies.

Four Rapid Radiations

Detailed sampling within and among subfamilies enabled us to delineate more precisely four apparent bursts of speciation (we will not discuss the near-polytomy in the Deomyinae in this context because three lineages do not constitute a significant radiation): (I) the basal radiation within the Eumuroida; (II) the basal radiation among the cricetid subfamilies; (III) the basal radiation among the South American sigmodontines (Oryzomyalia); and (IV) the basal radiation among the core murines (excluding *Batomys*). To varying degrees, the apparent rapidity may be an artifact of taxon sampling or extinction, but at least some probably represent meaningful evolutionary or biogeographic events. We will discuss each of these briefly in turn.

At least four lineages at the base of the Eumuroida (node I) diverged within an estimated 1 to 3 My. The confidence intervals on the dates for individual nodes expand that range, but every gene studied to date (GHR, RAG1, BRCA1, c-myc, vWF, LCAT) indicates very short internal branches. Whether this pattern reflects an increase in speciation rate or a product of extinction patterns is unclear.

Within the cricetid group (node II), five major lineages diverged in approximately 1 My. These include three New World lineages, a Holarctic lineage (Arvicolinae), and an Asian clade (Cricetinae). If Baskin (1986) and others are correct that the long-lived North American genus *Copemys* was the paraphyletic ancestor to both the Neotominae and the Sigmodontinae, then it should also have been the ancestor to the Arvicolinae and Cricetinae or have been very closely related to their ancestor. As this idea has not received direct support among paleontologists, it seems more likely to us that *Copemys* is ancestral to some or all of the Neotominae or the peromyscine group after diverging from sigmodontines.

The evidence for a rapid radiation is strongest for the radiation among the Oryzomyalia (node III). We have included representatives of most major lineages identified by DNA sequencing (Engel et al., 1998; Smith and Patton, 1999; D'Elía, 2003; Weksler, 2003) and morphology (Hooper and Musser, 1964; Reig, 1980; Musser and Carleton, 1993; Steppan, 1995). Despite the large amount of sequence data collected, branching order is still unclear. All of the lineages in this group are endemic to South America or have centers of diversity there although several North American fossils have been attributed to Oryzomyalia tribes (Jacobs and Lindsay, 1984; Baskin, 1986; Czaplewski, 1987). We suggest that the direct cause of this rapid radiation was the colonization of South America by a single sigmodontine lineage, followed by rapid range expansion, fragmentation, and adaptive divergence. It will be interesting to learn whether this pattern is confirmed by more extensive taxon sampling and whether a similar pattern develops for the murine radiation (node IV) or the murine radiation had fundamentally different causes. Resolution of these regions will likely require large amounts of additional data, although even then a strictly bifurcating tree may not be attainable if the time between speciation events was short enough that lineage sorting was common, yielding many gene tree/species tree conflicts. Ongoing studies are focused on increasing sampling within and around these key regions.

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APPENDIX 1

List of specimens sequenced. Abbreviations: Appalachian State University (ASU); Carnegie Museum of Natural History (CMNH); Field Museum of Natural History (FMNH); Louisiana State University Museum of Zoology (LSUMZ); Museum of Vertebrate Zoology, Berkeley (MVZ); Royal Ontario Museum (ROM); Texas Cooperative Wildlife Collection (TCWC); Transvaal Museum (TM); United States National Museum (USNM); Oklahoma State University Collection of Vertebrates

(OK). The collector numbers EAR refer to uncataloged specimens housed at FMNH and collected by Eric Rickart, numbers RA collected by Ronald Adkins and housed in the TCWC, and collector numbers H refer to uncataloged specimens housed in the TCWC and collected by members of the laboratory of Dr. Rodney Honeycutt. Tra (Andringitra Park), Mor (Morandavo Park), and Zah (Zahamena Park) are sample numbers assigned by Dr. Edward Louis of the Henry Doorly Zoo of Omaha, Nebraska.

Myoxidae: *Graphiurus murinus*. SP6067. South Africa, Cape Prov., Kabusi Forest. 32°31′S, 27°15′E. *Dryomys nitedula*. Collection of D. Kramerov, locality unknown.
Aplodontidae: *Aplodontia rufa*. H2370. MVZ 185228. USA, California.
Sciuridae: *Glaucomys volans*. LSUMZ M5762.USA, W.Virginia, Kanawha Co. *Sciurus stramineus*. LSUMZ M936. Peru, Piura Dept., Parinas, 7 km N, 15 km E Talara. *Scurus niger*. H2376. *Marmota monax*. ASU 16756. USA, North Carolina, no exact locality. 35°30′N82°30′W.
Pedetidae: *Pedetes capensis*. CMNH 95012. South Africa, Orange Free State, Benfontein, 17 km N, 25 km W Perdeberg, 28°50′S 24°49′E.
Dipodidae: *Alactaga sibirica*. USNM 449152. China, Qinghai Prov., Hainan State, Gonghe Co., Daotanghe, Hudong, E. shore, Qinghai Lake. *Zapus princeps*. FMNH 163053. USA, Utah, Grand Co., La Sal Mts, 0.5 km N, 0.2 km W Warner Lake. *Zapus hudsonius*. H939. USA, New Hampshire, Hillsboro Co., 2.3 km N, 2.3 km N Peterborough.
Muridae
Spalacinae: *Spalax ehrenbergi*. H150. Israel, South Golan Heights. No specific locality.
Rhizomyinae: *Rhizomys pruinosus*. MVZ 176525. China, Yunnan Prov., Yunnan Institute of Tropical Botany, ca. 2 km E Menglung, 65 km E Mengyang; elevation 600 m. *Tachyoryctes splendens*. TK33494.
Calomyscinae: *Calomyscus* sp. MVZ 191923. Iran, Kerman Prov., 30°19′S, 57°41′E.
Dendromurinae: *Dendromus mesomelas*. FMNH 153931. Tanzania, Kilimanjaro Region, Same Dist., South Pare Mts, Chome Forest Reserve, 3 km E, 0.7 km N Mhero. *Malacothrix typica*. TM39370. *Steatomys krebsi*. SP6328. South Africa, Cape Prov., Rockerpan Provincial Nature Reserve. 32°38′S, 18°18′E.
Cricetomyinae: *Beamys hindei*. FMNH 150099. Tanzania; Tanga Region; Muheza District, E Usambara Mts, 6 km NW Amani, Monga Tea Estate. *Cricetomys gambianus* FMNH 166654. Tanzania; Morogoro Region; Kilosa Dist., Ukaguru Mts, Mamiwa-Kisara Forest Reserve, 1 km E, 0.75 km S Mt. Munyera.
Petromyscinae: *Petromyscus monticularus*. RA14. Namibia, Karasburg District, Kanabeam, S28°07′17,″ E17°33′32.″
Nesomyinae: *Hypogeomys antimena*. Mor 149. Madagascar, Beroboka. S19°58′, E44°39′. *Brachytarsomys albicauda*. Zah 66, Madagascar, Zahamena Special Reserve, S17°29′, E48°44′. *Gymnuromys major*. Zah B666. Madagascar, Zahamena Special Reserve. *Nesomys audiberti*. Tra 213. Madagascar, Andringitra National Park, S22°13′, E47°01′. *Eliurus minor*. Tra 241. Madagascar, Andringitra National Park, S22°13′, E47°01′.
Murinae: *Rattus norvegicus*. Sprague-Dawley laboratory strain. *Rhabdomys pumilio*. RA23. Namibia, Karasburg District, Kanabeam, S28°07′17,″ E17°33′32.″ *Aethomys namaquensis*. RA12. Namibia, Karasburg District, Kanabeam, S28°07′17,″ E17°33′32.″ *Apomys hylocoetes* FMNH 147871. Philippines. Mindano Is. Bukidnon Prov., Mt. Katanglad Range, 16.5 km S, 4 km E Camp Phillips. *Chrotomys gonzalesi* USNM 458952, Luzon Is. Camarines Sur Prov., Mt. Isarog, 1350 m. *Rhynchomys isarogensis*. EAR1840, Luzon Is., Camarines Sur Prov., Mt. Isarog, 1750 m. *Mus musculus*. Lab colony, strain BALB/C. *Mastomys natalensis* FMNH 150104. Tanzania; Tanga Region; Muheza District, E. Usambara Mts, 4.5 km ESE Amani, Monga Tea Estate. *Mastomys hildebranti*. H783. *Batomys granti*. EAR 1822. Philippines, Luzon Is., Camarines Sur Prov., Mt. Isarog, 1750 m. *Arvicanthis somalicus*. H894. Kenya, Isiolo District, Buffalo Springs National Preserve, 1 km N Buffalo Springs. *Praomys taitae*. CMNH 102637. Kenya, Coast Region, Taita Dist., Ngangao Forest,. Taita Hills. 3°22′S, 38°21′E.
Otomyinae: *Parotomys* sp. H656.
Gerbillinae: *Meriones shawi*. H583. *Gerbillurus vallianus*. H675. *Tatera robusta*. FMNH 158105. Tanzania; Arusha Region; Babati District, Tarangire National Park, near Engelhardt Bridge.
Deomyinae: *Lophuromys flavopunctatus* FMNH 144777. Uganda; Western; Kasese Dist., Rwenzori Mts, Bujuku R, L bank, John Mate Camp. *Deomys ferrugineus*. FMNH 149427. Zaire, Haute Zaire, Ituri, Epulu, 2 km W, Wpulu R, rt bank. *Acomys ignitus*. CMNH 102383. Kenya, Coast Region, Kwale Dist., Shimba Hills Natl Reserve, 5 km S, 1 km W Kwale. 04°13′S 39°27′E.
Arvicolinae: *Microtus irene*. USNM 444173. China, Qinghai Prov., Yushu State, Nangqeng Co., Bei Zha Forestry Station. *Microtus pennsylvanicus*. USA, Massachusetts. *Clethriomomys gapperi* FMNH 145956. USA; Utah; Wasatch Co, Kamas, 1 mi N, 18 mi E. *Ondatra zibethicus*. Unaccessioned road kill. USA, Massachusetts, Hampshire County, Amherst, 0.3 km N University of Massachusetts on rd North Pleasant.
Cricetinae: *Phodopus sungorus*. Laboratory specimen. Voucher stored in laboratory of R. Adkins. *Mesocricetus auratus*. Laboratory specimen. Voucher stored in laboratory of R. Adkins. *Cricetulus migratorius* MVZ 191941. Iran, Kerman Prov., Zar Rud Bala, Aabshar-e Rayen, Kuh-ehazar, W of Rayen. 29°55′N, 57°30′E.
Neotominae: *Peromyscus leucopus*. OK 014. USA, Oklahoma, Payne Co. No specific locality. *Reithrodontomys fulvescens*. OK 325. USA, Oklahoma, Payne Co. No specific locality. *Neotoma floridana*. OK 107. USA, Oklahoma, Payne Co. No specific locality.
Tylomyinae: *Ototylomys phyllotis*. ROM CN101351. El Salvador, Ahuachapan, El Imposible. *Tylomys nudicaudus*. ROM CN103590. El Salvador, Ahuachapan, El Imposible.
Sigmodontinae: *Sigmodon hispidus*. TCWC AK9175 *Oryzomys couesi*. H678. *Phyllotis xanthopygus*. LSUMZ M1440. Peru, Arequipa Dept., Ca 53 rd km E Arequipa. AK13014. *Akodon boliviensis* FMNH 162754. Bolivia; Oruro; Basin E of Lago Poopo, 4 km by rd N Huancane. *Rhipidomys masticalis* MVZ 193037 Brazil, Espírito SantoReserva Florestal da Companhia Vale do Rio Doce, 30 km N (by road) of Linhares. *Irenomys tarsalis* MVZ 155839. Argentina, Prov. Rio Negro, Depto. Bariloche, Puerto Blest. *Reithrodon auritus* MVZ 182707 Argentina, Prov. Rio Negro, Depto. Pilcaniyeu, 10 km S Comallo. *Andinomys edax* FMNH 132647. Chile, Tarapaca, Parinacota, Arica, ca. 72 km E and Chapiquina, 10 km S. *Auliscomys sublimis* FMNH 162764. Bolivia, Oruro, Escuela Seccional Villa Ventilla, 181 km S Oruro. *Calomys lepidus* FMNH 162785. Bolivia, Oruro, Basin E of Lago Poop, 4 km N Huancane.

APPENDIX 2. Species examined in this study and their GenBank accession numbers.

	Gene region			
Species	GHR	RAG1	BRCA1	c-myc
Sciuridae				
Glaucomys volans		AY241472	AF284003	AY241514
Marmota monax		AY241492		AY241536
Sciurus niger/ *stramineus*	AF332032	AY241476	AF332044	AY241518
Myoxidae				
Graphiurus murinus	AF332031	AY294934	AF332046	AY294967
Dryomys	AY294896			
Aplodontidae				
Aplodontia rufa	AF332030	AY241468	AF332045	AY241510
Family Pedetidae				
Pedetes capensis	AF332025		AF332047	
Dipodidae				
Jaculus jaculus	AF332040			
Allactaga sibirica	AY294897	AY241467	AY294996	AY241509
Zapus hudsonius/ *princeps*	AF332041	AY294935		AY294968
Muridae				
Spalacinae				
Spalax ehrenbergi	AY294898			
Rhizomyinae				
Rhizomys pruinosus	AY294899			
Tachyoryctes splendens	AY294900			
Calomyscinae				
Calomyscus sp.	AY294901			

(Continued on next page)

APPENDIX 2. Continued

Species	Gene region			
	GHR	RAG1	BRCA1	c-myc
Dendromurinae				
Dendromus mesomelas	AY294902	AY241458	AY294997	AY241501
Malacothrix typica	AY294903			
Cricetomyinae				
Beamys hindei	AY294904	AY241459	AY294998	AY241502
Cricetomys gambianus	AY294905	AY294936		AY294969
Petromyscinae				
Petromyscus monticularus	AY294906	AY294937	AY294999	
Nesomyinae				
Hypogeomys antimena	AY294907			
Brachytarsomys albicauda	AY294908			
Gymnuromys major	AY294909			
Nesomys audiberti	AY294910			
Eliurus minor	AY294911			
Murinae				
Rattus norvegicus	X16726	AY294938	AF036760	AY294970
Rhabdomys pumilio	AY294913	AY294940		
Aethomys namaquensis	AY294914	AY294941		AY294972
Apomys hylocoetes	AY294915	AY294942	AY295000	AY294973
Chrotomys gonzalesi		AY294943		AY294974
Rhynchomys isarogensis		AY294944		AY294975
Mus musculus	M33324	AY241462	U31625	AY294976
Mastomys hildebranti/ natalensis	AY294916	AY294945	AY295001	AY294977
Batomys granti	AY294917	AY241461	AY295002	AY241504
Arvicanthis somalicus	AY294918	AY294946	AY295003	AY294978
Praomys taitae	AY294919			
Otomyinae				
Parotomys sp.	AY294912	AY294939		AY294971
Gerbillinae				
Meriones shawi/ unguiculatus	AF332021	AY294947	AF332048	
Gerbillurus vallianus	AF332022	AY294948		
Tatera robusta	AY294920	AY294949	AY295005	AY294979
Deomyinae				
Lophuromys flavopuntatus	AY294921	AY294950	AY295006	AY294980
Deomys ferrugineus	AY294922	AY241460	AY295007	AY241503
Acomys ignitus	AY294923	AY294951	AY295008	AY294981
Arvicolinae				
Microtus pennsylvanicus	AF540633	AY241463	AY295009	AY241505
Microtus irene	AY294924	AY241464		AY241506
Clethrionomys gapperi	AF540623	AY294952	AY295010	AY294982
Ondatra zibethicus	AY294925	AY294953	AY295011	AY294983
Cricetinae				
Phodopus sungorus	AF540640	AY294954	AY295012	AY294984
Mesocricetus auratus	AF540632	AY294955	AY295013	AY294985
Cricetulus migratorius	AY294926	AY294956		AY294986
Neotominae				
Peromyscus leucopus	AY294927	AY294957	AY295014	AY294987
Reithrodontomys fulvescens	AY294928	AY294958	AY295015	AY294988
Neotoma floridana		AY294959		
Sigmodontinae				
Sigmodon hispidus	AF540641	AY241465	AY295016	AY241507
Oryzomys couesi	AF332020		AF332043	
Phyllotis xanthopygus	AF332023	AY241466		AY241508
Akodon boliviensis		AY294960		AY294989
Rhipidomys masticalis	AY294929	AY294961		AY294990
Irenomys tarsalis		AY294962		AY294991
Reithrodon auritus	AY294930	AY294963		AY294992
Andinomys edax		AY294964		AY294993
Auliscomys sublimis		AY294965		AY294994
Calomys lepidus	AY294931	AY294966		AY294995
Tylomyinae				
Ototylomys phyllotis	AY294932		AY295018	
Tylomys nudicaudus	AY294933		AY295019	