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Phylogeny of muroid rodents: relationships within and among major lineages as determined by IRBP gene sequences

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Abstract

The rodent family Muridae is the single most diverse family of mammals with over 1300 recognized species. We used DNA sequences from the first exon (~1200 bp) of the IRBP gene to infer phylogenetic relationships within and among the major lineages of muroid rodents. We included sequences from every recognized muroid subfamily except Platacanthomyinae and from all genera within the endemic Malagasy subfamily Nesomyinae, all recognized tribes of Sigmodontinae, and a broad sample of genera in Murinae. Phylogenetic analysis of the IRBP data suggest that muroid rodents can be sorted into five major lineages: (1) a basal clade containing the fossorial rodents in the subfamilies Spalacinae, Myospalacinae, and Rhizomyinae, (2) a clade of African and Malagasy genera comprising the subfamilies Petromyscinae, Mystromyinae, Cricetomyinae, Nesomyinae, and core dendromurines, (3) a clade of Old World taxa belonging to Murinae, Otomyinae, Gerbillinae, Acomyinae, and Lophiomyinae, (4) a clade uniting the subfamilies Sigmodontinae, Arvicolinae, and Cricetinae, and (5) a unique lineage containing the monotypic Calomyscinae. Although relationships among the latter four clades cannot be resolved, several well-supported supergeneric groupings within each are identified. A preliminary examination of molar tooth morphology on the resulting phylogeny suggests the triserial murid molar pattern as conceived by Simpson (1945) evolved at least three times during the course of muroid evolution.
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Keywords: Murid rodents; Muroidea; Nesomyinae; Sigmodontinae; Murinae; IRBP; Phylogeny; Evolution

1. Introduction

Numerically, the family Muridae (Musser and Carleton, 1993) is the single most diverse family of mammals with over 1300 recognized species. These species are distributed world-wide where they occupy a broad range of habitats from humid tropical forests to arid deserts to tundra and have adopted an equally wide array of lifestyles, including semi-aquatic, arboreal, scansorial, and fossorial. Species of muroid rodents have been the focus of studies in a number of disciplines including ecology (e.g., Kelt et al., 1996; Kessing, 1998; Leirs et al., 1997), behavior (e.g., Jackson, 1999; Pellis and Iwaniuk, 1999; Pillay, 2000), and functional morphology (e.g., Satoh, 1999). The role that many muroid species play as viral reservoirs and vectors of human disease has given studies of their ecology and phylogeny immediate importance (Bowen et al., 1997; Glass et al., 2002; Heyman et al., 2002). Perhaps most notably, the fecundity and adaptability of certain taxa (hamsters, gerbils, mice, and rats) have made them popular model organisms in biomedical research.

Despite advances in systematic research on muroid rodents (reviewed in Carleton and Musser, 1984; Michaux et al., 2001; Musser and Carleton, 1993), many aspects of muroid systematics from species diagnosis to phylogenetic relationships within and among lineages remain poorly understood. The currently recognized species of muroid rodents are allocated to nearly 300 genera in 17 subfamilies (Musser and Carleton, 1993). Some of these subfamilies contain only one or a few species (e.g., Lophiomyinae, Otomyinae, and Petromyscinae), while others encompass a considerable

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number of forms (e.g., Murinae with over 500 species, Sigmodontinae with over 300). The taxonomic history of muroids was reviewed most recently in Carleton and Musser (1984); we provide a brief summary of influential classifications below and in Table 1 to provide the necessary context for this study.

Muroid rodents have been treated variously as a family, with the major groups arranged as subfamilies, or as a superfamily, with groups raised to familial status. Regardless of taxonomic rank, the crown group Muroidea is currently understood as a clade of rodents with myomorphic jaw structure that have lost the upper fourth premolar and that have a well-developed anterocone (-id) on the first molar (Flynn et al., 1985). While early classifications of rodents lacked such an explicitly phylogenetic concept of Muroidea, they

Table 1
Selected previous classifications of muroid rodents

Alston (1876)	
Lophiomyidae	
Muridae:	Sminthinae (equivalent to Dipodidae); Hydromyinae; Platacanthomyinae; Gerbillinae (includes *Mystromys*, *Otomys*, *Dasymys*); Phloeomyinae; Dendromyinae (includes *Lophuromys*); Cricetinae; Murinae (includes *Nesomys*, *Brachytarsomys*, sigmodontines); Arvicolinae; Siphneinae (includes *Ellobius*, *Siphneus* = *Myospalax*)
Spalacidae:	Spalacinae (includes *Spalax*, *Rhizomys*, *Heterocephalus*); Bathyerginae
Thomas (1896)	
Muridae:	Hydromyinae; Rhynchomyinae; Phloeomyinae; Gerbillinae; Otomyinae; Dendromurinae; Murinae; Lophiomyinae; Sigmodontinae (includes nesomyines); Neotominae; Microtinae; Myospalacinae
Spalacidae:	Rhizomyinae; Spalacinae
Tullberg (1899) Muriformes	
Spalacidae (includes *Rhizomys*, *Tachyoryctes*, *Spalax*, *Myospalax*)	
Nesomyidae	
Cricetidae	
Lophiomyidae	
Arvicolidae	
Hesperomyidae (= Sigmodontinae)	
Muridae:	Murini (includes *Dendromus*, *Saccostomus*, *Steatomys*); Phloeomyini; Otomyini
Gerbillidae	
Miller and Gidley (1918) Muroidea	
Muscardinidae	
Cricetidae:	Cricetinae (includes sigmodontines, cricetines, and nesomyines); Gerbillinae; Microtinae; Lophiomyinae
Platacanthomyidae	
Rhizomyidae	
Spalacidae:	Myospalacinae; Spalacinae
Muridae:	Dendromyinae; Murinae; Phloeomyinae; Hydromyinae
Ellerman (1940, 1941) Muroidea	
Muscardinidae	
Lophiomyidae	
Spalacidae	
Rhizomyidae	
Muridae:	Murinae (includes *Eliurus*, cricetomyines); Rhynchomyinae; Hydromyinae; Dendromyinae (includes *Petromyscus*); Deomyinae; Otomyinae; Cricetinae (includes *Hypogeomys*, *Macrotarsomys*, *Calomyscus*); Gymnuromyinae; Tachyoryctinae (includes *Brachyuromys*); Gerbillinae; Myospalacinae; Microtinae (includes *Brachytarsomys*)
Simpson (1945) Muroidea	
Cricetidae:	Cricetinae (includes Hesperomyini, Cricetini, Myospalacini); Nesomyinae; Lophiomyinae; Microtinae; Gerbillinae
Spalacidae	
Rhizomyidae	
Muridae:	Murinae; Dendromurinae; Otomyinae; Phloeomyinae; Rhynchomyinae; Hydromyinae
Chaline et al. (1977) Muroidea	
Cricetidae:	† Cricetodontinae (and other extinct taxa); Hesperomyinae; Cricetinae; Spalacinae; Myospalacinae; Lophiomyinae; Platacanthomyinae
Nesomyidae:	† Afrocricetodontinae; Nesomyinae; Otomyinae
Rhizomyidae:	† Tachyoryctoidinae; Rhizomyinae
Gerbillidae:	† Myocricetodontinae; Gerbillinae
Arvicolidae	
Dendromuridae:	Dendromurinae; Petromyscinae
Cricetomyidae	
Muridae:	Murinae; Hydromyinae

generally recognized that the core muroids comprise those lineages with a myomorphic zygomatic plate and three cheek teeth. The principal debates surrounding definition of the Muroidea have revolved around two issues: (1) whether the fossorial taxa *Spalax*, *Rhizomys*, and *Tachyoryctes* should be part of the crown group Muroidea (following Ellerman, 1940, 1941; Miller and Gidley, 1918; Simpson, 1945; Tullberg, 1899) or should be a separate lineage of equal rank to the core muroids (following Alston, 1876; Thomas, 1896), and (2) whether dipodoids (following Alston, 1876), myoxids (following Ellerman, 1940, 1941; Miller and Gidley, 1918), or neither (following Simpson, 1945; Thomas, 1896) should be included in a concept of Muroidea. Recent systematic studies of molecular (Adkins et al., 2001, 2003; DeBry and Sagel, 2001; Huchon et al., 1999) and morphological (Flynn et al., 1985; Klingener, 1964; Wilson, 1949) data for rodents have reached the consensus that the muroid crown group includes these fossorial lineages and that the sister taxon to muroids is Dipodidae.

Within Muroidea, several major groups have been consistently recognized across most classifications either as families or subfamilies. These include gerbils (Gerbillinae), voles (Arvicolinae), Old World hamsters (Cricetinae), New World cricetines (Sigmodontinae), and Old World mice and rats (Murinae) plus several smaller groups including nesomyines, dendromurines, myospalacines, and lophiomyines. These lineages have in turn been sorted into higher-level groupings using a variety of evolutionary interpretations of character systems. Alston (1876), Thomas (1896) and Tullberg (1899), did not recognize much higher-order structure within muroids, but retained the major groups as independent lineages. Miller and Gidley (1918) were the first to impose structure among muroids by sorting the core lineages into two major groups—Cricetidae and Muridae—based on the arrangement of cusps on the upper molars; this division in turn formed the basis of Simpson's (1945) influential classification of rodents. Ellerman (1940, 1941) attempted to classify rodents based on characters rather than interpretations of evolutionary trends, but his resulting classification so radically rearranged subfamilial content that it is not accorded much credibility today. Chaline et al. (1977) also did not retain the established murid/cricetid division and added an important paleontological perspective to classification. Although many of their hypothesized ancestor-descendant relationships are disputed, their classification of extant taxa provided the first useful counter-example to the widely adopted murid/cricetid dichotomy and in many aspects, resembles the explicitly non-hierarchical classification used today (Musser and Carleton, 1993).

The principal challenges facing students of muroid systematics include determining the reality of subfamilial groupings and the relationships within and among them. Molecular data have recently been applied to some of these problems with encouraging results. These studies have discovered new lineages of muroids (e.g., Acomyinae [Chevret et al., 1993b]), questioned the validity of others (e.g., Dendromurinae [Verheyen et al., 1996], Otomyinae [Chevret et al., 1993b], Sigmodontinae [Engel et al., 1998], Nesomyinae [Jansa et al., 1999]) and described several higher-order associations among groups (e.g., a clade of gerbils and murines [Dubois et al., 1999] and a clade comprising sigmodontines, cricetines, and arvicolines [Michaux et al., 2001]). Nonetheless, numerous phylogenetic problems remain. The rapid evolution of mitochondrial markers used in some studies yielded relatively poor support for hypotheses of relationship at deeper levels of divergence (e.g., Engel et al., 1998; Jansa et al., 1999). The limited number of taxa employed in studies of muroid phylogeny using slowly evolving nuclear genes (Michaux and Catzeflis, 2000; Michaux et al., 2001) has precluded examining the monophyly of several groups including Nesomyinae, Sigmodontinae, and Cricetomyinae and could not address the phylogenetic position of several enigmatic taxa including *Lophiomys*, *Rhynchomys*, and *Phloeomys*. Finally, certain well-supported relationships uncovered in these studies—notably the position of *Myospalax* as nested well within Old World cricetines (Michaux and Catzeflis, 2000; Michaux et al., 2001)—are so surprising that that they demand to be tested with additional material.

Although our taxonomic sampling is far from exhaustive, it is representative of the diversity of muroids and is the most extensive to date. We have included representatives of all subfamilies except Platacanthomyinae, and have included all genera of Nesomyinae and Cricetomyinae, all tribes of Sigmodontinae, and a broad sample of murine genera. Moreover, we include taxa whose relationships have never been examined with molecular data; most notably, we include sequence from the monotypic subfamily Lophiomyinae. To examine relationships among muroids, we sequenced part of the first exon (ca. 1200 bp) of the gene encoding the Interphotoreceptor Retinoid Binding Protein (IRBP) for selected species. This region of nuclear DNA has been used extensively to address questions of mammalian interordinal phylogeny (Springer et al., 1997, 1999; Stanhope et al., 1992, 1996), and appears to be useful for discerning relationships at lower taxonomic levels as well (Jansa and Voss, 2000; Weksler, in press; Yoder and Irwin, 1999). The primary goals of this study are: (1) To test the monophyly of currently recognized subfamilies, with particular focus on Nesomyinae, the New World sigmodontines, and Murinae, (2) to provide new information regarding the interrelationships within and among major muroid lineages, and (3) to explore the utility of the IRBP gene for phylogenetic studies of muroid rodents.

2. Methods

2.1. Taxon sampling

We sequenced specimens from every recognized muroid subfamily save one (Platacanthomyinae). Within subfamilies, our choice of taxa was guided partly by availability of specimens, but we made an effort to sample the recognized diversity of each. In particular, we have included all genera within the endemic Malagasy subfamily Nesomyinae in order to test the monophyly of this morphologically diverse group. We have also included representatives of all recognized tribes of Sigmodontinae (McKenna and Bell, 1997; Smith and Patton, 1999) as well some with no clear tribal affinities (e.g., *Delomys*, *Scolomys*, *Reithrodon*, *Irenomys*, *Wiedomys*, *Nyctomys*, and *Tylomys*). Moreover, we recognized the division within Sigmodontinae between North American taxa with a simple glans penis ("neotomine-peromyscines"), South American taxa with a complex glans (Hooper and Musser, 1964), and the enigmatic Central American tylomyines (Carleton, 1980), and sampled genera from each. The diversity of Murinae (over 500 recognized species) thwarts exhaustive taxonomic sampling, but we have tried to capture the geographic diversity of the group by sampling both African and Asian representatives including the morphologically distinct Philippine taxa *Rhynchomys* and *Phloeomys*.

In addition, we included taxa whose placement in current taxonomy has been questioned. For example, *Acomys* and *Lophuromys* are traditionally classified as part of Murinae, but molecular evidence (Chevret et al., 1993b; Michaux and Catzeflis, 2000; Michaux et al., 2001; Sarich, 1985) suggests that they are part of a unique lineage ("Acomyinae") that is more closely related to gerbils than to murines. Similarly, *Deomys* has never been securely placed in Dendromurinae (Carleton and Musser, 1984; Ellerman, 1940), and recent molecular evidence suggests that this genus is also part of an acomyine clade (Denys et al., 1995; Verheyen et al., 1996). We made a special effort to include the enigmatic *Lophiomys*, the sole member of Lophiomyinae, which has not been included in any molecular study to date.

To root our phylogeny, we chose representatives of all four major lineages of Dipodidae as outgroups. In total, we included sequence from 74 muroid genera and five dipodid outgroups; 72 of these specimens were sequenced by us, the remainder was retrieved from GenBank (Table 2).

2.2. DNA amplification and sequencing

For most specimens, DNA was isolated from heart, liver, or kidney tissue that had been frozen or preserved

Table 2
List of taxa included in this study, their museum voucher numbers, and their subfamilial membership according to current classification (Musser and Carleton, 1993)

Taxon	Voucher number[a]	Subfamily
Clethrionomys gapperi	UMMZ 162467	Arvicolinae
Eothenomys melanogaster	{AY163583}[b]	Arvicolinae
Microtus sikimensis	{AY163593}[b]	Arvicolinae
Calomyscus baluchi	{AY163581}[b]	Calomyscinae
Cricetulus longicaudatus	USNM 449102	Cricetinae
Mesocricetus auratus	{AY163591}[b]	Cricetinae
Phodopus sungorus	{AY163631}[b]	Cricetinae
Beamys hindei	FMNH 151225	Cricetomyinae
Cricetomys emini	ROM 199510	Cricetomyinae
Saccostomus campestris	MNHN 1999-438	Cricetomyinae
Dendromus nyikae	FMNH 155544	Dendromurinae
Deomys ferrugineus	AMNH 269864	Dendromurinae
Steatomys parvus	CMNH 98495	Dendromurinae
Allactaga sibirica	USNM 449152	Dipodidae
Dipus sagitta	{DSA427232}	Dipodidae
Napaeozapus insignis	UMMZ 172382	Dipodidae
Sicista tianshanica	{AF297288}	Dipodidae
Zapus princeps	{AF297287}	Dipodidae
Meriones unguiculatus	AMNH 269948	Gerbillinae
Tatera robusta	FMNH 151230	Gerbillinae
Lophiomys imhausi	USNM 291730	Lophiomyinae
Acomys spinosissimus	FMNH 153939	Murinae
Aethomys chrysophilus	FMNH 168101	Murinae
Grammomys macmillani	FMNH 153945	Murinae
Hylomyscus denniae	FMNH 155566	Murinae
Lophuromys flavopunctatus	FMNH 155575	Murinae
Mastomys natalensis	FMNH 155603	Murinae
Maxomys whiteheadi	UMMZ 174492	Murinae
Micromys minutus	{AB033710}	Murinae
Mus musculus	{AF126968}	Murinae
Niviventer confuscianus	USNM 574361	Murinae
Phloeomys cumingi	USNM 573332	Murinae
Praomys delectorum	FMNH 153977	Murinae
Rattus exulans	USNM 458836	Murinae
Rhabdomys pumilo	FMNH 168114	Murinae
Rhynchomys isarogensis	USNM 573900	Murinae
Sundamys muelleri	UMMZ 174436	Murinae
Tokudaia osimensis	{AB033712}	Murinae
Myospalax aspalax	MSB 100576	Myospalacinae
Mystromys albicaudatus	{AY163594}[b]	Mystromyinae
Brachytarsomys albicauda	USNM 449351	Nesomyinae
Brachyuromys betsileoensis	FMNH 156227	Nesomyinae
Eliurus myoxinus	UA [SMG 8679]	Nesomyinae
Gymnuromys roberti	FMNH 156614	Nesomyinae
Hypogeomys antimena	FMNH 154636	Nesomyinae
Macrotarsomys bastardii	UA [FH 28]	Nesomyinae
Monticolomys koopmani	FMNH 15663	Nesomyinae

Table 2 (*continued*)

Taxon	Voucher number[a]	Subfamily
Nesomys rufus	FMNH 151915	Nesomyinae
Voalavo gymnocauda	FMNH 154051	Nesomyinae
Otomys anchietae	FMNH 155623	Otomyinae
Petromyscus collinus	TTU 55218	Petromyscinae
Rhizomys pruinosus	MVZ 186547	Rhizomyinae
Tachyoryctes splendens	CMNH 98212	Rhizomyinae
Abrothrix longipilis	{AY163577}[b]	Sigmodontinae
Akodon azarae	{AY163578}[b]	Sigmodontinae
Calomys lepidus	{AY163580}[b]	Sigmodontinae
Delomys sublineatus	{AY163582}[b]	Sigmodontinae
Irenomys tarsalis	{AY163587}[b]	Sigmodontinae
Juliomys pictipes	{AY163588}[b]	Sigmodontinae
Nectomys squamipes	{AY163598}[b]	Sigmodontinae
Neotoma lepida	{AY163599}[b]	Sigmodontinae
Notiomys edwardsii	{AY163602}[b]	Sigmodontinae
Nyctomys sumichrasti	{AY163603}[b]	Sigmodontinae
Oligoryzomys flavescens	{AY163609}[b]	Sigmodontinae
Oryzomys macconnelli	{AY163620}[b]	Sigmodontinae
Peromyscus maniculatus	{AY163630}[b]	Sigmodontinae
Phyllotis xanthopygus	{AY163632}[b]	Sigmodontinae
Reithrodon auritus	{AY163634}[b]	Sigmodontinae
Rheomys raptor	{AY163635}[b]	Sigmodontinae
Rhipidomys nitela	{AY163636}[b]	Sigmodontinae
Scapteromys tumidus	{AY163637}[b]	Sigmodontinae
Scolomys ucayalensis	{AY163638}[b]	Sigmodontinae
Scotinomys teguina	{AY163639}[b]	Sigmodontinae
Sigmodon alstoni	{AY163640}[b]	Sigmodontinae
Thomasomys baeops	{AY163642}[b]	Sigmodontinae
Tylomys nudicaudus	{AY163643}[b]	Sigmodontinae
Wiedomys pyrrhorhinos	{AY163644}[b]	Sigmodontinae
Zygodontomys brevicauda	{AY163645}[b]	Sigmodontinae
Spalax zemni	{SZU48589}	Spalacinae

[a] Museums are recognized by institutional acronym as follows: AMNH = American Museum of Natural History; CMNH = Carnegie Museum of Natural History; FMNH = Field Museum of Natural History; MNHN = Museé National d'Histoire Naturelle, Paris; MSB = Museum of Southwestern Biology; MVZ = Museum of Vertebrate Zoology, UC Berkeley; ROM = Royal Ontario Museum; TTU = Museum of Texas Tech University; UA = University of Antananarivo; UMMZ = University of Michigan Museum of Zoology; USNM = National Museum of Natural History, Smithsonian Institution. Numbers in braces are Genbank accession numbers, numbers in square brackets are collector numbers for uncatalogued specimens.

[b] Sequences reported in Weksler (in press).

in the field. For *Lophiomys* and *Deomys*, DNA was extracted from muscle or skin tissue taken from dried museum specimens (collected in 1950 and 1996 respectively). DNA was extracted from all tissues using a QiaAmp extraction kit (Qiagen Inc.). Extracted DNA was used as a template in PCRs with various combinations of primers. For most specimens, the first exon of IRBP was amplified from genomic DNA using primers IRBPA and IRBPB (as published in Stanhope et al., 1992). To generate fragments of a suitable size for sequencing, this product was used in two subsequent reamplification PCRs, one using primer IRBPA paired with IRBPF and one using primers IRBPE and IRBPB (see Jansa and Voss, 2000 for additional primer sequences). DNA extracted from *Deomys* was amplified in small fragments using primer pairs A2/F, J/F, E/D, and E/B (Jansa and Voss, 2000). DNA extracted from *Lophiomys* was amplified in 100–200 bp fragments with primers given in Table 3.

Additionally, we amplified and sequenced exon 28 of the von Willebrand Factor (vWF) gene from two specimens of *Myospalax aspalax* to check the identity of the *Myospalax* specimen reported in a previous study based on this gene (Michaux et al., 2001). Amplifications of this gene region were accomplished using primers vWF-A3 (a version of vWF-A [Porter et al., 1996] modified by removing 13 bp from the 3′ end) and W1 (Huchon et al., 1999). Secondary amplifications were done by pairing vWF-A3 and W1 with internal primers R-660 (5′ATCT CATCCCTTCTCTGCTCCA) and F-561 (5′GAAGAA GGTCATTNTGATCCC), respectively.

Initial amplifications using genomic DNA as template were performed as 20 μL reactions using Ampli-Taq Gold polymerase (Perkin–Elmer) and recommended concentrations of primers, nucleotides, buffer, and $MgCl_2$. These reactions were performed on a Perkin–Elmer 9700 thermal cycler using a four-stage touchdown protocol. The first stage consisted of 5 cycles of denaturation at 95 °C for 20 s, annealing at 58 °C for 15 s and extension at 72 °C for 60 s. The second and third stages were identical to the first except for lowered annealing temperatures of 56 and 54 °C, respectively. The final stage consisted of 25 cycles

Table 3
Names and DNA sequence of primers used in PCR amplification and sequencing of *Lophiomys imhausi* dried muscle tissue

Primer name	Primer sequence
F36	5′-TGAGAACYTGATGGGRATGCA
F47	5′-TrGGAATGCAAGCrGCCATtga
F102	5′-CTCAGACCCTCAGACRCTGGC
F223	5′-cTCACCAACCTCACCCGAGAAGA
F387	5′-CATRGRCACCTCCTCCTTGGT
F531	5′-CACCACAGAGATCTGGACCTTGCC
F724	5′-GGYCAGTCCRACTTCTTCCT
F836	5′-CAGArcAGGCCCTGGAAAAGGC
R204	5′-MVCCTCVAGGGTACTGGGCTC
R395	5′-CCCATGAGCTGVCTCCACA
R551	5′-GGYAAGGTCCAGATCTCTGTGG
R610	5′-GNCCRCTGGTGAGGACNACCAC
R688	5′-TCCTCTCACCCACCACGATGG
R860	5′-CCTTTTCCAGGGCCTGCTCTG
R881	5′-CKRCGCAGGGTVAGGATGG
R1244	5′-ATYTGTCRAAGCGCAGGTAGCCCA
R1294	5′-CCTGCYGCAGCACATAAGGGCCCA

In the primer name, letters "F" and "R" refer to forward and reverse, respectively; numbers correspond to where the 3′ end of the sequence falls on the laboratory mouse sequence as numbered from the initiation codon.

with an annealing temperature of 52 °C. The reaction series was preceded by an initial denaturation at 95 °C for 10 min and followed by a 7 min extension at 72 °C. Products were purified via electrophoresis through a 2% low melting point agarose gel. The appropriate band was excised from the gel using a Pasteur pipette, and the gel plug was melted in 300 μL sterile water at 73 °C for 20 min.

The resulting gel-purified product was used as a template in 30–40 μL reamplification reactions with Taq polymerase (Promega). Reactions were performed for 30–35 PCR cycles using an annealing temperature of 52 °C. The resulting PCR products were cleaned using either a Geneclean II system (Bio 101) or a QiaQuick PCR purification kit (Qiagen). PCR products were sequenced in both directions using amplification primers and dye-terminator chemistry (either dRhodamine or BigDye Ready Reaction Kits, Applied Biosystems, Inc.). Reactions were cleaned using either Sephadex beads or an ethanol precipitation protocol and run an ABI 377 automated sequencer. Sequences were edited and compiled using Sequencher 4.1 (GeneCodes). Base-calling ambiguities between strands were resolved either by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. All sequences have been deposited in GenBank with Accession Nos. AY326074–AY326114.

2.3. Data analysis

The resulting IRBP exon sequences were aligned manually with reference to the translated amino acid sequence. Aligned sequences were subjected to phylogenetic analysis using parsimony, maximum likelihood, and Bayesian approaches. Parsimony analysis was implemented using PAUP* 4.0b10 (Swofford, 2002) with informative characters treated as unordered and equally weighted. In order to improve the chances of finding the globally most-parsimonious trees, we used the parsimony ratchet (Nixon, 1999) as implemented in PAUPRat (Sikes and Lewis, 2001) to generate a pool of minimum-length trees for subsequent branch swapping. We performed 200 iterations of the ratchet with 15% of the characters perturbed for each iteration. This procedure generated a pool of 200 trees (all of which had identical lengths) that were then subjected to TBR branch swapping in PAUP. Bootstrap values (Felsenstein, 1985) for the parsimony analysis were calculated using 1000 pseudoreplicates with heuristic searches employed within each replicate (5 random addition replicates, TBR branch swapping, no more than 500 trees saved per replicate). Bremer support values (Bremer, 1994) were calculated by searching for the shortest tree(s) not consistent with a constraint tree containing a particular node. TreeRot (Sorenson, 1999) was used to create the constraint files and PAUP* commands for Bremer support calculations.

For maximum likelihood analysis, the best-fit model of nucleotide substitution was determined by evaluating the likelihood of various substitution models optimized on a neighbor-joining tree calculated from Jukes–Cantor corrected distances (Jukes and Cantor, 1969). We assessed the relative fit of eight models of nucleotide substitution: Jukes and Cantor (1969; JC69), Felsenstein (1981; F81), Hasegawa et al. (1985; HKY), Tamura and Nei (1993; TrN), Kimura (1980; K2P), Kimura (1981; K3P), Zharkikh (1994; SYM), and Rodriguez et al. (1990; GTR). We also assessed whether including parameters for site-specific rate heterogeneity (Γ-distributed rate parameter; Yang, 1994) and for a proportion of invariant sites (I) improved the fit of the model to the data. Lastly, we evaluated whether enforcing a molecular clock provided a better fit to the data than allowing substitution rates to vary across branches of the tree. The best-fit model was the one for which additional parameters no longer significantly improved the log-likelihood score, as determined with a likelihood-ratio test (Goldman, 1993; Huelsenbeck and Rannala, 1997). Subsequent to model evaluation and selection, the maximum-likelihood tree was determined using a heuristic search in PAUP* in which the parameter values under the best-fit model were fixed and a neighbor-joining tree was used as a starting point for TBR branch swapping. The resulting tree topology and new parameter estimates were used in a second round of branch swapping to provide the final maximum-likelihood tree; reported parameter values were estimated on this tree. Bootstrap support for nodes in the maximum-likelihood tree was evaluated for 100 pseudoreplicates using SPR branch swapping on starting trees obtained by neighbor joining.

Bayesian analysis was performed using MrBayes ver. 2.01 (Huelsenbeck and Ronquist, 2001). Because the starting conditions for Bayesian analysis can affect the probability of becoming trapped on local optima (Huelsenbeck et al., 2002), we performed four independent runs of 10 heated Markov chains each. For each run, we specified a model with six categories of base substitution, a gamma-distributed rate parameter, and a proportion of invariant sites. Uniform interval priors were assumed for all parameters except base composition, which assumed a Dirichlet prior. Runs were allowed to proceed for 2×10^5, 3×10^5, 1.2×10^6, and 5×10^6 generations, respectively, and trees were sampled every 100 generations for each run. To check that each run converged on a stable log-likelihood value, we plotted the log-likelihood values against generation time for each. We used the final (longest) run to calculate posterior probabilities for each node. We discarded the first 75,000 generations (750 trees) as burn-in, and constructed a 50% majority-rule consensus tree of the remaining trees to obtain posterior probability estimates for each node.

3. Results

3.1. Sequence characteristics

Alignment of the protein-coding IRBP sequences was straightforward. In total, seven insertion–deletion events were postulated in order to align the sequences to each other. Based on outgroup comparison, six of these are autapomorphic, including three single-codon insertions, one single-codon deletion, and two deletions of two adjacent codons. The seventh is a three-codon deletion that was present in both *Napaeozapus* and *Zapus* but absent in all other taxa. All insertion–deletion events were coded as missing data ("?") for purposes of phylogenetic analysis.

Because base compositional heterogeneity is known to affect phylogenetic inference (Galtier and Gouy, 1998; Galtier et al., 1999; Lockhart et al., 1994; Yang and Roberts, 1995), we examined base composition of all taxa prior to phylogenetic analysis. The average base composition for IRBP across taxa is reasonably even, with a slight bias towards G and C (Table 4). We evaluated each taxon for departures from the average base-composition across all three codon positions using a χ^2 goodness of fit test with the alpha value Bonferroni-adjusted for multiple tests ($\alpha = 0.00065$). None of the taxa showed a significant departure from expected base-composition values for first or second codon positions (for all taxa $\chi^2 < 17.18$; df = 3); however, two taxa showed significant departures from expected base composition values for third positions (*Mystromys*: $\chi^2 = 23.71$, $p = 2.9 \times 10^{-5}$; *Dipus*: $\chi^2 = 25.4$, $p = 4.4 \times 10^{-16}$; df = 3 for both tests). Because these taxa did not cluster as sister taxa in our phylogenetic analyses, we do not consider these deviations from base-compositional stationarity to be problematic for our data.

3.2. Phylogenetic analysis

Our data matrix consisted of 79 taxa scored for 1248 nucleotide positions. Parsimony analysis of these data resulted in 10,608 minimum-length trees, the strict consensus of which is shown in Fig. 1A. Consistent with our ingroup-outgroup dichotomy, muroids and dipodids form distinct, well-supported groups and we have rooted this tree between the two clades. The strict-consensus tree is highly resolved, with 60 of a possible 78 nodes retained. Polytomies are distributed throughout the tree and do not appear to be clustered either at the tips or base, suggesting that the IRBP data provide phylogenetic signal throughout the hierarchy. In addition, most nodes in the strict-consensus tree are well supported, and support is also distributed evenly throughout the tree. Of 60 resolved nodes, 54 were recovered in $\geqslant 50\%$, 45 in $\geqslant 75\%$, and 30 in $\geqslant 95\%$ of bootstrap replicates (Fig. 1A). Tree statistics associated with this topology are given in Table 5.

Log-likelihood tests among competing models identified the GTR + I + Γ model with no molecular clock as the best fit for these data; parameter estimates for trees resulting from analysis under this model are given in Table 5. The tree resulting from maximum likelihood analysis is entirely consistent with the tree resulting from Bayesian analysis under the same model; therefore, we show only the maximum likelihood tree in Fig. 1B and report both maximum likelihood bootstrap values and Bayesian posterior probabilities on this tree. The likelihood tree is missing five nodes (node numbers 27, 28, 31, 51, and 53) that were present in the strict consensus of most-parsimonious trees and contains 14 nodes (61–74) that were not present in the parsimony analysis. None of these conflicting nodes have bootstrap support $\geqslant 50\%$ in either the likelihood or parsimony analysis or have Bayesian posterior probabilities $\geqslant 95\%$ (Table 6). One notable exception concerns node 65 (Fig. 1B), which defines the position of the monotypic subfamily Lophiomyinae relative to other Old World taxa. This node is not present in the strict consensus parsimony tree (Fig. 1A; though it is present in 93% of the minimum length trees), but is recovered in 65 and 84% of bootstrap replicates in the parsimony and likelihood analyzes, respectively, and receives a significantly high posterior probability of 99% in the Bayesian analysis (Table 6). These results suggest that there is a small amount of conflicting signal in the IRBP dataset that prevents this node from being unambiguously resolved in the parsimony analysis. This conflict is apparently mitigated by applying an explicit model of nucleotide substitution, and this node is well supported by both likelihood bootstrap values and Bayesian posterior probabilities.

In general, nodes that are well supported in the likelihood analysis are also well supported in the parsimony analysis. Bootstrap support values calculated

Table 4
Average and range (in parentheses) of base composition (%) for each codon position across all ingroup and outgroup taxa

Position	A	C	G	T
First	21.5 (20.0–23.0)	28.4 (26.9–30.0)	38.0 (36.3–39.6)	12.1 (10.6–13.6)
Second	26.7 (26.8–27.6)	24.5 (23.2–26.8)	18.3 (17.3–19.2)	30.6 (30.0–32.4)
Third	15.4 (9.2–18.7)	33.2 (28.7–39.3)	32.5 (29.0–38.2)	18.9 (12.2–22.7)
All	21.2 (18.8–22.8)	28.7 (26.7–30.9)	29.6 (27.9–32.0)	20.6 (18.0–22.1)

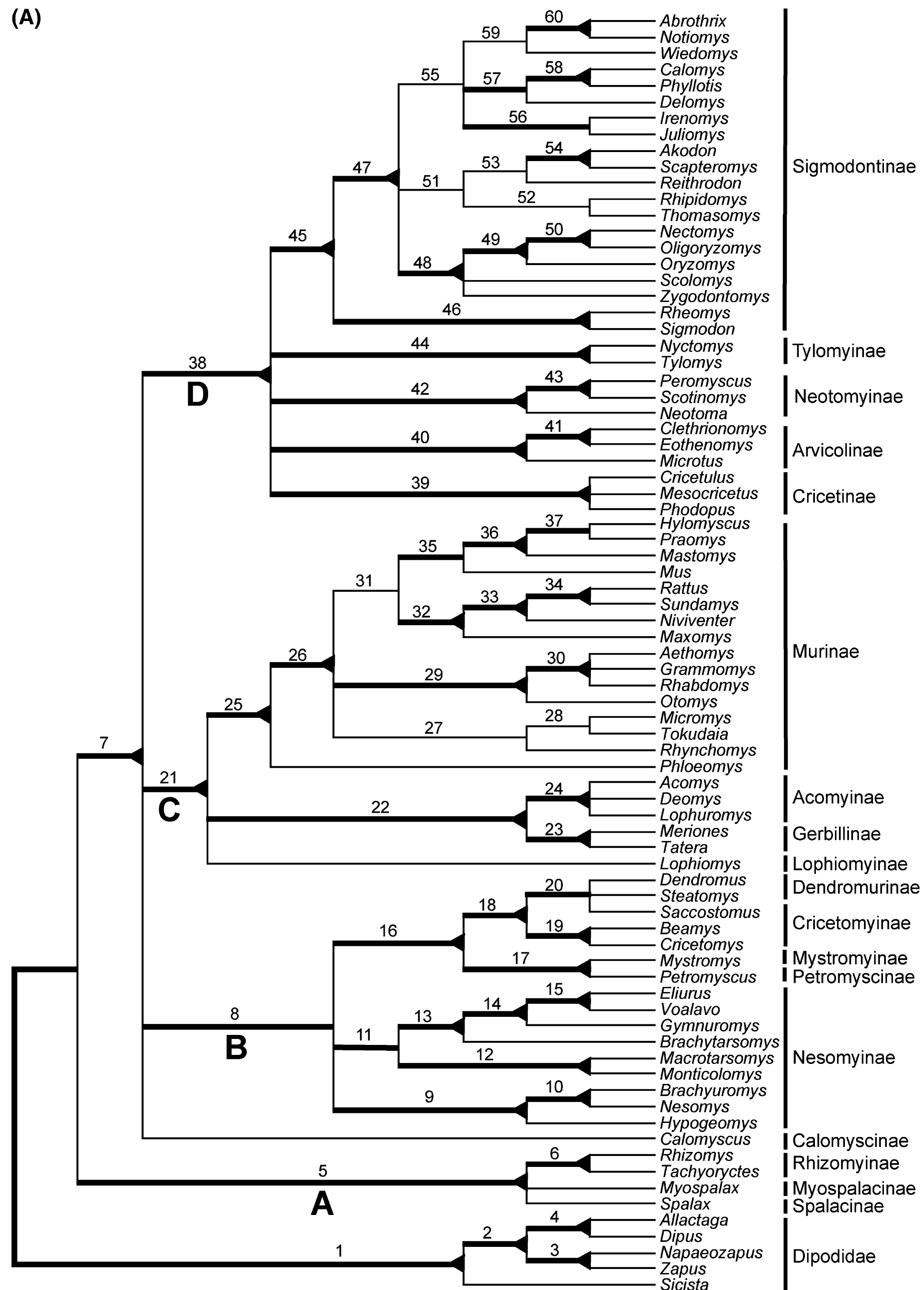

Fig. 1. Trees obtained from phylogenetic analysis of muroid IRBP sequences: (A) Strict consensus of 10,608 minimum-length trees resulting from parsimony analysis (length = 3171, CI = 0.38, RI = 0.60) and (B) the tree resulting from maximum likelihood analysis ($-\ln L = 17773.04$; parameter estimates given in Table 5). Numbers on the branches refer to node numbers in Table 5, letters A–D define clades discussed in the text. Branch thickness and color indicates nodal support as measured by bootstrap percentage (BP) or Bayesian posterior probabilities. For tree A, a thickened branch indicates 50% < BP < 75%, a thickened branch with a terminal buttress indicates BP ⩾75%. For tree B, a thickened branch indicates 50% < BP < 75% a thickened branch with a terminal buttress indicates BP ⩾75%; a black heavy line indicates Bayesian posterior probability ⩾95%, a gray heavy line indicates Bayesian posterior probability <95%.

under both parsimony and maximum-likelihood are significantly correlated as determined by a Spearman rank-correlation test ($r_s = 0.96$; $p < 0.0001$; df = 72). Only two nodes (52 and 61) receive bootstrap support greater than 50% in the likelihood analysis that are not equivalently supported in the parsimony analysis (Table 6); however, neither of these nodes would be considered well-supported, as they are recovered in less than 75% of bootstrap replicates in the likelihood analysis. Four nodes (11, 20, 56, and 62) are recovered with bootstrap support greater than 50% in the parsimony analysis that do not have comparable support from the likelihood bootstrap analysis; however, none of these are recovered with bootstrap support > 75%

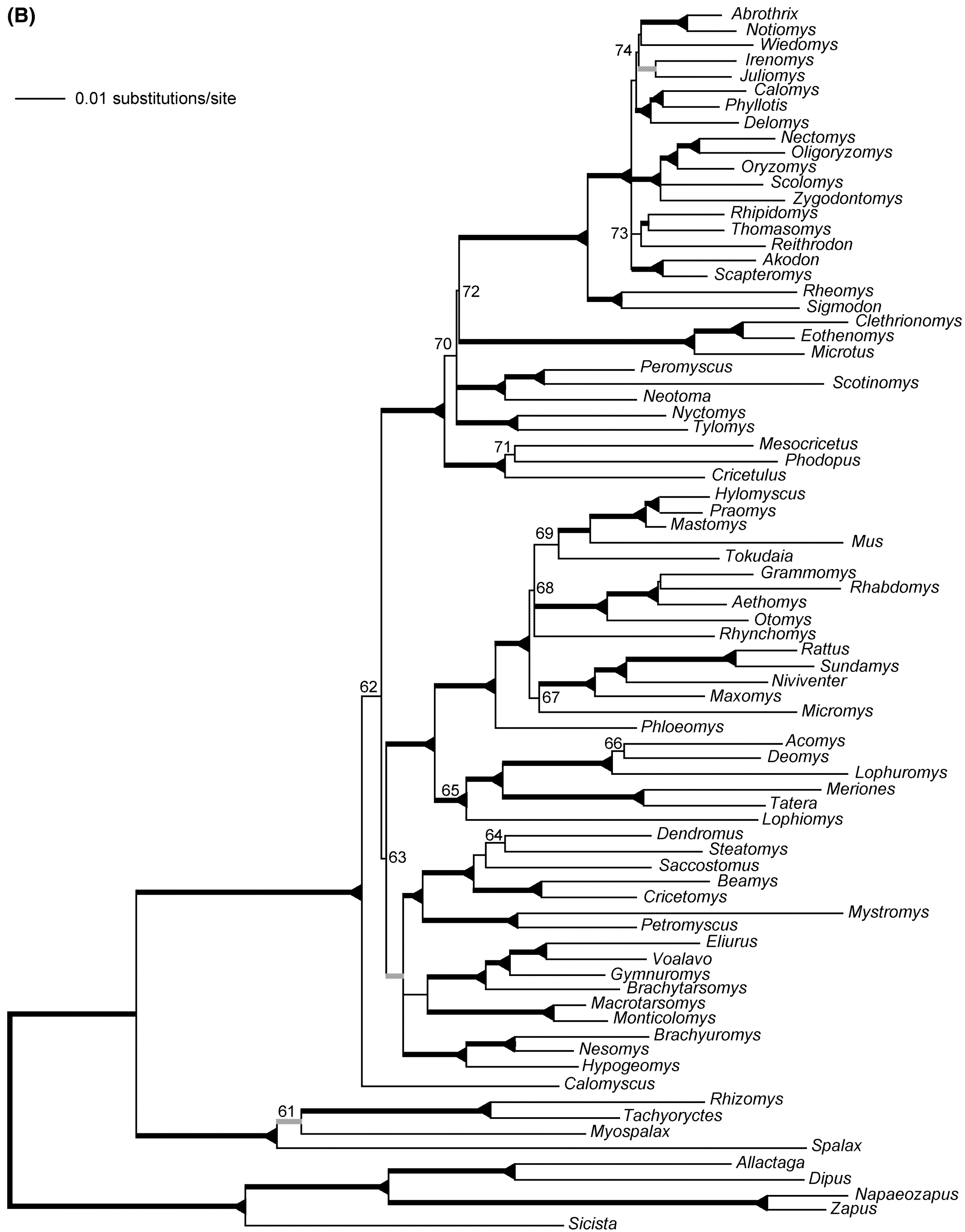

Fig. 1. (*continued*)

under either optimality criterion and cannot be considered robust.

There has been recent concern that Bayesian methods are too liberal and result in posterior probabilities that overestimate nodal support (Suzuki et al., 2002; but see Alfaro et al., 2003). We find no evidence of this phenomenon in our dataset. Bayesian posterior probability values are significantly correlated with both parsimony and likelihood bootstrap values as assessed with a Spearman rank-correlation test ($r_s = 0.852$, $p < 0.0001$, df = 72 and $r_s = 0.856$, $p < 0.0001$, df = 72, respectively). Moreover, only six nodes have significantly high posterior probabilities (⩾95%), but show parsimony bootstrap support less than 75% (node numbers 35, 37,

Table 5
Tree statistics (parsimony) and estimated substitution parameters (maximum likelihood) for the IRBP data

Parsimony tree statistics	
No. informative characters	590
No. minimum length trees	10608
Tree length[a]	3171 (3000)
CI[a]	0.378 (0.342)
RI	0.596
Likelihood parameter estimates	
r_{AC}	1.638
r_{AG}	6.294
r_{AT}	1.073
r_{CG}	0.726
r_{CT}	7.237
% A	0.219
% C	0.283
% G	0.295
% T	0.203
P_{inv}	0.313
α	1.285
$-\ln L$	17773.040

[a] Values excluding uninformative characters are given in parentheses.

49, 52, 57, and 65). Of these, only one (52) is recovered with bootstrap percentage less than 50%. Using a likelihood criterion, only two nodes (35 and 52) receive significantly high posterior probabilities, but are recovered with bootstrap support less than 75%. All of these nodes describe relationships among genera within well-supported clades and are subtended by relatively short branches. We suspect that with denser taxon sampling and additional sequence data, these mid-level relationships among genera will stabilize and support values from likelihood bootstrapping and Bayesian methods will converge.

3.3. Phylogenetic relationships

Five major lineages are common to both our parsimony and likelihood trees; four of these are labeled A, B, C, and D in Fig. 1A, *Calomyscus* forms the fifth. The most basal clade of muroids (clade A) securely unites the fossorial rodents currently assigned to the three subfamilies Spalacinae, Myospalacinae, and Rhizomyinae (node 5; Table 6). Although the monophyly of Rhizomyinae is well supported, relationships among the three lineages of fossorial muroids cannot be unequivocally resolved. The sister-group relationship between rhizomyines and myospalacines is consistent with 90% of the minimum length trees and is recovered with only marginal support in the likelihood analysis (node 61; Table 6).

Clade B contains the African and Malagasy genera referred to as "archaic muroids" (Carleton and Musser, 1984; Jansa and Carleton, 2003). This clade is the least well supported of the four major clades we identify and does not receive Bayesian posterior probability $\geqslant 95\%$ (node 8, Table 6). Within this clade, however, we identify four, well-supported lineages. The first (node 16) unites taxa distributed on mainland Africa that are assigned to the subfamilies Cricetomyinae, Petromyscinae, Mystromyinae, and Dendromurinae. The three remaining well-supported lineages (nodes 9, 12, and 13) describe relationships among the nine genera of muroids native to Madagascar. An additional node (node 11) describing relationships among the Malagasy taxa is recovered in both the likelihood and the parsimony analysis; however, this node receives negligible support (Table 6). A monophyletic Nesomyinae is neither supported nor contradicted by our data; the basal polytomy in clade B cannot be resolved in the maximum likelihood analysis and the three possible resolutions of this trichotomy are equally represented among the minimum-length trees.

Clade C contains the Old World taxa belonging to the recognized subfamilies Murinae, Otomyinae, Gerbillinae, and Lophiomyinae in addition to a recently discovered clade containing *Acomys*, *Deomys*, and *Lophuromys* (Chevret et al., 1993b; Verheyen et al., 1996). Our trees nest *Otomys* well within the Murinae; therefore, we cease to recognize the subfamilial rank of Otomyinae and include *Otomys* in Murinae. So defined, Murinae is a well-supported clade (node 25, Table 6), with the enigmatic Philippine endemic *Phloeomys* securely seated as its most basal member (node 26, Table 6). The other Philippine endemic included in this study, *Rhynchomys*, is less securely placed. The parsimony and likelihood analyses differ with respect to its position within Murinae, and neither alternative (nodes 27 and 68, respectively) is well supported (Table 6). Similarly, the Ryukyu island endemic *Tokudaia* (nodes 28 and 69) and the broadly-distributed Eurasian *Micromys* (nodes 28 and 67) cannot be securely placed within Murinae. In contrast, we recover three, extremely robust clades within Murinae. First, *Otomys* joins the African genera *Grammomys*, *Rhabdomys*, and *Aethomys* in a well-supported grouping (node 29, Table 6). Second, we recover a group uniting genera with a predominantly southeast Asian distribution (node 32). Finally, a second robust clade of African genera (node 36) joins with *Mus* to form a group with reasonable bootstrap support and 100% posterior probability (node 35; Table 6).

Also within clade C, we find a tight association between gerbils (node 23) and a clade corresponding to Acomyinae (node 24) containing genera that were, until recently, classified as murines (*Acomys* and *Lophuromys*) or dendromurines (*Deomys*). The monotypic subfamily Lophiomyinae joins this clade with 99% posterior probability and is recovered in 84% of the likelihood bootstrap replicates. Although this node (node 65) is not recovered in the parsimony tree, it is recovered in 65% of

Table 6
Metrics of nodal support for trees obtained under parsimony, maximum likelihood, and Bayesian analyses

Node	BPP	BPL	EPP	BSI
1	100	100	100	37
2	98	100	100	10
3	100	100	100	45
4	100	100	100	13
5	99	100	100	11
6	100	100	100	21
7	100	100	100	26
8	56	60	93	1
9	98	100	100	6
10	100	100	100	9
11	56	49	67	1
12	100	100	100	22
13	100	100	100	11
14	82	91	100	2
15	99	98	100	6
16	79	85	100	3
17	98	100	100	12
18	86	98	100	4
19	98	94	100	9
20	58	48	60	1
21	92	97	100	4
22	86	96	100	3
23	100	100	100	23
24	100	100	100	14
25	96	98	100	5
26	88	96	100	3
27	40	28	49	3
28	49	46	77	3
29	100	100	100	13
30	97	98	100	8
31	32	35	64	2
32	100	100	100	10
33	91	98	100	3
34	100	100	100	18
35	68	70	100	3
36	99	99	100	8
37	70	76	99	1
38	96	100	100	6
39	84	96	100	3
40	100	100	100	34
41	93	99	100	4
42	84	93	100	4
43	95	96	100	5
44	96	96	100	7
45	100	100	100	20
46	100	100	100	7
47	98	95	100	6
48	88	95	100	5
49	75	92	100	2
50	89	95	100	4
51	34	15	36	1
52	43	69	95	1
53	32	37	40	1
54	87	83	100	3
55	28	26	69	1
56	56	50	77	2
57	70	83	100	2
58	83	83	100	2
59	48	46	66	1
60	100	100	100	9
61	40	73	77	NA
62	53	48	62	NA
63	17	33	27	NA
64	21	38	55	NA
65	65	84	99	NA
66	44	45	55	NA
67	10	0	12	NA
68	0	25	14	NA
69	28	40	20	NA
70	28	46	57	NA
71	45	49	38	NA
72	28	22	44	NA
73	28	31	57	NA
74	23	0	40	NA

BPP = parsimony bootstrap percentage; BPL = likelihood bootstrap percentage; EPP = estimated Bayesian posterior probabilities (×100); BSI = Bremer support index. Node numbers refer to Fig. 1.

the parsimony bootstrap replicates, and the alternative resolution as the sister taxon to Murinae is recovered in only 7% of the minimum-length trees.

Clade D unites the subfamilies Sigmodontinae (sensu Musser and Carleton, 1993), Arvicolinae, and Cricetinae and is impressively corroborated by high Bremer, bootstrap, and Bayesian posterior probability values (node 38; Table 6). Within this clade, we recover several groups that correspond to commonly recognized tribes or subfamilies. Aside from the non-controversial subfamilies Arvicolinae (node 40) and Old World Cricetinae (node 39), we recover three separate, well-supported clades of New World mice and rats (nodes 42, 44, and 45) corresponding to neotomines, tylomyines, and sigmodontines, respectively (following Reig, 1984). Relationships among these three clades remain unresolved in the strict consensus parsimony tree. The likelihood tree disrupts monophyly of a broadly-defined Sigmodontinae (sensu Musser and Carleton, 1993) by inserting the arvicolines as the sister group to the core sigmodontines; however, nodal support for this arrangement is negligible (node 72; Table 6). Within the core sigmodontines, we find a well-supported basal dichotomy between a clade uniting *Rheomys* and *Sigmodon* (node 46) versus the remaining sigmodontines (node 47), but little structure among the tribes of Neotropical sigmodontines.

The fifth lineage contains the monotypic subfamily Calomyscinae. The phylogenetic position of *Calomyscus* was not resolved in the parsimony analysis, although the likelihood analysis places it as the most-basal divergence in the clade arising from node 7 (Fig. 1B). This position of *Calomyscus* among muroids is recovered in 90% of the minimum-length trees; the alternative resolutions with *Calomyscus* sister to clade D and sister to clade B are each consistent with 5% of the minimum-length trees. However, these two alternative resolutions do not have significantly different log-likelihood values from the most-likely topology as evaluated by a Shimodaira–

Hasegawa test (1000 RELL replicates; $\delta = 7.49$; $p = 0.270$ and $\delta = 8.51$; $p = 0.237$, respectively).

4. Discussion

The IRBP sequences analyzed for this study display many desirable qualities for phylogenetic inference among muroid genera. First, these protein-coding sequences are easily aligned, which makes positional homology statements unproblematic. Second, we find no compelling evidence for departure from base-compositional stationarity that might otherwise cause spurious attraction among unrelated lineages. Finally, the trees resulting from unweighted parsimony and maximum likelihood analyses are well-resolved and highly congruent, and nodes that differ between the two analyses are not strongly supported in either analysis, suggesting that these data are robust to the various assumptions made under the different optimality criteria. These characteristics bolster our confidence in the following principal phylogenetic conclusions of this study.

4.1. Clade A: A basal clade of fossorial rodents

One of the outstanding debates in muroid systematics is whether the burrowing muroids form a monophyletic group, or whether the morphological adaptations associated with underground life (reduced eyes and pinnae, reduced limbs, well-developed fore claws, fusiform body shape; Stein, 2000) have evolved independently. The three lineages of subterranean myomorphs—myospalacines, rhizomyines, and spalacines—have been recognized in most major classifications; however, the higher-level systematics among these groups has remained fluid. Nearly all possible combinations of taxa in the three groups have been suggested by various workers depending on which character systems were investigated and whether traits were interpreted as independently derived or not (Table 1).

The phylogenetic position of *Myospalax* has been particularly problematic. Most classifications have recognized it either as a unique lineage (Ellerman, 1940, 1941; Thomas, 1896) or as having close ties to other burrowing rodents (Miller and Gidley, 1918; Tullberg, 1899). Certain workers have commented on the similarities between myospalacine and arvicoline tooth structure (Alston, 1876; Ellerman, 1940; Hinton, 1926; Tullberg, 1899) but Lawrence (1991) dismissed these characteristics as parallel adaptations for chewing of fibrous plant material. Simpson (1945) nested *Myospalax* with the Old and New World hamsters in his subfamily Cricetinae "... supposing [*Myospalax*] to have been of cricetid ancestry and not highly divergent" (op. cit. p. 208). Following Simpson's views, Carleton and Musser (1984) considered *Myospalax* a primitive cricetid that secondarily adopted a fossorial lifestyle, but they conservatively retained Myospalacinae as an independent lineage in their classifications (Carleton and Musser, 1984; Musser and Carleton, 1993).

Recent analyses of sequences from the LCAT gene (Michaux and Catzeflis, 2000) and combined sequences from LCAT and vWF (Michaux et al., 2001) appear to confirm the position of *Myospalax* as part of the Old World cricetine radiation and not as a member of a clade including the subterranean spalacines and rhizomyines. This conclusion is in stark contrast to our IRBP results, which place all three of these lineages in a single, well-supported, basal clade. The discrepancy between our results and those based on other nuclear loci (Michaux and Catzeflis, 2000; Michaux et al., 2001) could result from the evolutionary dynamics of the IRBP locus in mammals with reduced eyes. A non-functional copy of the IRBP gene has been found in the blind marsupial mole (*Notoryctes*; Springer et al., 1997); it might be reasonable to expect the gene to have become non-functional or severely modified in these underground rodents as well. We believe we can dismiss this explanation of our results for the following reasons: (1) all of our IRBP sequences translate to open reading frame including those from *Spalax*, *Myospalax*, and the rhizomyines, suggesting that this gene is expressed in these rodents, (2) none of these fossorial lineages show an elevated rate of replacement substitutions, as would be expected if this gene were under selection or otherwise evolving rapidly in this lineage (results not shown, but see branch lengths in Fig. 1B).

Rather, we suspect that the specimen of "*Myospalax*" sequenced by Michaux and Catzeflis (2000) and Michaux et al. (2001) is not a representative of this genus but is in fact a true cricetine. To test this suspicion, we confirmed the identity of our *Myospalax* samples by examining voucher material (skin and skull preparations) and sequenced the vWF gene from our two tissue samples of this genus. The vWF sequences we obtained from these two specimens are identical to each other but differ from the sequence reported by Michaux et al. (2001) by 13.9% (uncorrected sequence divergence). Moreover, re-analysis of the existing vWF data with our *Myospalax* sequence included confirms that this genus is part of a well-supported basal lineage of burrowing muroids and not a derived member of Cricetinae (Fig. 2).

4.2. The unique lineage Calomyscinae

Calomyscus has been variously associated with New World sigmodontines (Pavlinov, 1980) and Old World cricetines (Vorontsov and Potapova, 1979) or as a unique lineage that does not tie in well with any extant muroid group (Carleton and Musser, 1984). Certain

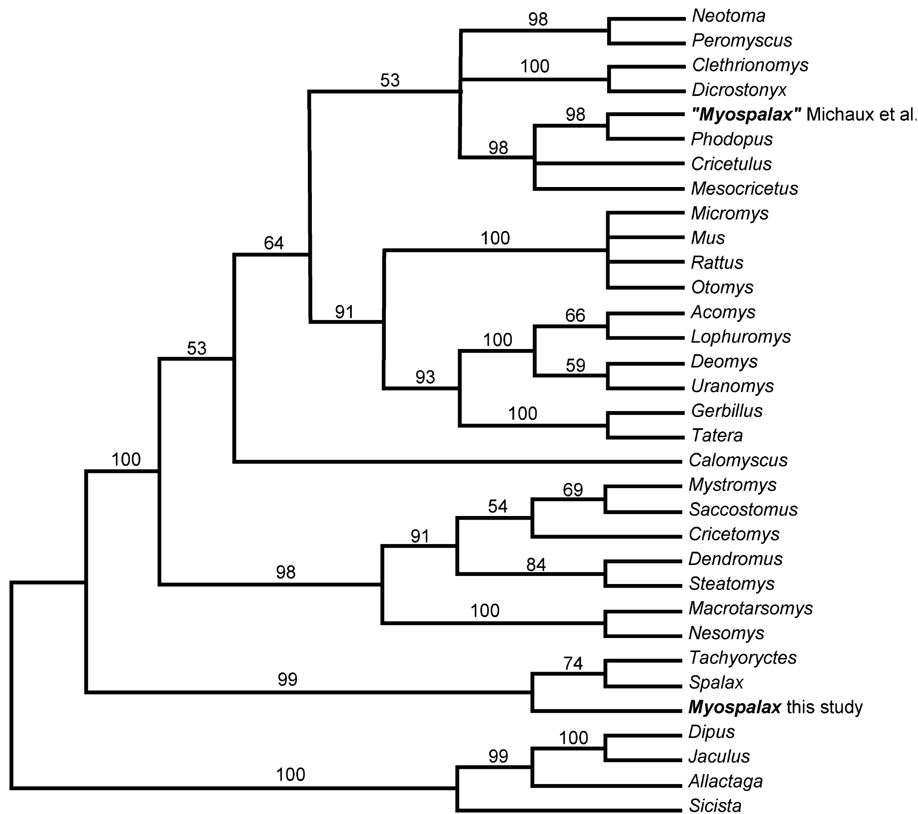

Fig. 2. Strict consensus of four minimum-length trees resulting from parsimony analysis of vWF sequences as reported by Michaux et al. (2001) including the new vWF sequence from *Myospalax* generated for this study. Our vWF sequence from *Myospalax* differs from the one reported by Michaux et al. (2001) and clusters with *Spalax* and *Tachyoryctes* rather than the Old World cricetines, indicating probable misidentification of "*Myospalax*" sequences included in their study. Numbers above branches are bootstrap percentages (1000 reps).

authors (Fahlbusch, 1969; Carleton and Musser, 1984) suggest that its affinities may lie with the Miocene *Democricetodon*, in which case *Calomyscus* may be the sole living member of the cricetodontines. The uniqueness of *Calomyscus* is confirmed by our IRBP sequence data and by other studies employing DNA sequence data (Michaux and Catzeflis, 2000; Michaux et al., 2001). These studies all place *Calomyscus* as an isolated lineage within the muroid radiation, but none can securely place it among the various muroid lineages. In contrast, an analysis of the LCAT and vWF genes (Michaux et al., 2001) identifies the archaic muroids as the most basal lineage of core muroids and places *Calomyscus* as sister to the remaining taxa, but support for this arrangement is slight. Therefore, none of these nuclear genes are able to convincingly resolve the position of *Calomyscus* within the core muroid radiation when analyzed separately or in combination. Assuming that the basal radiation of muroid rodents was not so rapid as to be irresolvable, it is clear that considerable quantities of sequence data from nuclear genes will be required to unambiguously resolve the position of *Calomyscus* among the core muroid lineages.

4.3. Clade B: A separate radiation of African and Malagasy muroids

The IRBP data recover a clade of taxa that are confined to sub-Saharan Africa and Madagascar (Clade B; Fig. 1A). Most accounts of muroid evolution have emphasized the ancient derivation of taxa in this clade (Major, 1896, 1897; Simpson, 1945; reviewed in Jansa and Carleton, in press). The IRBP data cannot resolve the position of this clade relative to the other major groupings of muroids, but it is clear that none of these genera have a recent origin, as has been suggested by some workers (Ellerman, 1941). Lavocat (1973, 1978) and Chaline et al. (1977) provided the most explicit scenarios for the evolution of archaic African and Malagasy muroids, whereby the genera included in Nesomyinae, Dendromurinae, Petromyscinae, Cricetomyinae, and Mystromyinae were considered relicts of a cricetodontine stock present in Africa since the early Miocene. Carleton and Musser (1984) suggested that, with future research, the genera currently included in these subfamilies could be arrayed as lineages in an inclusive archaic Afro-Malagasy clade, but that the limits of these lineages would most likely not correspond to

current subfamilial definitions, a prediction that seems to have been realized by recent studies.

Although the IRBP data provide scant support for this archaic Afro-Malagasy clade, other molecular studies (Michaux and Catzeflis, 2000; Michaux et al., 2001) have recovered a robust group including mystromyines, cricetomyines, dendromurines, and two nesomyine taxa. The lack of support for this clade from the IRBP data is disappointing and is not simply an artifact of our denser sampling of muroid taxa. When we reduce the number of muroid taxa in our dataset to agree with the sample used by Michaux et al. (2001), we still recover this clade with low bootstrap support. These results suggest that support for this clade will be bolstered by combining the IRBP data with vWF, LCAT, and other nuclear genes from the same dense sampling of taxa.

Within this clade, however, several groupings are well supported. The monotypic Petromyscinae and Mystromyinae join to form a secure clade as was previously suggested, but not supported by an analysis of cytochrome *b* sequences (Jansa et al., 1999). It is now well-established that Dendromurinae (sensu Musser and Carleton, 1993) is not a natural grouping: several molecular datasets (including this one) support the result that *Deomys*, which is traditionally classified as a dendromurine, is part of Acomyinae. However, ours is the first study to test the monophyly of Cricetomyinae by including all three genera ascribed to the group. The IRBP data suggest that this subfamily may not be monophyletic as *Saccostomus* groups with dendromurines. Support for this grouping is weak, however, and trees consistent with cricetomyine monophyly are not significantly different from the most likely tree (Shimodaira–Hasegawa test; 1000 RELL replicates, $\delta = 7.25$, $p = 0.235$).

In an effort to test the monophyly of Nesomyinae, we included sequences from all nine genera, but could neither confirm nor refute monophyly of these native Malagasy rodents. Despite the ambiguity of nesomyine monophyly, several groupings among the nine genera are well-supported by this dataset. Three of these clades (nodes 10, 12, and 15) are consistent with prior analysis of nesomyine phylogeny using the cytochrome *b* gene (Jansa et al., 1999). Moreover, the IRBP data reveal additional, well-supported groupings among the Malagasy rodents that were not apparent with the mitochondrial dataset. Whereas cytochrome *b* was unable to resolve the position of *Hypogeomys*, *Gymnuromys*, or *Brachytarsomys* with any confidence, the IRBP data place each of these genera securely with other nesomyines (Fig. 1).

4.4. Clade C: Murines, acomyines, gerbils, and *Lophiomys*

Although traditional classifications have placed *Acomys*, *Uranomys*, and *Lophuromys* as true murines (Carleton and Musser, 1984; Musser and Carleton, 1993), molecular data have consistently recovered a unique group including these three genera plus the dendromurine *Deomys* (Chevret et al., 1993b; Denys et al., 1995; Michaux and Catzeflis, 2000; Michaux et al., 2001; Sarich, 1985; Verheyen et al., 1996). Results from our IRBP data are consistent with those from other nuclear genes (Dubois et al., 1999; Michaux et al., 2001) in supporting a clade of acomyines and gerbils that is sister to true murines. Moreover, our study provides the first molecular evidence to place the monotypic Lophiomyinae as the sister group to the Acomyinae.

4.4.1. *Lophiomys* is not a cricetine

The position of Lophiomyinae within clade C deserves special attention as this is the first time the phylogeny of this taxon has been addressed using molecular data. The phylogenetic affinities of *Lophiomys* have puzzled systematists due to the simplified tooth structure and unusual skull architecture exhibited by this genus. Most workers have either recognized *Lophiomys* as a unique lineage apart from other muroids (Ellerman, 1941; Thomas, 1896; Tullberg, 1899) or grouped it with cricetids (Chaline et al., 1977; Miller and Gidley, 1918; Simpson, 1945). Lavocat (1973) departed from these classifications and placed *Lophiomys* in the family Nesomyidae, reflecting his opinion that this genus is part of the early African radiation of muroids that descended from the Afrocricetodontidae. In contrast to these classifications, the IRBP sequences place *Lophiomys* in a clade with Murinae, Acomyinae, and Gerbillinae. The parsimony analysis is unable to unambiguously resolve the position of *Lophiomys* among these lineages, but the likelihood analysis strongly supports its position as sister to the Acomyinae + Gerbillinae (node 65; Table 6). The IRBP data also significantly reject the alternative traditional placements of *Lophiomys* as the sister taxon to clade D, to the Old World cricetines, or to clade B (Shimodaira–Hasegawa test: 1000 RELL replicates; $\delta = 23.99$, $p = 0.005$; $\delta = 42.50$, $p < 0.005$; $\delta = 23.22$, $p = 0.006$, respectively).

To our knowledge, this placement of *Lophiomys* has not been suggested previously and no morphological studies exist to support this hypothesis. We suspect the lack of nonmolecular support for a clade including *Lophiomys* with acomyines and gerbils is simply because these taxa have never been rigorously compared to each other. Because *Lophiomys* has only been considered closely related to cricetines or extinct cricetids, morphologists have never looked beyond these groups for comparative material (e.g., Aguilar and Thaler, 1987; Lavocat, 1973; Wahlert, 1984). Moreover, the morphological distinctiveness of gerbils has perhaps discouraged their inclusion in broad-scale comparative studies, thus masking any close ties to acomyines or *Lophiomys*. Our IRBP findings suggest that a fresh

assessment of lophiomyine morphology including comparative material from gerbils, acomyines, and true murines is in order.

4.4.2. Relationships within Murinae

Current classifications (Carleton and Musser, 1984; Musser and Carleton, 1993) retain the genera *Otomys* and *Parotomys* as a separate subfamily (Otomyinae), reflecting the uncertain placement of these genera in previous classifications of muroids (e.g., Chaline et al., 1977; Lavocat , 1978; Misonne, 1974; Simpson, 1945). In contrast, our IRBP results agree with recent molecular (Chevret et al., 1993a; Michaux et al., 2001; Watts and Baverstock, 1995) and paleontological (Pocock, 1976; Senegas and Avery, 1998) studies that *Otomys* is nested within Murinae, and that a subfamilial rank for otomyines is unwarranted. While this conclusion is now uncontroversial, the phylogenetic position of *Otomys* among other murine genera has been difficult to resolve with any certainty. Carleton and Musser (1984) suggested that its affinities would be found with one or another arvicanthine genera, while Pocock (1976) postulated that otomyines were descended from a *Pelomys* -like ancestor. DNA-hybridization data (Chevret et al., 1993a) showed that *Otomys* was more closely related to *Arvicanthis* and *Oenomys* than to *Mus*, *Rattus*, or *Acomys*, but this study lacks a broad range of murine taxa. In contrast, a recent analysis of mitochondrial DNA from a suitably broad taxonomic sample found *Otomys* to be the basal member of a more inclusive "African lineage" consisting of arvicanthines, *Dasymys*, *Hybomys*, *Aethomys*, *Grammomys*, and *Otomys* (Ducroz et al., 2001). Whereas mtDNA data provided very little support for this arrangement, the IRBP data provide convincing evidence for such an "African lineage" and further confirm that *Otomys* is the basal member of this clade.

Delineation of natural groupings within true murines has been a notoriously difficult phylogenetic problem. Among murines, the giant cloud rat *Phloeomys* has historically been considered unique to the extent that most workers have placed it in its own lineage apart from other murines (Miller and Gidley, 1918; Thomas, 1896) or with other enigmatic genera (Simpson, 1945). In their revision of Philippine rodents, Musser and Heaney (1992) commented on the number of primitive character states retained by *Phloeomys*. Our IRBP results at least partially confirm these observations. *Phloeomys* is securely seated as the basal member of the murine radiation, a result that corroborates those from microcomplement fixation studies (Watts and Baverstock, 1995) and morphology (Heaney and Rickart, 1990). Unfortunately, the other endemic Philippine taxon included in this study—the shrew rat *Rhynchomys*—cannot be placed with any certainty in the present phylogenetic scheme beyond the conclusion that it is not closely related to *Phloeomys*. Evaluation of the suggestion that phylogenetic affinities of *Phloeomys* and *Rhynchomys* lie with other Old Endemic rodents east of the Sunda Shelf (Musser, 1981a; Musser and Heaney, 1992) awaits completion of ongoing studies (Jansa and Heaney, in prep.).

Splitting *Rattus* into several well-defined genera has done much to improve our understanding of murine diversity and evolution (Musser, 1981a,b; Musser and Newcomb, 1983). Three of the genera included in this study—*Maxomys*, *Sundamys*, and *Niviventer*—were each split from an inclusive *Rattus* (sensu Ellerman, 1940, 1941), but phylogenetic relationships among these taxa and other murine rodents have not been rigorously tested. The IRBP data place these four genera in a clade to the exclusion of African taxa and the two Philippine genera included in our study. To the extent that our taxon sampling can address relationships among southeast Asian murines, our results tentatively confirm the suggestion that there are at least two separate groupings in southeast Asia: the Old Endemics, here represented by *Phloeomys* and *Rhynchomys*, and the New Endemics, represented here by the clade including *Rattus*, *Sundamys*, *Niviventer*, and *Maxomys*. Clearly, additional taxon sampling throughout Australasia will be required to rigorously test this hypothesis.

In addition to this Asian murine clade, we recover two well-supported clades of murines with primarily African distribution. The first (node 36, Fig. 1) unites genera in the *Praomys* group (Chevret et al., 1994), the second (node 30, Fig. 1) ties together *Rhabdomys*, our sole representative of the *Arvicanthis* division (sensu Musser, 1987), with the African murines *Aethomys* and *Grammomys*. Both of these clades are recovered with high nodal support, and neither can be considered particularly controversial. At various times, *Hylomyscus* and *Mastomys* have been considered subgenera of *Praomys* (Davis, 1962; Misonne, 1969), and DNA hybridization show these three as a tightly-knit unit apart from *Mus* and *Rattus* (Chevret et al., 1994). Although the contents of an *Arvicanthis* division have varied (compare Misonne, 1974 with Musser, 1987), there is general consensus that arvicanthines cluster with *Aethomys* and possibly *Grammomys*, a result that was also suggested (but with scant support) by a recent analysis of mitochondrial DNA (Ducroz et al., 2001).

The phylogenetic position of *Mus* has been historically difficult to resolve. There is general agreement that *Mus* is not particularly closely related to *Rattus* (Jouvin-Marche et al., 1988; She et al., 1990; Lundrigan et al., 2002; Musser and Newcomb, 1983), but few studies have included a broad enough sampling of taxa to definitively address the position of *Mus* among other murines. The one phylogenetic study to include a comprehensive sample of murine genera (Ducroz et al., 2001) was unable to unequivocally resolve the placement of *Mus* among the included taxa, a result that was probably due

to the rapid evolution of the mitochondrial genes used in that study. Our study of the slowly-evolving nuclear IRBP exon includes a reasonably broad sample of murine taxa and provides convincing evidence that *Mus* is the sister-taxon to the *Praomys* group (Fig. 1). Given that the IRBP data are able to confidently resolve relationships among several murine genera, it appears that the lack of resolution apparent in the mitochondrial DNA is not due to a rapid radiation among murines as Ducroz et al. (2001) tentatively suggest.

4.5. Clade D: "Cricetidae"

The clade comprising sigmodontines (sensu Musser and Carleton, 1993), arvicolines, and 'true' cricetines (i.e., excluding *Calomyscus*, *Mystromys*, and *Myospalax*) is one of the strongest higher-level groupings to emerge from this study (Clade D; Fig. 1). A number of recent molecular studies have recovered this clade as well (Conroy and Cook, 1999; Dubois et al., 1999; Engel et al., 1998; Michaux and Catzeflis, 2000; Michaux et al., 2001; Robinson et al., 1997), though none has included as broad a range of muroid taxa as are included herein. While molecular data seem to be unambiguous in recovering this group, morphological evidence for this clade is equivocal.

Traditionally, sigmodontines, cricetines, and arvicolines have been placed in a group along with various other taxa exhibiting a biserial arrangement of cusps on the molars. Miller and Gidley (1918) were the first to propose a definition of Cricetidae that included cricetines, sigmodontines, and arvicolines together with *Mystromys*, *Calomyscus*, *Lophiomys*, gerbils, and nesomyines. Simpson (1945) retained this arrangement, but also included *Myospalax* in his Cricetidae. Later classifications maintained a broadly inclusive Cricetidae, but placed arvicolines in a separate family (Chaline et al., 1977; Honacki et al., 1982; Reig, 1980). Some authors have restricted Cricetidae to include only cricetines, sigmodontines, and myospalacines (Jacobs and Lindsay, 1984) or have included sigmodontines and cricetines with several fossil taxa (Flynn et al., 1985). Recent classifications (Musser and Carleton, 1993) place cricetines, sigmodontines, and arvicolines in separate groups of equal rank. Unfortunately, there has been no explicit phylogenetic analysis to date of morphological data focused on muroid higher level relationships involving a comprehensive sampling of all (or most) muroid subfamilies. The present study, coupled with strong results from other molecular studies, provides a robust framework for re-evaluating the morphological evolution within this clade.

4.5.1. Relationships within "Cricetidae"

While the IRBP data provide convincing evidence for a single clade of Palearctic hamsters, Holarctic voles, and New World rats and mice, relationships among the major lineages within this clade cannot be confidently resolved. Analysis of the IRBP data recovers the two non-controversial clades Arvicolinae and Cricetinae; however, the monophyly of New World Sigmodontinae has been more difficult to establish. Sigmodontinae, as employed in recent classifications (e.g., McKenna and Bell, 1997; Musser and Carleton, 1993; Nowak, 1999), generally refers to a group encompassing all non-arvicoline, New World muroids, following the definition of the subfamily given by Carleton and Musser (1984). The IRBP data, however, cannot recover monophyly of Sigmodontinae in this sense. Rather, three separate monophyletic groups containing sigmodontine taxa are recognized within clade D: a clade containing the predominantly South American taxa (node 45), one including neotomines and peromyscines (node 42), and one comprising tylomyines (node 44). Although the IRBP data cannot convincingly recover monophyly of a broadly defined Sigmodontinae in either parsimony or likelihood analyses, this clade is still consistent with our data: a monophyletic Sigmodontinae appears among the minimum length trees from the parsimony analysis and is not significantly different from the maximum likelihood tree (Shimodaira–Hasegawa test; 1000 RELL replicates, $\delta = 0.036$; $p = 0.397$).

The division of sigmodontines into three main groups is consistent with the taxonomic arrangement proposed by Reig (1984), who designated subfamilies for each of the three clades recovered here: Sigmodontinae, Neotominae, and Tylomyinae. Although Reig (1984) based his division of the evolutionary interpretation of various morphological characters, the only phylogenetic evidence for a tripartite division among sigmodontines comes from a parsimony analysis of the mitochondrial NADH gene (Engel et al., 1998). As with our IRBP study, these mitochondrial data were unable to convincingly resolve relationships among these three groups; however, the molecular evidence suggests that each should be recognized as a formal taxonomic entity.

First, the IRBP data corroborate the monophyly of neotomine-peromyscines, referred to hereafter as Neotominae (following Reig, 1984). This result corroborates the assessment that the simple phallic structure is a synapomorphy for this group (Hooper, 1958, 1959, 1960; Hooper and Musser, 1964). Second, we recover a well-supported clade including *Nyctomys* and *Tylomys*, which we refer to as Tylomyinae (Reig, 1984). Both *Nyctomys* and *Tylomys*, and the associated genera *Otonyctomys* and *Ototylomys*, have been tentatively placed in various separate groups throughout muroid taxonomic history (Arata, 1964; Carleton, 1980; Hershkovitz, 1944, 1962; Hooper and Musser, 1964; Voss and Linzey, 1981). The IRBP data firmly support a clade corresponding to Tylomyinae, but further molecular studies should include *Ototylomys* and *Otonyctomys* as well. Finally, we recover a clade of New World cricetines

that are distributed predominantly in the Neotropical region (node 45; Fig. 1) and restrict the name Sigmodontinae (following Reig, 1984) to this group. Studies based on several data sources including morphology (Carleton, 1980; Hooper and Musser, 1964), micro-complement fixation (Sarich, 1985), and mitochondrial DNA sequences (Engel et al., 1998) also recover this group, and it is consistent with the taxonomy of associated endo- and ecto-parasites (Slaughter and Ubelaker, 1984; Wenzel and Tipton, 1966). The only study to notably fail in recovering a monophyletic Sigmodontinae is Steppan's (1995) analysis of morphological characters; however, he acknowledges that this result was probably due to homoplasy in several of his characters.

While the IRBP data consistently recover recognized tribes of sigmodontines (Oryzomyini, Thomasomyini, Akodontini, Phyllotini, and Abrothrichini; nodes 48, 52, 54, 58, and 60, respectively), relationships among these tribes and several enigmatic sigmodontines (*Wiedomys*, *Delomys*, *Irenomys*, *Juliomys*, and *Reithrodon*) cannot be convincingly resolved. Failure to resolve relationships among sigmodontine tribes is not a novel result. Morphological (Steppan, 1995) and mitochondrial (Engel et al., 1998; Smith and Patton, 1999) data, as well as a study of IRBP with a denser sampling of sigmodontines (Weksler, in press) all fail to recover well-supported relationships among sigmodontine tribes. The only higher-level relationship among sigmodontines recovered with strong support, apart from tribal groupings, is the basal split between the clade including *Rheomys* + *Sigmodon* and the remaining sigmodontines. The basal position of *Sigmodon* among sigmodontines is consistent with analyses of mitochondrial data (Engel et al., 1998; Smith and Patton, 1999); however, only one other molecular study has addressed the phylogenetic position of the ichthyomyine *Rheomys* among other sigmodontine taxa (Weksler, in press). Because this study was also based on IRBP sequence data, it is not surprising that it supports the same sister-taxon relationship between *Rheomys* and *Sigmodon* recovered here (node 46; Fig. 1A).

4.6. A reevaluation of the murid vs. cricetid molar plan

Early classifications of muroids established a fundamental division among taxa based on the arrangement of the principal cusps of the upper molars (Schaub, 1958; Simpson, 1945; Stehlin and Schaub, 1951). These studies established two principal molar patterns: the cricetid plan, characterized by two longitudinal series of cusps, and the murid plan, with three such series. Although sigmodontines, cricetines, and arvicolines all display the cricetid plan, several other groups also have this pattern, including calomyscines, mystromyines, gerbils, lophiomyines, myospalacines, and some nesomyines. The murid plan is displayed most clearly in murine rodents (including acomyines), but some workers (Ellerman, 1941; Simpson, 1945) have considered the triserial arrangement of cusps in dendromurines an example of the murid condition as well (but see Lavocat (1964) for an interpretation of the lingual cingular cusp as independently derived from the cricetid condition). Similarly, cricetomyines have been shuffled back and forth between murids (Ellerman, 1941) and cricetids (Petter, 1966), reflecting alternative interpretations of the third row of lingual cusps on their upper molars.

To examine the evolution of molar structure among muroids, we coded each taxon as displaying either the cricetid molar condition (two longitudinal rows of cusps), the murid condition (three rows), or an indeterminate condition (i.e., the condition exhibited in rhizomyines, *Spalax*, and the outgroup taxa). Our coding thus corresponds to the concept of the murid and cricetid molar plans as used by Simpson (1945). Optimization of these character states on the maximum-likelihood topology reveals that a triserial arrangement of cusps (the murid condition) has evolved at least three times among muroids: once in true murines, once in acomyines, and once in the clade including cricetomyines and true dendromurines (Fig. 3). Various interpretations of the evolution of molar morphology have generally emphasized the primitive nature of the cricetid pattern (Miller and Gidley, 1918; Simpson, 1945; Petter, 1966). Whereas none of these interpretations was grounded in a phylogenetic framework, our explicitly phylogenetic study supports the conclusion that the cricetid plan is the primitive condition among the core muroids (Fig. 3). Moreover, this molar morphology also seems to have evolved at least twice: once in *Myospalax* and once in the lineage leading to the core muroids.

As this study reveals, interrelationships among muroids appear to be far more complex than this simple division between cricetids and murids. Our phylogenetic analysis suggests that a grouping of all taxa with the cricetid molar plan, as suggested most notably by Simpson (1945), defines a polyphyletic assemblage that would indefensibly be based on the symplesiomorphic condition of a single character. Nonetheless, there is apparently more phylogenetic structure among the lineages of muroids than is suggested by recent classifications (e.g., Musser and Carleton, 1993). The available molecular evidence (this study and that of Michaux et al., 2001) suggests that muroid rodents can be divided into five major lineages (clades A through D plus *Calomyscus*; Fig. 1), one of which (clade A) could defensibly be excluded from Muroidea. The pattern of relationships among these lineages is still an open question, and we refrain from naming them pending further morphological and molecular studies of these taxa.

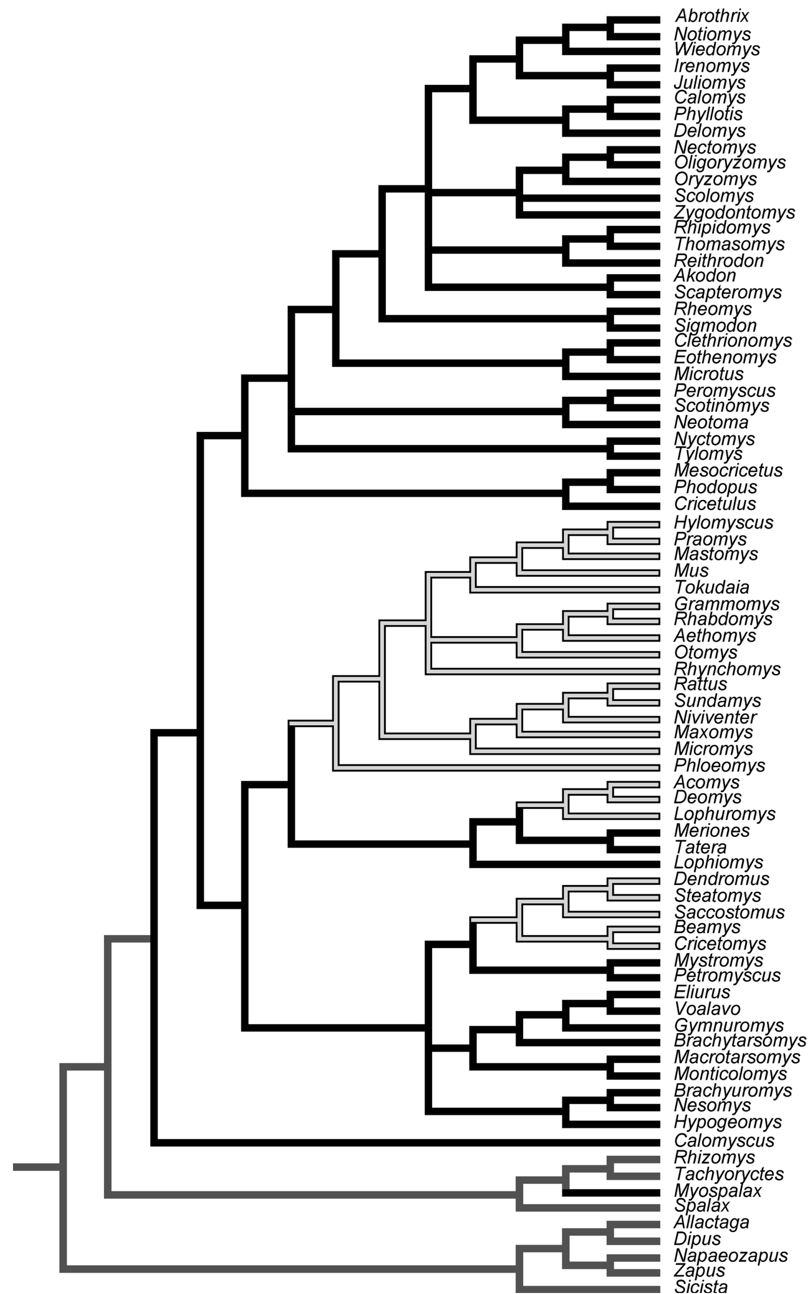

Fig. 3. Molar tooth morphology optimized on the maximum likelihood topology from Fig. 1B. Molar tooth patterns were coded as either cricetid (black), murid (pale gray outlined in black), or neither cricetid nor murid (dark gray).

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