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Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (*Spermophilus*)

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Abstract

The squirrel family, Sciuridae, is one of the largest and most widely dispersed families of mammals. In spite of the wide distribution and conspicuousness of this group, phylogenetic relationships remain poorly understood. We used DNA sequence data from the mitochondrial cytochrome *b* gene of 114 species in 21 genera to infer phylogenetic relationships among sciurids based on maximum parsimony and Bayesian phylogenetic methods. Although we evaluated more complex alternative models of nucleotide substitution to reconstruct Bayesian phylogenies, none provided a better fit to the data than the GTR + G + I model. We used the reconstructed phylogenies to evaluate the current taxonomy of the Sciuridae. At essentially all levels of relationships, we found the phylogeny of squirrels to be in substantial conflict with the current taxonomy. At the highest level, the flying squirrels do not represent a basal divergence, and the current division of Sciuridae into two subfamilies is therefore not phylogenetically informative. At the tribal level, the Neotropical pygmy squirrel, *Sciurillus*, represents a basal divergence and is not closely related to the other members of the tribe Sciurini. At the genus level, the sciurine genus *Sciurus* is paraphyletic with respect to the dwarf squirrels (*Microsciurus*), and the Holarctic ground squirrels (*Spermophilus*) are paraphyletic with respect to antelope squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), and marmots (*Marmota*). Finally, several species of chipmunks and Holarctic ground squirrels do not appear monophyletic, indicating a need for reevaluation of alpha taxonomy.

Keywords: Bayesian phylogeny; cytochrome *b*; Marmotini; Sciuridae; *Spermophilus*; Squirrels; Taxonomy

1. Introduction

The squirrel family, Sciuridae, is one of the most diverse and widely distributed families of mammals. With more than 270 species belonging to 50 genera, members of this family occur on all continents except Antarctica and Australia (Hoffmann et al., 1993; McKenna and Bell, 1997). Most species are diurnal and are conspicuous members of the diverse array of ecological communities they inhabit. In spite of the significance of this group, phylogenetic relationships within the family remain poorly understood. Substantial questions remain regarding the monophyly of genera, the relationships among genera, and the validity of the current taxonomy at virtually all levels. In particular, the species content and monophyly of many recognized genera have not been comprehensively evaluated.

Two subfamilies of squirrels are currently recognized: Pteromyinae, the flying squirrels; and Sciurinae, consisting of nine tribes of arboreal and terrestrial squirrels (McKenna and Bell, 1997). Multiple independent origins have been proposed for the Pteromyinae based on fossil and immunological data (Black, 1963; Engesser, 1979; Hight et al., 1974; James, 1963; Mein, 1970). Studies based on nucleotide and anatomical data, however, have suggested monophyly of the subfamily (Mercer and Roth, 2003; Oshida et al., 1996; Steppan et al., in press; Thorington, 1984). A close relationship between the flying squirrels (whether monophyletic or polyphyletic) and the tribe Sciurini has been inferred based on analyses of fossil and DNA data (Black, 1963, 1972; Mercer and Roth, 2003; Oshida et al., 1996; Steppan et al., in press). Several other studies, though, have supported an early divergence between the

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Pteromyinae and the remaining sciurids (Ellis and Maxson, 1980; Engesser, 1979; Hafner, 1984; Hafner et al., 1994; Hight et al., 1974; James, 1963; Forsyth Major, 1893; Mein, 1970; Wade and Gilbert, 1940).

Within the Sciurinae, the tribe Marmotini includes the antelope squirrels (*Ammospermophilus*), prairie dogs (*Cynomys*), marmots (*Marmota*), and Holarctic ground squirrels (*Spermophilus*). Based on fossil data, Bryant (1945) suggested that the chipmunks (*Eutamias*, *Neotamias*, and *Tamias*) were close relatives of the Marmotini, and Moore (1959) considered them a subtribe (Tamiina) of the Marmotini. Later, fossil evidence led Black (1963) to elevate chipmunks to tribal status (Tamiini). Historically, chipmunks have been variously assigned to one genus, sometimes with three subgenera (Bryant, 1945; Ellerman et al., 1940; Levenson et al., 1985; Nadler et al., 1969, 1977); two genera (Ellis and Maxson, 1979; Hall, 1981; Hall and Kelson, 1959; Nadler, 1964; White, 1953); or three genera (Jameson, 1999; Piaggio and Spicer, 2001).

The Marmotini and Tamiini were traditionally thought to be closely related to the tribe Sciurini, based on characters of the skull, the baculum, and the number of mammae (Moore, 1959, 1961; Pocock, 1923; Wade and Gilbert, 1940). Analyses of nuclear (Steppan et al., in press) and combined nuclear and mitochondrial (Mercer and Roth, 2003) DNA, however, recovered no particularly close relationship between the Marmotini + Tamiini and the Sciurini, finding instead that the Marmotini and Tamiini belong to a clade also including the Chinese rock squirrel *Sciurotamias* and the tribes Xerini (African, North African, and long-clawed ground squirrels), Protoxerini (African palm, African giant, and sun squirrels), and Funambulini (African tree squirrels) minus *Funambulus*. Moore (1959) considered the Xerini basal to the Protoxerini (concluding that similarities between the Marmotini + Tamiini and the Xerini were due to convergence), and suggested a close relationship between the Ratufini (Asian giant squirrels) and the Nannosciurini (Oriental tree squirrels), and between the Protoxerini and the Funambulini.

Placement of the American red squirrels, genus *Tamiasciurus*, which along with *Sciurotamias* make up the Tamiasciurini, has historically been problematic. The unique genital tract of this group (Mossman et al., 1932; Pocock, 1923) led Pocock (1923) to place it in a separate tribe, the Tamiasciurini. Simpson (1945) retained this designation, but Layne (1952) argued that the vestigial baculum was insufficient to warrant tribal status for *Tamiasciurus*. Moore (1959) suggested affinities between *Tamiasciurus* and the Xerini, and a distant relationship with the Sciurini. However, close affinities with *Sciurus* have been supported by studies based on fossils, protein electrophoresis, and immunological data (Black, 1963; Bryant, 1945; Ellis and Maxson, 1980; Hafner et al., 1994; Hight et al., 1974). Analyses of nucleotide data (Mercer and Roth, 2003; Steppan et al., in press) found *Tamiasciurus* basal among the Sciurini (minus *Sciurillus*). Mercer and Roth (2003) found *Sciurotamias* basal to a clade including the Marmotini, Tamiini, Protoxerini, and Funambulini (minus *Funambulus*), while Steppan et al. (in press) found *Sciurotamias* nested within this clade, sister to the Marmotini.

Two recent studies have provided considerable insight regarding relationships among and generic content of tribes and have presented evidence for intergeneric relationships. Mercer and Roth (2003) inferred relationships for all but one extant sciurid genus using one nuclear (IRBP; interphotoreceptor retinoid binding protein) and two mitochondrial (12S and 16S rRNA) genes. The deepest divergence within the family in their reconstruction was that of *Sciurillus*, followed by *Ratufa*, from the other sciurids. The remaining genera were resolved as three major lineages: the Pteromyinae, Tamiasciurini, and Sciurini (except *Sciurillus*); the Nannosciurini plus *Funambulus*; and the Marmotini, Tamiini, Protoxerini, and Xerini plus *Sciurotamias* and most members of the Funambulini. Steppan et al. (in press) used two nuclear genes (c-myc and RAG1) to infer relationships among 23 squirrel genera. Their results were in broad agreement with those of Mercer and Roth (2003), but differ in branching order and in their placement of *Sciurotamias*. Neither of these studies, however, was designed to investigate the specific content and monophyly of genera.

In this study, we use mitochondrial cytochrome *b* (cyt *b*) gene sequences from a large and densely sampled array of scuirid taxa to further clarify the evolutionary relationships within the Sciuridae. We aimed at complementing the taxon sampling design of Mercer and Roth (2003) and Steppan et al. (in press) by densely sampling from the intraspecific level to the tribal level. This approach allows evaluation of the monophyly of genera and, in some cases, species. Additionally, we reexamine hypotheses for higher-level relationships within the Sciuridae and the monophyly of the family. We use both maximum parsimony and Bayesian phylogenetic methods to reconstruct the phylogeny of sciurids. For Bayesian phylogenetic reconstruction we test conventional and more complex models of nucleotide substitution to identify a best fitting model for the data. Estimated phylogenies are used to evaluate the validity of the current taxonomy at the generic level, as well as for higher taxa.

2. Materials and methods

2.1. Taxon sampling

Within the family Sciuridae, our taxonomic sampling (Table 1) included representatives of 12 species in six

Table 1
Taxa used in this study

Tribe/Family/Subfamily	O.T.U.	Accession number	Sequence length (bp)
Aplodontidae	*Aplodontia rufa*	AJ389528	1140
Myoxidae	*Eliomys quercinus*	EQAJ5030	1140
	Glis glis	AJ225031	1140
	Graphiurus murinus	AJ225115	1140
Anomaluridae	*Anomalurus* sp.	AJ389526	1140
	Idiurus macrotis	AJ389525	1140
Pedetidae	*Pedetes surdaster*	AJ389527	1140
Castoridae	*Castor fiber*	AJ389529	1140
Dipodidae	*Allactaga elater*	AJ389534	1140
Caviidae	*Cavia porcellus*	NC000884	1140
Pteromyinae	*Belomys pearsonii*	AB030262	1068
	Glaucomys sabrinus	AF030390	1140
	Glaucomys sabrinus	AF271668	1140
	Glaucomys sabrinus	AF359211	1140
	Glaucomys sabrinus	AF359221	1140
	Glaucomys sabrinus griseifrons	AF359238	1140
	Glaucomys volans	AJ389531	1140
	Glaucomys volans	AF157921	1140
	Hylopetes phayrei	AB030259	1068
	Petaurista alborufus	AB023902	1068
	Petaurista elegans	AB047380	1068
	Petaurista leucogenys	AB023906	1068
	Petaurista petaurista	AB023909	1068
	Petaurista philippensis	AB023907	1068
	Petinomys setosus	AB030260	1068
	Pteromys momonga	AB030263	1068
	Pteromys volans	AB023910	1068
Funambulini	*Paraxerus cepapi*	PCU59179	714
Marmotini	*Ammospermophilus harrisii*	AF157926	1140
	Ammospermophilus harrisii	AHU46172	528
	Ammospermophilus interpres	AIU46174	528
	Ammospermophilus leucurus	ALU46175	528
	Cynomys gunnisoni	AF157930	1140
	Cynomys leucurus	AF157879	1140
	Cynomys ludovicianus	AF157890	1140
	Cynomys mexicanus	AF157847	1140
	Cynomys parvidens	AF157922	1140
	Marmota baibacina centralis	AF143915	1140
	Marmota baibacina kastchenkoi	AF143914	1140
	Marmota bobak bobak	AF143916	1140
	Marmota bobak bobak	AF143917	1140
	Marmota broweri	AF143919	1140
	Marmota caligata caligata	AF143920	1140
	Marmota camtschatica camtschatica	AF143921	1140
	Marmota camtschatica doppelmayri	AF143922	1140
	Marmota caudata aurea	AF143925	1140
	Marmota caudata caudata	AF143923	1140
	Marmota flaviventris luteola	AF143926	1140
	Marmota flaviventris obscura	AF143927	1140
	Marmota himalayana robusta	AF143928	1140
	Marmota marmota	AF100711	1140
	Marmota marmota marmota	AF143930	1140
	Marmota marmota marmota	AF143929	1140
	Marmota menzbieri zachidovi	AF143931	1140
	Marmota monax monax	AF143934	1140
	Marmota monax ochracea	AF143932	1140
	Marmota olympus	AF111182	810
	Marmota sibirica sibirica	AF143937	1140
	Marmota vancouverensis	AF143939	1140
	Spermophilus adocetus	AF157844	1140
	Spermophilus annulatus	AF157851	1140
	Spermophilus armatus	AF157901	1140
	Spermophilus atricapillus	AF157945	1140

Table 1 (*continued*)

Tribe/Family/Subfamily	O.T.U.	Accession number	Sequence length (bp)
	Spermophilus beechyi	AF157918	1140
	Spermophilus beldingi creber	AF157951	1140
	Spermophilus brunneus brunneus	AF157952	1140
	Spermophilus citellus	AF157859	1140
	Spermophilus columbianus columbianus	AF157939	1140
	Spermophilus columbianus ruficaudus	AF157943	1140
	Spermophilus dauricus	AF157899	1140
	Spermophilus elegans elegans	AF157891	1140
	Spermophilus erythrogenys	AF157855	1140
	Spermophilus erythrogenys	AF157875	1140
	Spermophilus franklinii	AF157893	1140
	Spermophilus franklinii	AF157894	1140
	Spermophilus fulvus	AF157913	1140
	Spermophilus lateralis trepidus	AF157887	1140
	Spermophilus lateralis trepidus	AF157950	1140
	Spermophilus madrensis	AF157946	1140
	Spermophilus madrensis	AF157947	1140
	Spermophilus major	AF157903	1140
	Spermophilus major	AF157905	1140
	Spermophilus mexicanus mexicanus	AF157848	1140
	Spermophilus mexicanus parvidens	AF157853	1140
	Spermophilus mojavensis	AF157928	1140
	Spermophilus mollis	AF157938	1140
	Spermophilus musicus	AF157904	1140
	Spermophilus pallicaudata	AF157869	1140
	Spermophilus parryi	AF157896	1140
	Spermophilus parryi kenicotti	AF157931	1140
	Spermophilus parryi plesius	AF157927	1140
	Spermophilus perotensis	AF157948	1140
	Spermophilus pygmaeus	AF157907	1140
	Spermophilus pygmaeus	AF157910	1140
	Spermophilus relictus	AF157862	1140
	Spermophilus relictus	AF157867	1140
	Spermophilus relictus	AF157876	1140
	Spermophilus richardsoni	AF157915	1140
	Spermophilus saturatus	AF157917	1140
	Spermophilus spilosoma	AF157846	1140
	Spermophilus spilosoma marginatus	AF157911	1140
	Spermophilus suslicus	AF157895	1140
	Spermophilus suslicus	AF157898	1140
	Spermophilus tereticaudus	AF157941	1140
	Spermophilus townsendii idahoensis	AF157949	1140
	Spermophilus townsendii townsendii	AF157935	1140
	Spermophilus townsendii vigilis	AF157889	1140
	Spermophilus tridecemlineatus arenicola	AF157877	1140
	Spermophilus undulatus	AF157906	1140
	Spermophilus undulatus	AF157912	1140
	Spermophilus variegatus	AF157854	1140
	Spermophilus variegatus utah	AF157878	1140
	Spermophilus washingtoni	AF157936	1140
	Spermophilus xanthoprymnus	AF157909	1140
Nannosciurini	*Callosciurus erythraeus*	AB043876	1080
	Callosciurus erythraeus	AB043877	1080
	Callosciurus finlaysonii	AB043878	1080
	Callosciurus nigrovittatus	AB043882	1080
	Callosciurus prevostii	AB043879	1080
	Callosciurus prevostii	AB043880	1080
Sciurini	*Microsciurus flaviventer*	MFU46169	528
	Sciurillus pusillus	SPU46179	528
	Sciurus aberti barberi	SAU10177	1134
	Sciurus aberti chuscensis	SAU10176	1134
	Sciurus aberti kaibabensis	SAU10182	1140
	Sciurus aestuans	SAE389530	1140
	Sciurus carolinensis	SCU46167	1140

Table 1 (*continued*)

Tribe/Family/Subfamily	O.T.U.	Accession number	Sequence length (bp)
	Sciurus lis	AB030024	1040
	Sciurus lis	AB043881	1080
	Sciurus niger	SNU10180	1140
	Sciurus stramineus	AB030025	1040
	Sciurus vulgaris	AB030028	1040
	Sciurus vulgaris	AJ238588	1140
Tamiasciurini	*Tamiasciurus douglasii*	AF322953	402
	Tamiasciurus hudsonicus	AB030029	1040
	Tamiasciurus mearnsi	AF322954	402
Tamiini	*Eutamias sibiricus*	AF147666	1139
	Neotamias amoenus	AF147630	1140
	Neotamias amoenus	AF147632	1140
	Neotamias bulleri	AF147634	1140
	Neotamias canipes	AF147635	1140
	Neotamias cinericollis	AF147636	1140
	Neotamias cinericollis	AF147638	1133
	Neotamias dorsalis	AF147639	1133
	Neotamias dorsalis	AF147641	1138
	Neotamias durangae	AF147642	1140
	Neotamias merriami	AF147644	1140
	Neotamias minimus	AF147645	1131
	Neotamias minimus	AF147650	1140
	Neotamias obscurus	AF147651	1139
	Neotamias ochrogenys	AF147654	1136
	Neotamias palmeri	AF147655	1103
	Neotamias panamintinus	AF147656	1140
	Neotamias quadrimaculatus	AF147657	1140
	Neotamias quadrivittatus	AF147658	1140
	Neotamias quadrivittatus	AF147660	1140
	Neotamias ruficaudus	AF147661	1140
	Neotamias rufus	AF147662	1140
	Neotamias rufus	AF147663	1140
	Neotamias senex	AF147665	1140
	Neotamias siskiyou	AF147668	1140
	Neotamias sonomae	AF147669	1118
	Neotamias townsendii	AF147674	1140
	Neotamias townsendii	AF147676	1140
	Neotamias umbrinus	AF147677	1070
	Tamias striatus	AF147673	1139
Xerini	*Spermophilopsis leptodactylus*	AF157865	1140
	Xerus inauris	AY452689	1140
	Xerus rutilus	AY452690	1140

genera of the subfamily Pteromyinae and 102 species in 15 genera of the subfamily Sciurinae. Two or more individuals were included for 37 species, with multiple nominal subspecies used where available. Multiple, successive outgroups (Smith, 1994) were used to root phylogenies and to examine the monophyly of the Sciuridae with respect to the Aplodontidae, the hypothesized sister family to the Sciuridae (Adkins et al., 2001; DeBry and Sagel, 2001; Huchon et al., 1999, 2000; Lavocat and Parent, 1985; Montgelard et al., 2002; Nedbal et al., 1996; Steppan et al., in press; Wahlert, 1985). Aplodontidae contains only a single extant representative, the mountain beaver (*Aplodontia rufa*).

In addition to *Aplodontia* (left as a member of the ingroup), outgroup taxa from other rodent families included two members of the Anomaluridae (*Idiurus macrotis* and *Anomalurus* sp.), one member of the Castoridae (*Castor fiber*), one member of the Dipodidae (*Allactaga elater*), three members of the Gliridae (*Eliomys quercinus*, *Glis glis* and *Graphiurus murinus*), one member of the Pedetidae (*Pedetes surdaster*), and a hystricomorph rodent (*Cavia porcellus*). The majority of sequences used in this study (167 of 169 total sequences) have been previously published (Table 1). Most of these sequences were originally published in genus- or species-level phylogenies (e.g., Arbogast et al., 2001; Oshida et al., 2000; Oshida and Masuda, 2000; Oshida et al., 2001; Piaggio and Spicer, 2001; Steppan et al., 1999; Wettstein et al., 1995) or as direct submissions to GenBank. This study is the first time these sequences have been combined in one analysis to explore higher-level relationships. This paper provides two new complete cyt *b* sequences (*Xerus inauris* and *X. rutilus*), which have been deposited in GenBank (see Table 1).

2.2. DNA sequencing and sequence alignment

Total DNA was extracted from 95% ethanol-preserved liver samples from *X. inauris* and *X. rutilus* using standard proteinase K digestion followed by phenol/chloroform/isoamyl alcohol organic separation methods as described in Sambrook and Russell (2001). The cyt *b* gene was PCR-amplified (Saiki et al., 1988) using the primers L14725 (Irwin et al., 1991) and XerusREV (5′-TTNGGTTTA-CAAGACCAAGT-3′, this study), located in the flanking tRNA-Glutamine and tRNA-Threonine genes. PCR amplification was carried out using an initial denaturation at 94 °C for 3 min 45 s; 45 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 2 min; and a final extension at 72 °C for 7 min. Products from PCR were excised from 1.4% agarose electrophoresis gels and purified using the MinElute Gel Extraction Kit (Qiagen). Purified PCR products were sequenced in both directions using the amplification primers and the internal primer XerusFctb (5′-TGAG GRCAAATATCCTTCTGAGG-3′, this study) with the CEQ Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter) and run on a Beckman CEQ2000 automated sequencer according to the manufacturer's protocols. Raw sequence chromatographs from novel sequences were edited in Sequencher 3.1 (Gene Codes).

All sequences were aligned manually in GeneDoc 2.6.002 (Nicholas and Nicholas, 1997). Aligned DNA sequences were translated to amino acid sequences to confirm alignment and check for the presence of stop codons or open reading frame shifts (neither of which were detected), which might have indicated pseudogenes. Since the cyt *b* gene is protein coding, indels were infrequent and occurred only in units representing the gain or loss of complete codons. Only two indels were observed, both 3 bp in length and both inferred as deletions in *Sciurus aberti barberi* and *S. a. chuscensis*. The exact position of one of these, beginning at position 19 or 22, could not be confidently determined, so three nucleotides were conservatively coded as missing data for these taxa. The second inferred deletion occurred at positions 694–696, and alignment was otherwise unambiguous. The alignment (in Nexus format) is available at TreeBASE (http://treebase.bio.buffalo.edu/treebase) under the matrix Accession No. M1630.

2.3. Phylogenetic reconstruction

Phylogenetic relationships were estimated using both maximum parsimony (MP) and Bayesian Metropolis-coupled Markov Chain Monte Carlo phylogenetic methods (MCMC). Unweighted MP searches were conducted in PAUP* v4.0b10 (Swofford, 2002) with gaps treated as missing. The heuristic search strategy was used to search for optimal MP trees with 100 random taxon addition sequence replicates and starting trees obtained via stepwise addition. Settings for MP analyses included tree bisection–reconnection branch swapping, steepest descent off, and MULTREES option on (Swofford, 2002). Clade support was assessed with 300 bootstrap pseudoreplicates, each with 10 random addition sequence replicates. Additional parameters employed for the bootstrap analysis were identical to those described above.

All MCMC phylogenetic reconstructions were conducted using MrBayes 2.10 (Huelsenbeck and Ronquist, 2001) with uniform priors and model parameters estimated as part of the analyses. Three heated chains and a single cold chain were used in all MCMC analyses, and runs were initiated with random trees. Trees were sampled every 100 generations, and majority rule consensus phylograms and posterior probabilities for nodes were assembled from all post-burn-in sampled trees. The data were subjected to MCMC analyses under multiple evolutionary models that differed in the way they parameterized among-site rate variation. Under each model of evolution, four independent MCMC runs were conducted, each with 1.4 million generations per run.

We used ModelTest 3.0 (Posada and Crandall, 1998) to infer the simplest, best-fit model of evolution based on hierarchical log-likelihood ratio tests comparing successively more complex models (Huelsenbeck and Crandall, 1997; Posada and Crandall, 2001). The most complex (parameter rich) model that ModelTest 3.0 can evaluate is a General Time Reversible (GTR, Tavaré, 1986) model with a parameter (gamma) estimating among-site rate variation (Yang, 1993) and another parameter (I) describing the proportion of invariant sites. This GTR + G + I model was selected by ModelTest, but we also wished to explore the effects of more complex models on topology and optimized tree likelihood scores (used to represent model fit to data).

MrBayes is capable of employing more complex models than this GTR + G + I model. In particular, MrBayes allows among-site rate variation to be partitioned among defined sites (site-specific gamma) as well as allowing the use of an autocorrelation parameter to account for autocorrelation of rates among sites (Kimura, 1986; Nielsen, 1997; Schöniger and Von Haeseler, 1994; Yang, 1995). These two types of modifications to simple parameterization of among-site rate variation using a single gamma shape parameter may be used independently as well as simultaneously in a given model. We tested the performance of four alternative models, all employing a single GTR substitution matrix, which differed only with respect to how among site rate variation was parameterized, including: GTR + G + I (chosen by Modeltest), GTR + AG (GTR model with autocorrelated among-site rate variation), GTR + partG (GTR model with site-specific partitions of gamma, partitioned as described below), and GTR + partAG (GTR with site-specific partitions of gamma with autocorrelated rates).

The structure of the cyt *b* protein has been well characterized (Howell, 1989), and variation of evolutionary rates across functional domains has been analyzed for birds and mammals (Griffiths, 1997; Irwin et al., 1991). For the site-specific gamma parameter partition models (GTR + partG and GTR + partAG) we assigned sites to one of three partitions, according to the structural model of the cyt *b* gene presented by Griffiths (1997) for the Falconidae (Aves). Because the cyt *b* gene is 1143 bp long in Falconidae and only 1140 bp in most sciurids, an alignment was created in Genedoc (Nicholas and Nicholas, 1997) including several bird and squirrel reference sequences. A single 3 bp indel at positions 4–6 accounted for the difference in length, and so we divided the squirrel gene into the following partitions: intermembrane (1–90, 316–336, 613–678, 925–954, and 1111–1140), transmembrane (91–165, 238–315, 337–399, 532–612, 679–744, 856–924, 955–1023, and 1051–1110), and matrix (166–237, 400–531, 745–855, and 1024–1050).

To explore whether these three partitions in fact appear to evolve under different patterns of among-site rate variation (justifying a model to account for this), we conducted MCMC runs in MrBayes independently on each partition. These runs employed a GTR + G model and were run in triplicate to guard against any one run failing to reach a global optimum of likelihood stationarity. Details of MCMC analyses were the same as those described previously.

2.4. Hypothesis testing

We examine two types of hypotheses with alternative Bayesian MCMC analyses: (1) hypotheses regarding which model of evolution best fits the data (alternative models of evolution explored are discussed above) and (2) null hypotheses of monophyly of taxa that were recovered as paraphyletic in the unconstrained MCMC reconstruction. To test alternate topological hypotheses, we conducted independent MCMC analyses in which a particular group was constrained as monophyletic based on the current taxonomy as described by McKenna and Bell (1997). To evaluate both types of hypotheses, we compared the 95% credibility interval (CI) of MCMC chain likelihood scores between the null and alternative hypotheses. To calculate the 95% CI, we ranked all post-burn-in tree estimates by ln likelihood and included the most likely 95% (Felsenstein, 1968; Huelsenbeck et al., 2002). Since a 95% Bayesian credibility interval is similar to a 95% confidence interval of classical statistics (Huelsenbeck et al., 2002), the degree of overlap observed between null and alternative ln likelihood CIs was used to test hypotheses. In testing null hypotheses based on current taxonomy, the null constrained hypotheses were rejected if the 95% CI of constrained MCMC chain likelihood scores did not overlap with the 95% CI of unconstrained MCMC chain likelihood scores.

3. Results

3.1. Maximum parsimony phylogenetic analysis

Of the 1140 aligned bases (380 codons), 446 were invariant (iv), 97 variable characters were parsimony uninformative (vpu), and 597 were parsimony informative (pi). Among the three codon positions, the first position contained 197 iv, 37 vpu, and 146 pi characters; the second position contained 247 iv, 58 vpu, and 75 pi characters; and the third position contained 2 iv, 2 vpu, and 376 pi characters. The observed base composition for the L-strand was: A = 28.42%; C = 27.07%; G = 12.63%; and T = 31.88%. Uncorrected percent pairwise sequence divergence was highest between *C. porcellus* and *P. surdaster* (27.54%). Within the Sciuridae, the highest observed uncorrected pairwise divergence was between *Spermophilus dauricus* and *Paraxerus cepapi* (25.82%).

The unweighted MP reconstruction resulted in 208 equally parsimonious trees with length = 9289 steps, consistency index = 0.137, retention index = 0.667, rescaled consistency index = 0.091, and homoplasy index = 0.863. A strict consensus of all 208 optimal MP trees is shown in Fig. 1, with bootstrap percentages (BP) above 50% indicated. Three major clades are evident among sciurids. The first is a weakly supported clade including *Aplodontia*, *Sciurillus*, and *Paraxerus* as sister to a monophyletic *Callosciurus* (BP = 99%). The second includes a weakly supported tribe Xerini, the Pteromyinae, the Tamiasciurini (BP = 100%), and the Sciurini minus *Sciurillus* (BP = 92%). Monophyly of the genera *Xerus* (BP = 100%), *Petaurista* (BP = 94%), *Glaucomys* (BP = 100%), *Pteromys* (BP = 100%), and *Tamiasciurus* (BP = 100%) received strong support. *Sciurus* was resolved as paraphyletic with respect to *Microsciurus*. The third clade shows the tribe Tamiini sister to the Marmotini (BP = 76%).

Among the species for which two or more individuals were included, the following were not resolved as exclusive monophyletic groups (i.e., individuals of the same species failed to group together) in the MP analysis: *Glaucomys sabrinus*, *Neotamias cinericollis*, *N. dorsalis*, *N. minimus*, *Ammospermophilus harrisii*, *Spermophilus citellus*, *S. major*, *S. mexicanus*, *S. pygmaeus*, *S. spilosoma*, and *S. townsendi*. *Neotamias rufus* was part of an unresolved polytomy with *N. quadrivittatus* and a clade including *N. cinericollis*, *N. dorsalis*, *N. palmeri*, and *N. umbrinus*.

3.2. Bayesian phylogenetic analyses

Based on hierarchical log-likelihood ratio tests of successively more complex models of sequence evolution, Modeltest indicated that the simplest, best-fit model for the dataset was the GTR + G + I model with the following

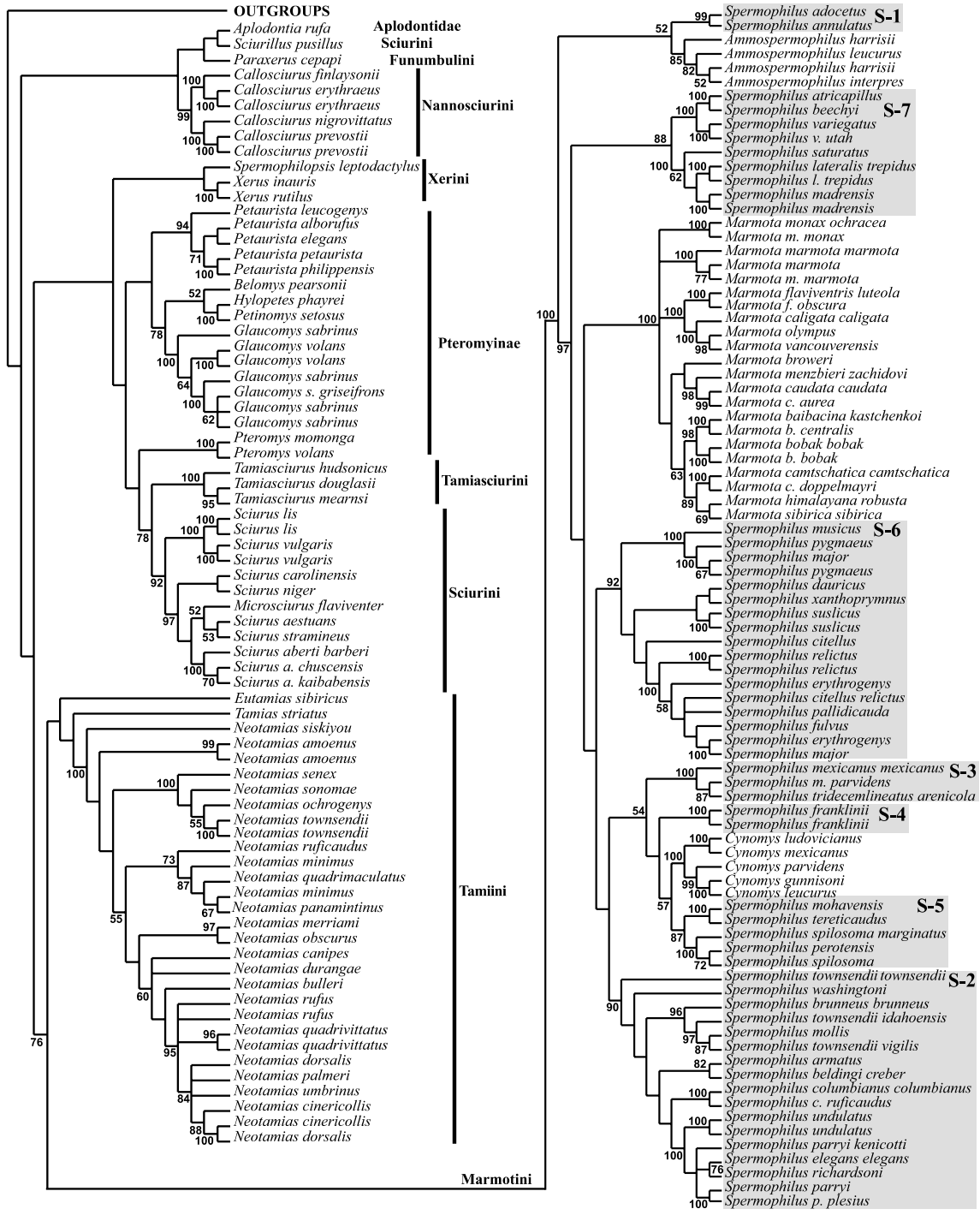

Fig. 1. Strict consensus of all 208 equally optimal trees based on unweighted maximum parsimony search. Bootstrap percentages above 50% are provided. Higher taxa are also given. Seven clades representing independent lineages containing members of the genus *Spermophilus* are indicated by sequentially labeled shading (S-1 to S-7).

parameter estimates: [A–C] = 0.209, [A–G] = 10.666, [A–T] = 0.413, [C–G] = 0.752, [C–T] = 4.817, [G–T] = 1.000, gamma parameter [G] = 0.5826, proportion of invariant sites [I] = 0.342.

Independent MCMC on the defined partitions (to initially detect domain-specific patterns of among-site rate variation) all converged to a common stationary value across replicate runs. Estimates of the gamma shape parameter for each domain did show domain-specific differentiation in the mean values (but overlap of 95% credibility interval) for gamma parameter estimates as follows (gamma shape parameter estimates reported as means with the minimum and maximum estimated values within the 95% credibility interval of trees):

intermembrane partition = 0.3197 (0.2222–0.4292), transmembrane partition = 0.3279 (0.2730–0.3804), matrix partition = 0.3030 (0.2459–0.3638).

The pooled mean ln likelihood score for the post-burn-in trees recovered by the four MCMC runs using the GTR + AG model was −37,999.54 (95% credibility interval −38,030.14 to −37,970.18). That of the GTR + partG model was −38,037.56 (−38,068.62 to −38,008.20), and that of the GTR + partAG model was −37,997.51 (−38,036.15 to −37,967.36) (Fig. 2). These three models have equal (GTR + AG) or greater numbers of model parameters as the GTR + G + I model. Despite being equally or more complex models, all these alternative models resulted in lower MCMC chain likelihood score values than did MCMC chains employing the GTR + G + I model (mean −37,934.99; 95% credibility interval −37,870.63 to −37,960.82; Fig. 2). Because these equally (GTR + AG) or more complex models yielded less optimal likelihood scores for chains than the GTR + G + I model, we chose to report our conclusions based on the latter (Fig. 3). The burn-in plots of the four independent MCMC runs using the GTR + G + I model (Fig. 4) show that all four runs reached stationarity prior to 350 000 generations. Thus, our burn-in time of 400 000 generations was sufficient. All four runs of the GTR + G + I model converged with regard to chain likelihood scores (Fig. 4), topology (50% majority rule consensus identical among runs), and

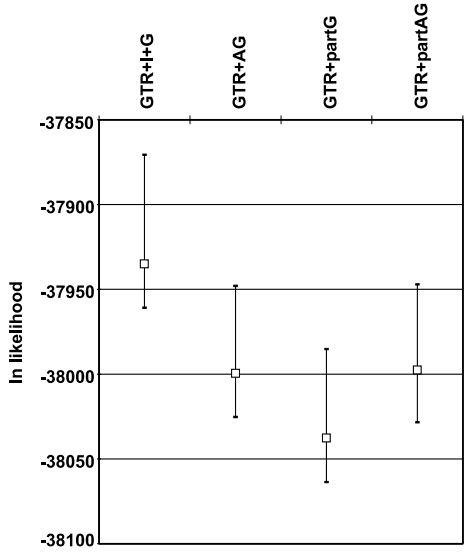

Fig. 2. Comparison of MCMC chain likelihood scores across alternative models of evolution evaluated. GTR + G + I = General Time Reversible (GTR) with a gamma distribution of among-site rate variation and a proportion of sites invariant; GTR + AG = GTR with autocorrelated gamma; GTR + partG = GTR with site-specific partitions of gamma; GTR + partAG = GTR with site-specific partitions of gamma with autocorrelated rates. Likelihood scores for each model are reported as the mean (box) and 95% credibility interval (bars) based on the combined distribution of 4 million post-burn-in generations (from four independent MCMC runs per model).

posterior probability estimates for clade support. Also, parameter plots for all four independent runs show overlapping estimates of substitution model parameters at stationarity (Fig. 5). Parameter estimates based on the combination of all post-burn-in generations from the four independent MCMC runs under the GTR + G + I model, reported as means with the minimum and maximum estimated values within the 95% credibility interval of trees, were: [A–C] = 0.242 (0.529–0.146), [A–G] = 13.171 (21.265–8.455), [A–T] = 0.479 (0.910–0.234), [C–G] = 0.864 (1.797–0.338), [C–T] = 5.851 (11.812–3.443), [G–T] = 1, gamma parameter [G] = 0.584 (0.672–0.499), and proportion of invariant sites [I] = 0.294 (0.347–0.237).

Based on the evidence for convergence among runs, we pooled all post-burn-in trees (representing a total of 4 million post-burn-in generations) to estimate the posterior probabilities of clades. A 50% majority rule consensus phylogram (branch lengths averaged over all post-burn-in trees) is presented in Fig. 3 with posterior probabilities from all post-burn-in generations of the GTR + G + I model runs indicated. *Aplodontia* appears sister to the Sciuridae with 99% Bayesian posterior probability (PP). The earliest divergence within the Sciuridae is that of *Sciurillus*, followed by the Xerini, *Paraxerus*, and *Callosciurus*. Among the remaining squirrels, three major clades are apparent: the Tamiasciurini, Sciurini (minus *Sciurillus*), and Pteromyinae (PP = 98%); the Tamiini (PP = 99%); and the Marmotini (PP = 100%). Monophyly of flying squirrels is strongly supported (PP = 99%), as is that of the genera *Ammospermophilus* (PP = 90%), *Callosciurus* (PP = 100%), *Cynomys* (PP = 100%), *Marmota* (PP = 100%), *Sciurus* (PP = 100% including *Microsciurus*), *Neotamias* (PP = 100%), and *Tamiasciurus* (PP = 100%). *Microsciurus* is deeply nested within the *Sciurus* clade.

As in the MP reconstruction, the MCMC analysis recovered *Spermophilus* (*Ictidomys*) *mexicanus* and *S.* (*I.*) *tridecemlineatus* as sister species (PP = 100%, BP = 100%) basal to the clade (PP = 93%, BP <50%) containing *S.* (*Poliocitellus*) *franklinii*, *Cynomys*, *S.* (*Xerospermophilus*) *mohavensis*, *S.* (*X.*) *tereticaudus*, and the remaining members of the subgenus *Ictidomys*, *S.* (*I.*) *perotensis* and *S.* (*I.*) *spilosoma*. The remaining members of subgenus *Ictidomys* are sister to subgenus *Xerospermophilus* (PP = 100%, BP = 87%). The subgenus *Spermophilus* forms two distinct clades, one consisting entirely of Old World taxa (PP = 100%, BP = 92%) and one of New World taxa plus *S. undulatus* (PP = 99%, BP = 90%). As in the MP reconstruction, multiple members of the same species failed to group together (i.e., the same species did not appear monophyletic) as in the MCMC analysis, with the addition of *N. rufus*.

Based on the resolution of clades from the GTR + G + I model, we identified seven relationships

Fig. 3. Majority rule consensus phylogram (with branch lengths averaged over all trees) resulting from Bayesian phylogenetic analysis using a GTR + G + I model of evolution. Posterior probabilities estimated from a total of 4 million post-burn-in generations (from four independent runs) are indicated for major nodes. Higher taxon names are also given. Seven clades representing independent lineages containing members of the genus *Spermophilus* are indicated by sequentially labeled shading (S-1 to S-7).

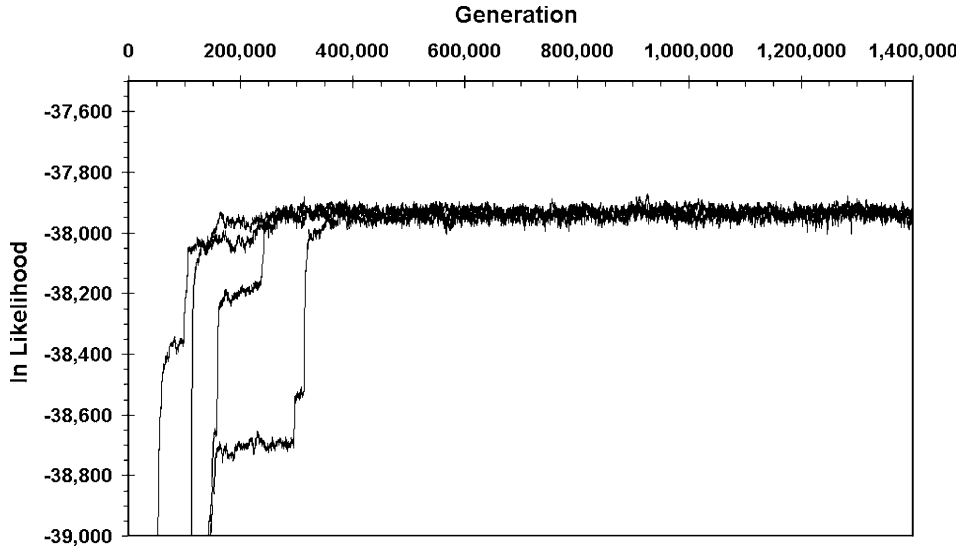

Fig. 4. Burn-in plot of ln likelihood scores of MCMC chains across 1.4 million generations for four independent runs under the GTR + G + I model of evolution.

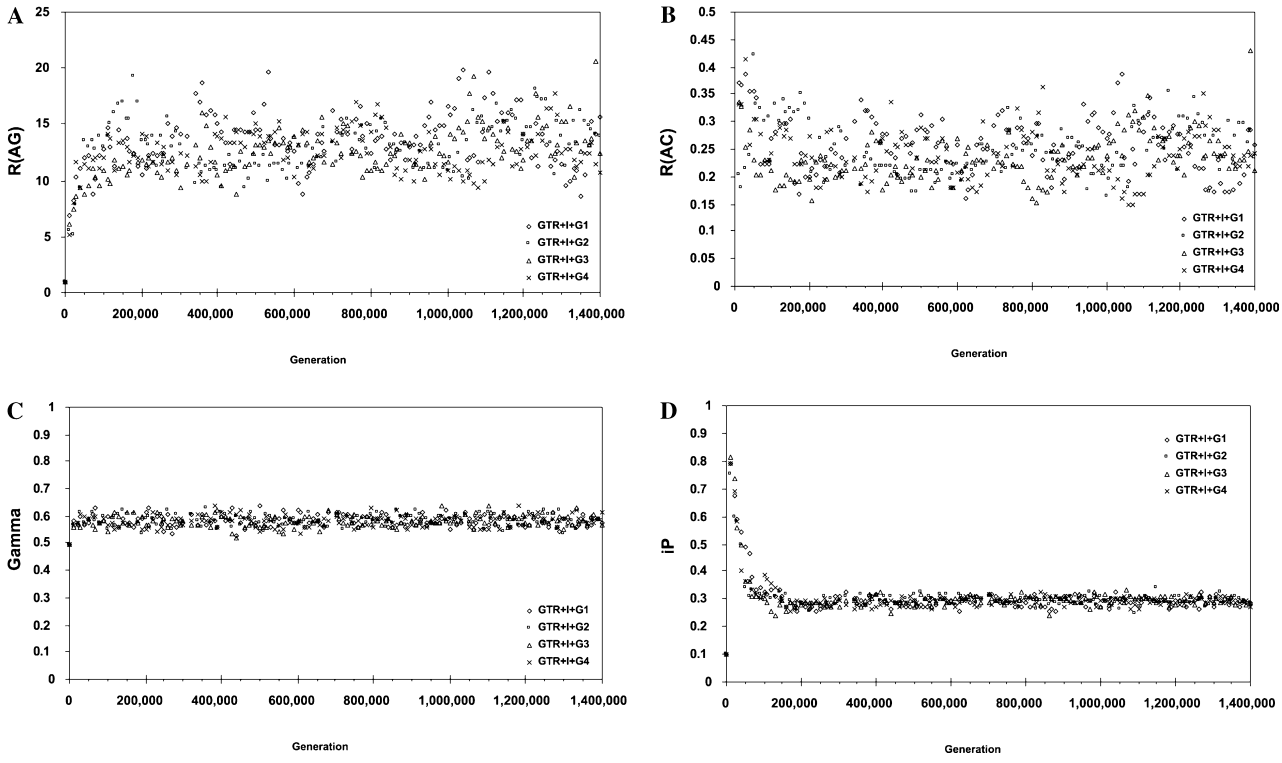

Fig. 5. Plots of selected parameters of independent MCMC runs under the GTR + G + I model through 1.4 million generations. All four independent runs are plotted per graph to show common burn-in rates and similar, overlapping parameter estimates. (A) Plot of the parametric estimates of r(A–G) from the GTR rate matrix. (B) Plot of the parametric estimates of r(A–T) from the GTR rate matrix. (C) Plot of the gamma parameter (G) estimates. (D) Plot of the proportion of invariant sites parameter (I) estimates.

that contradicted the null hypothesis of monophyly of conspicuous taxonomic groups. These included the paraphyly of the tribe Sciurini, the genera *Sciurus* and *Spermophilus*, and the subgenera *Ictidomys*, *Spermophilus*, and *Otospermophilus*. Finally, the Old World species *Spermophilus undulatus* is nested within the clade containing the New World members of the subgenus *Spermophilus*. A comparison between MCMC chain likelihood scores of the unconstrained GTR + G + I runs and MCMC runs where these particular relationships

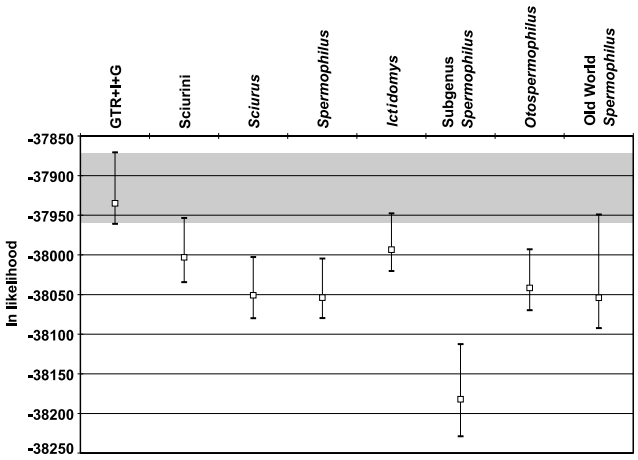

Fig. 6. Comparison between the ln likelihood score for MCMC chains for the unconstrained GTR + G + I model runs and those of alternative runs (with the same model) where particular relationships were constrained. Names of constrained runs represent the groups restricted to monophyly. Likelihood scores are reported as the mean (box) and 95% credibility interval (bars). The shaded area represents the likelihood score range spanned by the 95% credibility interval of the unconstrained MCMC analyses, and represents the region where null hypotheses of constrained relationships would not be rejected.

were constrained is provided in Fig. 6. The MCMC searches constrained to monophyly of the genera *Sciurus* and *Spermophilus* and of the *Spermophilus* subgenera *Spermophilus* and *Otospermophilus* resulted in chain likelihood scores with 95% CIs below the lower bound of that of the unconstrained runs (Fig. 6). Given these results, we reject the null hypothesis of monophyly for of each of these groups. Although we could not reject monophyly of the tribe Sciurini, the *Spermophilus* subgenus *Ictidomys*, and the Old World members of the subgenus *Spermophilus*, these groups did not appear monophyletic in either the MP or the unconstrained MCMC analyses.

4. Discussion

4.1. Models of evolution

Our evaluation of alternative complex models of nucleotide substitution for reconstructing phylogenies with Bayesian MCMC methods yielded unexpected, interesting results. Previous authors have estimated rates and patterns of nucleotide substitution for different domain-encoding regions of the cyt *b* gene, and have found domain-specific rates of substitution (Griffiths, 1997; Irwin et al., 1991). Our initial exploratory independent MCMC estimation of the among-site rate parameter (gamma) for each of the three partitions did provide evidence for at least weakly differentiated domain-specific patterns of among-site rate variation. We used this preliminary evidence as justification for testing the GTR + partG and GTR + partAG models that attempt to rescale a general gamma shape parameter to adjust fit across the three partitions. We expected this to yield models of nucleotide evolution that more realistically accounted for patterns of nucleotide substitution by allowing partition-specific scaling of the gamma shape parameter. Instead, we found that these structurally partitioned models (GTR + partG and GTR + partAG) were a poorer fit to the data than the standard GTR + G + I model. Krajewski and King (1996) found that although divergence among cytochrome *b* regions differed by as much as fivefold, variation was not correlated with structural domains. Their findings, together with the results of our model evaluation, suggest that while certain characteristics of molecular evolution are almost certainly related to the structure of the protein encoded by a gene, patterns of among-site rate variation are do not appear specific to particular domains or groups of domains (at least within the cyt *b* gene in sciurids). In addition to partitioned gamma models, the GTR + G + I model outperformed the GTR + AG model. These results seem to support the strong utility of the GTR + G + I model and, in particular, the ability of a simple gamma parameter to explain among-site rate variation when the proportion of invariant sites is estimated.

4.2. Relationships and taxonomy of the Sciuridae

A sister-group relationship between the Sciuridae and the Aplodontidae is supported by analyses of mitochondrial (Montgelard et al., 2002; Nedbal et al., 1996) and nuclear (Adkins et al., 2001; DeBry and Sagel, 2001; Huchon et al., 1999, 2000) gene sequences as well as by morphological data (Lavocat and Parent, 1985; Wahlert, 1985). Most recently, Mercer and Roth (2003) and Steppan et al. (in press) also provided strong evidence for Aplodontidae being the sister taxon to a monophyletic Sciuridae. While our MP reconstruction did not recover this relationship, our MCMC phylogeny did support this relationship between families, but failed to find strong support (PP = 41%) for the monophyly of the Sciuridae. Because our analyses do not contradict those of Mercer and Roth (2003) and Steppan et al. (in press), both of which employed more slowly evolving nuclear genes, we consider a sister-group relationship between Aplodontidae and a monophyletic Sciuridae to be the favored hypothesis.

In interpreting the results of this study, it is important to consider taxonomic sampling biases and how they may affect perspectives regarding the major relationships among sciurids. Our sampling includes six of the 16 extant genera within the Pteromyinae, and 15 of the 37 extant genera in seven of the nine recognized tribes within the Sciurinae (McKenna and Bell, 1997). Among the sciurine tribes, our generic taxon sampling lacks the

Protoxerini and the monotypic Ratufini and is deficient within the tribes Funambulini (one of four) and Nannosciurini (one of thirteen). Our generic sampling is, however, complete for the tribes Marmotini, Tamiasciurini, and Tamiini and nearly complete for the Xerini (two of three) and Sciurini (three of four). The basal position of the Ratufini, along with the diversity of the Nannosciurini suggested by Mercer and Roth (2003) and Steppan et al. (in press), implies that our taxon sampling may limit conclusions regarding the deepest relationships among sciurids.

Subfamily classification within the family Sciuridae has been a major taxonomic problem. Several studies have suggested that the Sciurinae are paraphyletic with respect to the only other recognized subfamily, the Pteromyinae (Black, 1963; Engesser, 1979; Hight et al., 1974; James, 1963; Mein, 1970). The analyses of Mercer and Roth (2003) and Steppan et al. (in press) supported monophyly of the flying squirrels, and found them to be sister to the tribes Sciurini (minus *Sciurillus*) and Tamiasciurini. Our MCMC analysis supports these findings, showing a well-supported clade of flying squirrels (99% BPP) and a sister-group relationship with Sciurini + Tamiasciurini (98% BPP). The MP analysis shows a monophyletic Sciurini + Tamiasciurini + flying squirrel clade (<50% BS), but places *Pteromys* sister to Sciurini + Tamiasciurini (<50% BS). The present study, along with those of Mercer and Roth (2003) and Steppan et al. (in press), suggests that retention of Pteromyinae as a subfamily is not justified unless the Sciurinae is subdivided into multiple subfamilies.

Current subfamily-level designations are clearly in need of revision. Steppan et al. (in press) propose promotion of each of the five major clades recovered in their analysis to subfamily rank. The recovery of the same five clades by Mercer and Roth's (2003) analysis suggests that this reorganization is justified. However, our results demonstrate two major disagreements with the proposed classification. First, the analyses of Mercer and Roth (2003) and Steppan et al. (in press) show the tribe Xerini within the lineage (clade IV in Mercer and Roth; clade E in Steppan et al.) including the Tamiini, Marmotini, Protoxerini, and Funambulini (minus *Funambulus*). Our results suggest a much earlier divergence (more basal placement) of the Xerini. Second, their analyses show *Paraxerus* within this same clade, while our analyses instead suggest a more basal divergence of *Paraxerus*. Based on phylogenetic hypotheses presented here, together with those of Mercer and Roth (2003) and Steppan et al. (in press), the classification proposed by Steppan et al. (in press) is at least a positive step in the direction of a phylogenetic subfamilial taxonomy for the Sciuridae. However, discrepancies among all three recent phylogenetic estimates for the Sciuridae merit further verification before a stable taxonomic solution is reached. Future phylogenetic studies with increased taxonomic representation among the basal taxa will likely be required to fully resolve this issue. Presently, a comprehensive assessment of the content of (and relationships among) the newly defined subfamilies is a major goal for Sciurid systematics. Included in this goal, efforts focused on the definition of synapomorphies supporting (or rejecting) the newly erected subfamilies are paramount.

The phylogenetic position of the monotypic genus *Sciurillus*, the Neotropical pygmy squirrel, differs between our two analyses, with neither agreeing with the current taxonomy. Based on characters of the baculum (Anthony and Tate, 1935) and skull (Moore, 1959), *Sciurillus* has been placed within the tribe Sciurini, but in its own subtribe, the Sciurillina (McKenna and Bell, 1997; Moore, 1959). This genus is unique in terms of skull characters (Moore, 1959) and jaw musculature (Ball and Roth, 1995; Thorington and Darrow, 1996), and Moore (1959) noted that it is "...one of the most distinctive genera of squirrels in the world." Both of our analyses place *Sciurillus* well outside of the remaining members of the tribe Sciurini (*Sciurus* and *Microsciurus*). In the MCMC analysis, *Sciurillus* is sister to the remaining Sciuridae (PP = 41%), the monophyly of which is well supported (PP = 93%). In the MP analysis, *Sciurillus* appears sister to *Aplodontia* (BP < 50%). The combination of molecular evidence and morphological distinctiveness suggest that assignment of *Sciurillus* to the tribe Sciurini may be in error, and *Sciurillus* may in fact represent a basal lineage among the Sciuridae, as suggested by Mercer and Roth (2003) and Steppan et al. (in press). Unfortunately, only a partial cyt *b* sequence (528 bp) was available for this taxon, making the confidence of this diagnosis tentative.

The proposed close relationship between *Microsciurus*, the dwarf squirrel, and *Sciurus* (Hafner et al., 1994; Mercer and Roth, 2003; Steppan et al., in press) is confirmed by both analyses. Moore (1959) considered *Microsciurus* a member of the subtribe Microsciurina, along with *Leptosciurus*, *Simosciurus*, *Syntheosciurus*, and *Mesosciurus*. Because no sequence data from these genera were included in this study, the validity of their status as a subtribe cannot be evaluated, but *Microsciurus* clearly falls within a well-supported, otherwise monophyletic *Sciurus* (PP = 100%, BP = 92%). The designation of *Microsciurus* as a genus distinct from *Sciurus* clearly requires further attention including additional sampling of taxa within the genus *Microsciurus*.

Our analyses confirm the close relationship between *Tamiasciurus* and the Sciurini suggested by Mercer and Roth (2003) and Steppan et al. (in press). *Tamiasciurus* appears sister to *Sciurus* (including *Microsciurus*) with strong support (PP = 100%, BP = 78%). Either retention at the tribal level of the Tamiasciurini or inclusion of *Tamiasciurus* within the Sciurini appears justified. In either case, the analyses of Mercer and Roth (2003) and Steppan et al. (in press) show that *Sciurotamias* does not

share a close enough relationship to *Tamiasciurus* to justify its retention in the same tribe.

Piaggio and Spicer (2000, 2001) recommend recognition of three genera for the chipmunks: *Tamias* for *T. striatus*, *Eutamias* for *E. sibiricus*, and *Neotamias* for the remaining chipmunks. Our MP and MCMC analyses are essentially in agreement with the hypotheses of Piaggio and Spicer (2000, 2001), supporting a monophyletic ancestry for chipmunks (PP = 99%, BP < 50%), monophyly of the genus *Neotamias* (PP = 100%, BP = 100%), and the uniqueness of *Tamias* and *Eutamias*. The MCMC analysis recovered *E. sibiricus* sister to *T. striatus* (PP = 72%), and this pair sister to the remaining chipmunks (*Neotamias*), whereas the MP analysis resolved *E. sibiricus* basal to all chipmunks and *T. striatus* sister to *Neotamias*.

In both MP and MCMC analyses, the genus *Spermophilus* appears paraphyletic with respect to *Cynomys*, *Marmota*, and possibly *Ammospermophilus*, and some current subgeneric designations (*sensu* Hall, 1981) are not natural groupings. The close relationship among these genera is in agreement with previous analyses of fossil (Black, 1963) and immunological (Hafner, 1984) data, which suggest that they are all descendants of the Miocene *Miospermophilus*. Results from our analyses, however, differ substantially from traditional taxonomy with respect to relationships within this group. Hafner (1984) considered *Ammospermophilus* to have diverged first from the remaining marmotines. Our data do not completely contradict this, but suggest that two species of *Spermophilus* (*S. adocetus* + *S. annulatus*) share an exclusive common ancestor with *Ammospermophilus*. Fossil (Black, 1963) and chromosomal (Nadler et al., 1971) evidence suggest that *Cynomys* is nested within *Spermophilus* and closely allied with the subgenus *Spermophilus*. Our analyses agree that *Cynomys* is derived from within the currently prescribed genus *Spermophilus*, but reject a close relationship with the subgenus *Spermophilus*.

Our phylogenetic hypotheses demonstrate the multiple polyphyletic origins of the genus *Spermophilus*. As currently defined, this genus contains a minimum of seven well-supported, independent clades in the MCMC analysis (Fig. 3, clades S-1 to S-7). Despite some rearrangements among the deepest internodes within the Marmotini, the MP analysis resolved the same seven clades (Fig. 1, clades S-1 to S-7). Clade S-1 (PP = 100%, BP = 99%) includes two members of subgenus *Otospermophilus*, *S. adocetus* and *S. annulatus*, and is sister to the antelope squirrels (PP = 60%, BP = 52%). Clade S-2 (PP = 99%, BP = 90%) is made up of the New World members of the subgenus *Spermophilus* plus the Old World *S. (S.) undulatus*. Clade S-3 (PP = 100%, BP = 100%) includes two members of subgenus *Ictidomys*, *S. mexicanus* and *S. tridecemlineatus*. Clade S-4 includes the only member of subgenus *Poliocitellus*, *S. franklinii*. Clade S-5 (PP = 100%, BP = 87%) includes the remaining members of subgenus *Ictidomys*, *S. perotensis* and *S. spilosoma*, and both members of subgenus *Xerospermophilus*, *S. mohavensis* and *S. tereticaudus*, and is sister to the prairie dogs (PP = 94%, BP = 57%). Clade S-6 (PP = 100%, BP = 92%) is made up of the Old World members of subgenus *Spermophilus*, except *S. undulatus*. Clade S-7 (PP = 100%, BP = 88%) includes the remaining members of subgenus *Otospermophilus*; *S. atricapillus*, *S. beechyi* and *S. variegatus*; and the three members of subgenus *Callospermophilus*; *S. lateralis*, *S. madrensis* and *S. saturatus*; and is sister to the marmots in the MCMC analysis (PP = 78%, MP does not support).

The taxonomic status of the genus *Spermophilus* is clearly a major problem given our conclusion that it appears to include a total of at least seven independent lineages. All of these clades (S-1 to S-7 in Figs. 1 and 3) are well supported and are sensible candidates for unique generic designations. Given the complex taxonomic history of the Sciuridae, the problem of generic taxonomy requires a comprehensive treatment including a redefinition of available generic names. The type species of the genus *Spermophilus* Cuvier 1825 is *S. citellus*. Thus, the name *Spermophilus* is appropriate for the species included in the Old World clade of the subgenus *Spermophilus* (clade S-6 on Figs. 1 and 3; BP = 92%, PP = 100%). These include *S. citellus*, *S. dauricus*, *S. erythrogenys*, *S. fulvus*, *S. major*, *S. musicus*, *S. pallicauda*, *S. pygmaeus*, *S. relictus*, *S. suslicus*, and *S. xanthoprymnus*.

In summary, our results have demonstrated that the current taxonomy of the Sciuridae poorly represents the phylogeny at several levels. Although many taxonomic problems remain, potential solutions are finally becoming apparent. The analyses of Mercer and Roth (2003) and Steppan et al. (in press) provided the basis for a new classification at the subfamilial and tribal levels, but well-supported differences among our phylogenetic hypotheses and theirs must be addressed by future studies that incorporate both a diversity of genetic loci and a more comprehensive representation of taxa. At the tribal level, their analyses found the Funambulini and the Tamiasciurini polyphyletic, and both their analyses and ours show that *Sciurillus* does not belong within the tribe Sciurini. At the genus level, the widely polyphyletic origins of *Spermophilus* suggest a need for further studies that similarly densely sample large genera within this diverse family. Finally, although not the focus of this study, the presence of several species that do not appear monophyletic in either of our reconstructions underscores the need for careful consideration of alpha taxonomy among squirrels.

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