Molecular Phylogeny of the Springhare, *Pedetes capensis,* Based on Mitochondrial DNA Sequences

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The phylogenetic position of the Pedetidae, represented by a single species *Pedetes capensis,* is controversial, reflecting in part the retention of both Hystricomorphous and Sciurognathous characteristics in this rodent. In an attempt to clarify the species' evolutionary relationships, mtDNA gene sequences from 10 rodent species (representing seven families) were analyzed using phenetic, parsimony, and maximum-likelihood methods of phylogenetic inference; the rabbit, *Oryctolagus cuniculus* (Order Lagomorpha), and cow, *Bos taurus* (Order Artiodactyla), were used as outgroups. Investigation of 714 base pairs of the protein-coding cytochrome *b* gene indicate strong base bias at the third codon position with significant rate heterogeneity evident between the three structural domains of this gene. Similar analyses conducted on 816 base pairs of the 12S rRNA gene revealed a transversion bias in the loop sections of all taxa. The cytochrome *b* gene sequences proved useful in resolving associations between closely related species but failed to produce consistent tree topologies at the family level. In contrast, phylogenetic analysis of the 12S rRNA gene resulted in strong support for the clustering of Pedetidae/Heteromyidae/Geomyidae and Muridae in one clade to the exclusion of the Hystricidae/Thryonomyidae and Sciuridae, a finding which is concordant with studies of rodent fetal membranes as well as reproductive and other anatomical features.

Introduction

The Rodentia, represented by some 32 families, 350 genera, and about 2,000 species, is the most diverse of the extant mammalian orders (Wilson and Reeder 1993). There is no absolute agreement on the classification of this group, especially at the family level (Luckett and Hartenberger 1985*a*; Vaughan 1986), with several factors having been suggested for the lack of consensus (Luckett and Hartenberger 1985*a*): (1) an explosive Eocene-Oligocene radiation, (2) an incomplete fossil record, (3) the large number of rodent species and families make it difficult to study and evaluate any character complex in most or all rodent taxa simultaneously, (4) a failure to distinguish between symplesiomorphies, autapomorphies, and convergent characters, and (5) emphasis has traditionally been placed on the discovery of ancestor–descendant relationships during rodent evolution, while less attention has been devoted to the assessment of sister group relationships among taxa.

In the past, the order Rodentia was divided into three suborders, the Sciuromorpha, Myomorpha, and Hystricomorpha (Brandt 1855; Simpson 1945; Anderson 1967). This classification system was based on the zygomasseteric complex and the associated size of the infraorbital foramen. The protrogomorphous condition observed in Eocene paramyids (Ischyromyoidea) resulted in this classification system being abandoned due to the convergence of characters (Wood 1962, 1974). Subsequently, Carleton (1984; following Tullberg 1899), suggested two suborders for the rodents, the Sciurognathi (squirrels, gophers, beavers, mice), which includes most taxa previously attributed to the Sciuromorpha and Myomorpha, and the Hystricognathi (porcupines, molerats, canerats), which consists largely of members of the Hystrocomorpha *sensu* Brandt (1855).

Although contributing significantly to the clarification of rodent relationships, this classification was confounded by the placement of several families, especially the African Pedetidae. This family, represented by a single species the springhare, *Pedetes capensis,* is regarded as falling within the Sciurognathi (Vaughan 1986), an association based on the primitive condition of the lower jaw (Jaeger 1988). This treatment is, however, equivocal since the species exhibits Hystricognathi characteristics which include multiserial gliriform incisors (Von Koenigswald 1985) and a medial masseter which passes through a greatly enlarged infraorbital foramen (Vaughan 1986). Subordinal affinities aside, familial relationships are equally inconsistent. Springhares share similarities with the Caviidae (eye lens proteins—De Jong 1985), the Anomaluridae (middle ear features—Lavocat and Parent 1985; carotid arterial patterns—Bugge 1985), the Geomyidae, and the Ctenodactylidae (weak paleontological evidence—Hartenberger 1985).

In an attempt to resolve the phylogenetic position of the Pedetidae within the Sciurognathi and Hystricognathi, we analyzed sequences from two mitochondrial DNA (mtDNA) genes, cytochrome *b* and 12S rRNA. Although data from these genes have been used independently to resolve some rodent evolutionary relationships (Allard and Honeycutt 1992; Sudman and Hafner 1992; Da Silva and Patton 1993; Smith and Patton 1993; Thomas and Martin 1993; Nedbal, Allard, and Honeycutt 1994; Myers, Lundrigan, and Tucker 1995), few investigations have been attempted using sequences from both genes. The exception has been Sullivan, Holsinger, and Simon (1995) and, in this instance, there were inconsistencies in the phylogenies that were retrieved. However, since mtDNA genes are inherited as a single locus, concordant results from linked mtDNA sequences can be of special significance in testing phylogenetic accuracy (Miyamoto et al. 1994) and we were

Key words: springhare, *Pedetes capensis,* Rodentia, mtDNA, 12S rRNA, cytochrome *b,* phylogeny.

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Table 1
Specimens Analyzed, Their Common Names and Familial Designations, Geographic Origins (Where Known), and Corresponding GenBank Accession Numbers

Species (Common Name)	Family	Accession Number		Locality
		12S rRNA	Cyt-*b*	
Bos taurus (cow)	Bovidae	J01394[a]	J01394[a]	Unknown
Oryctolagus cuniculus (rabbit)[b]	Leporidae	U59264[c] & X54172[d]	U0756[e]	Unknown
Hystrix africaeaustralis (porcupine)	Hystricidae	U12448[f]	X70674[g]	Unknown
Thryonomys swinderianus (canerat)	Thryonomidae	M63570[h]	U59181	Natal, South Africa
Paraxerus cepapi (tree squirrel)	Sciuridae	U59175	U59179	North Transvaal, South Africa
Sciurus niger (fox squirrel)	Sciuridae	U59174	U10180[i]	Georgia, U.S.A.
Mus musculus (mouse)	Muridae	J01420[j]	J01420[j]	Unknown
Rattus norvegicus (rat)	Muridae	X14848[k]	X14848[k]	Unknown
Cratogeomys castanops (gopher)	Geomyidae	U59172	L11902[l]	Texas, U.S.A.
Dipodomys ordii (kangaroo rat)	Heteromyidae	U59173	U59180	Texas, U.S.A.
Pedetes capensis (springhare 1)	Pedetidae	U59169	U59176	North Transvaal, South Africa
Pedetes capensis (springhare 2)	Pedetidae	U59170	U59177	North Cape, South Africa
Pedetes capensis (springhare 3)	Pedetidae	U59171	U59178	Masai Mara, Kenya

[a] Anderson et al. (1982).
[b] The 12S rRNA sequence is a composite of two GenBank entries.
[c] Halanych and Robinson (unpublished).
[d] Mignotte (unpublished).
[e] Irwin and Árnason (1994).
[f] Ma et al. (1993).
[g] Nedbal, Allard, and Honeycutt (1994).
[h] Allard and Honeycutt (1992).
[i] Wettstein et al. (1995).
[j] Bibb et al. (1981).
[k] Gadaleta et al. (1989).
[l] De Walt et al. (1993).

hopeful that by targeting two genes a more informative picture would emerge. Moreover, this approach allowed us to assess the effects of rate heterogeneity between the study taxa as well as between different structural domains within the 12S rRNA and cytochrome *b* genes (Irwin, Kocher, and Wilson 1991; Muse and Weir 1992; Springer, Hollar, and Burk 1995).

Materials and Methods

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from eight specimens representing five rodent families (table 1) and target sequences amplified by the polymerase chain reaction (PCR). Corresponding sequences for two additional families, the Muridae and the Hystricidae, were obtained from GenBank.

Standard phenol/chloroform/iso-amyl alcohol DNA extractions were done using either fresh or DMSO/NaCl-preserved heart tissue (Amos and Hoelzel 1991). "Universal" end-primers were employed to amplify approximately 818 bp of the mitochondrial 12S rRNA (L82 and H900, Allard and Honeycutt 1992) and 770 bp of the cytochrome *b* gene (L14724, Pääbo and Wilson 1988; H15494, complement of L15513—Kocher et al. 1989); the numbers for cytochrome *b* primers correspond to the human sequence (Anderson et al. 1981) and those for the 12S rRNA gene to the mouse (Bibb et al. 1981). Both end-primers, as well as five internal primers, were used for sequencing. The cytochrome *b* internal primers were L14841 (Kocher et al. 1989), L15162 (Pääbo and Wilson 1988), and H15149 (Kocher et al. 1989); those for the 12S rRNA region were H618 and L509 (Allard and Honeycutt 1992). To facilitate ssDNA sequencing, one of the end-primers was biotinylated.

The 50-μl PCR reaction mixture contained approximately 0.05 μg of total genomic DNA, 5 μl of 10 × polymerase buffer, 5 μl 25 mM $MgCl_2$, 5 μl of 2 mM dNTPs, 25 pmol of appropriate primers and 1 unit of thermostable DNA polymerase (Advanced Biotechnologies). The final cocktail was layered with 50 μl mineral oil. Cycling parameters were: 4 min at 95°C for initial denaturation (one cycle), 45 s at 94°C for denaturation, 45 s at 50°C for annealing of specific primers, 90 s at 72°C for the extension (35 cycles), and 5 min at 72°C for final extension. The PCR products were separated in 2% low-melting-point Nusieve agarose and extracted using a commercial purification system (Cleanmix, Talent). Single-stranded biotinylated products were captured magnetically using Dynabeads (Dynal, Oslo, Norway). Sequencing followed routine procedures (Sequenase kit v 2.0; U.S. Biochemical), with the chain terminated DNA fragments being separated using 7% polyacrylamide gels. All sequences were verified by sequencing both the light and heavy strands

Sequence Analyses and Outgroups Used in this Study

Sequences were aligned using CLUSTAL version V (Higgins, Bleasby, and Fuchs 1992) and the 12S rRNA segments were manually corrected using the secondary structure model. The alignments can be obtained

from EBI (accession numbers for the cytochrome *b* sequences = DS27132 and 12S rRNA sequences = DS26902). Given the uncertainty concerning rodent monophyly (Graur, Hide, and Li 1991; Ma et al. 1993; Luckett and Hartenberger 1993; Nedbal, Allard, and Honeycutt 1994; Frye and Hedges 1995; D'Erchia et al. 1996; Porter, Goodman, and Stanhope 1996) as well as the questionable sister taxon relationship between rodents and lagomorphs (Eastel 1990; Novacek 1993; Meng et al. 1994; Graur, Duret, and Gouy 1996), sequences of the rabbit (*Oryctolagus cuniculus*) and the cow (*Bos taurus*) were used together and independently as outgroups to polarize the characters (table 1). For the phylogenetic illustrations, we used the rabbit as outgroup because of its traditional placement as sister taxon to the order Rodentia (Honeycutt and Adkins 1993; Novacek 1993; Meng et al. 1994). However, when the cow was used as outgroup, either independently or with the rabbit, the main conclusion of this study remained unaltered.

Parsimony analysis of the aligned sequences was done using heuristic and branch-and-bound search options in PAUP (v. 3.1.2d5; Swofford 1993). Ten thousand random trees were evaluated to obtain the g_1 statistic. Because the cytochrome *b* and the 12S rRNA genes operate under different molecular constraints, various analyses were employed to enhance the phylogenetic signal of the sequence data. Consequently, nucleotide sequences were partitioned into different regions that are known to evolve at different rates (Hillis, Allard, and Miyamoto 1993). Differential weighting of transitions (TI) and transversions (TV) was employed in all parsimony analyses (unweighted, 1:2, and 1:3). Empirical TI : TV ratios and ranges were calculated using the "state changes and stasis" option in MacClade (Maddison and Maddison 1992) for 100 random trees (Halanych 1996).

Neighbor-joining analysis (Saitou and Nei 1987) was performed using PHYLIP v.3.5 (Felsenstein 1993). Because the order of taxa in the data set can affect the results of a neighbor-joining tree (Ferris et al. 1995), we analyzed each data set 10 times using a randomized input order. The Kimura two-parameter distance correction was used to generate the distance statistics, while topologies based on transversions only were derived from the Jukes-Cantor corrected values. Maximum-likelihood trees (Felsenstein 1981) were obtained using DNAML in PHYLIP (Felsenstein 1993) applying a 1:2 TI : TV value.

Nodal support was assessed using bootstrap analyses. In both parsimony and neighbor-joining analyses, 1,000 bootstrap iterations were performed while only 100 iterations were employed for the maximum-likelihood estimates.

Rate Heterogeneity Test

We used a likelihood ratio test (Muse and Weir 1992) to compare relative rates of evolutionary change between the rodent taxa included in this study. This approach, which is largely unaffected by saturation of transitions (Muse, personal communication), allowed us to distinguish between unequal substitution rates along paths of descent leading to two species. The rabbit was used as reference taxon for the pairwise comparisons.

Cytochrome b *Analysis*

Parsimony, neighbor-joining and maximum-likelihood analyses were performed including all characters. Additionally, we excluded third-position transitions and also analyzed the data using transversions only. Furthermore, the cytochrome *b* sequence data set was translated to protein codons and partitioned into the three structural domains: the inner membrane (Q_i), the outer membrane (Q_o), and the transmembrane (Q; Irwin, Kocher, and Wilson 1991). Codon changes within these regions were scored as fast- or slow-evolving sites. Fast-evolving sites were scored when at least two changes were evident at a specific codon (Irwin, Kocher, and Wilson 1991). In order to improve phylogenetic resolution, emphasis was placed on those regions characterized by more conservative changes (Irwin, Kocher, and Wilson 1991). Rate differences between these cytochrome *b* domains were tested using the (2 × 2)-independence test and the significance of the obtained values was determined using χ^2 tables.

12S rRNA Analysis

Since the 12S rRNA gene is often marked by compensatory substitutions in the stem sites and is therefore nonindependent (Dixon and Hillis 1993; Funk et al. 1995; Springer, Hollar, and Burk 1995), the ribosomal gene sequences were partitioned into stem and loop sections using the mammalian secondary structure model for the 12S rRNA gene (Springer, Hollar, and Burk 1995). First, the stem and loop characters were analyzed separately. Second, the complete data set was analyzed and stem characters were down-weighted (0.6; Springer, Hollar, and Burk 1995). Finally, down-weighting was ignored and all characters were treated equally. Neighbor-joining analyses were conducted including all characters, as well as using transversions only, while the complete data set was used for maximum-likelihood analyses.

Combined Analysis

Hillis (1987) suggested that all relevant data should be included to obtain the best estimate of phylogeny. Although this idea is currently widely supported, different ideas have been put forward as to how data from multiple data sets should be combined in phylogenetic analyses (Bull et al. 1993; De Queiroz 1993; Chippindale and Wiens 1994). In this study, we analyzed our data sets separately (see above) and in combination. We applied equal weighting of TIs and TVs to each gene segment, and we also used a TI : TV weighting of 1:2 and excluded the saturated third codon position of the protein-coding gene (see Chippindale and Wiens 1994).

Results and Discussion

Rate Heterogeneity

The presence of a constant molecular clock in rodents has been questioned at both the interordinal level

(Li, Tanimura, and Sharp 1987) and, pertinent to this investigation, between families of the Rodentia (De Walt et al. 1993). The application of a likelihood ratio test (Muse and Weir 1992) to our sequence data revealed differences in the rate of evolution between some rodent families but not others (table 3). *Cratogeomys* and *Pedetes* show significant cytochrome *b* rate differences in comparison with *Mus,* thereby confirming an earlier report with respect to *Cratogeomys* (De Walt et al. 1993), while *Thryonomys* differed with respect to *Mus, Rattus, Sciurus,* and *Hystrix.* In general it seems that the cytochrome *b* rate differences are restricted to two rodent taxa used in this study, *Mus* and *Thryonomys* (table 3). In contrast, perhaps reflecting the different evolutionary constraints under which these two genes have evolved, the 12S rRNA data failed to provide evidence of rate heterogeneity between these taxa. The only exceptions to this were the Geomyidae (*Cratogeomys*) and Heteromyidae (*Dipodomys*), which were significantly different to all rodent taxa with the exception of *Paraxerus* (table 3).

The rate differences detected between some rodent taxa (table 3) highlight difficulties in identifying factors responsible for causing rate heterogeneity between taxa. It has been hypothesized that DNA mutation rate could be proportional to metabolic rate and life history patterns (Britten 1986; Catzeflis et al. 1987; Li et al. 1996). However, in this study taxa such as *Mus* and *Rattus* (both small rodents with similar life history patterns) do not show the same cytochrome *b* rate differences when compared to the other rodents. This finding suggests that differences in rate between taxa are not simply due to metabolic rate and life history, emphasizing the danger of generalizing across species even within the same family (see Zhang and Ryder 1995).

Characteristics of the Cytochrome *b* Data Set

The cytochrome *b* data set consisted of 714 characters of which 284 were parsimony sites. GenBank accession numbers are given in table 1. Of the variable sites, 62 (21.8 %) were at the first, 23 (8.1 %) were at the second, and 199 (70.1 %) were at the third codon position. These values did not change significantly with the exclusion of the outgroup. Translation of the cytochrome *b* sequence into amino acids resulted in 238 characters of which 55 were phylogenetically informative.

The TI : TV value depended heavily on which data were examined. The 100 random trees obtained for the complete cytochrome *b* data set illustrated 512–582 transitions and 488–633 transversions which correspond to a mean TI : TV value of 0.94. This value was similarly obtained for first codon positions alone (0.97); in contrast, the conserved second codon showed a TI : TV bias of 1.56 while the third positions were more saturated (0.89) and probably contribute most to the homoplasy present in the cytochrome *b* data set (see Hillis, Allard, and Miyamoto 1993). The first- and second-position bias in nucleotides was similar to that recorded for other mammals (Irwin, Kocher, and Wilson 1991) but, by comparison, the third-position bias tends to be lower in the rodent families included in this study (table 2).

The cytochrome *b* structural domains showed a similar pattern to that reported for mammals (Irwin, Kocher, and Wilson 1991). The Q_i consisted of 66 amino acid codons of which 20 were fast evolving, while 46 were considered to be slow-evolving codons. The Q section contained 102 codons of which 32 were fast and 70 slow. The Q_o was considerably more conserved with 7 of 70 codons considered fast and the remainder as slow codons. Significant rate heterogeneity is evident when comparing Q_o and Q ($\chi^2 = 9.63$, $P < 0.005$) as well as Q_o and Q_i ($\chi^2 = 7.57$, $P < 0.01$). There is no significant difference between Q and Q_i ($\chi^2 = 0.0006$, $P < 0.99$), indicating that the cytochrome *b* outer surface membrane sequence evolves slowly in comparison with the other two regions.

The g_1 value of the cytochrome *b* data set was significant ($g_1 = -1.06$; $P < 0.01$, Hillis and Huelsenbeck 1992). A nonsignificant value of -0.27 was obtained when only one representative of each rodent family was included, clearly indicating that most of the phylogenetic signal is due to recent evolutionary events (within the rodent families).

Cytochrome *b* Phylogeny

Branch-and-bound unordered parsimony analysis resulted in two trees that were 1,065 steps long (consistency index [CI] = 0.53; CI excluding uninformative characters = 0.49; retention index [RI] = 0.41). The strict consensus is presented in figure 1*a.* Differential weighting of TI : TV, the exclusion of the homoplasic third codon characters, and translation to proteins, as well as down weighting (0.5; 0.25) the more variable Q_i and Q domains of cytochrome *b* did not improve the resolution which was characterized by considerable branch swapping between taxa. Additionally, most internal nodes collapsed (<50% support) during bootstrap analysis.

By comparison, a single concordant topology was obtained from both the neighbor-joining (irrespective of taxa input order) and the maximum-likelihood analyses which resulted in strong bootstrap support for some well-established rodent phylogenetic associations (fig. 1*b*). These included the close relationship between *Mus* and *Rattus* (taxa within the Muridae) and between *Dipodomys* and *Cratogeomys* (both representatives of the superfamily Geomyoidae; see Luckett and Hartenberger 1985*a* and references therein). Between families, however, the only node that was well supported by bootstrap analysis was that supporting the Muridae (suborder Sciurognathi) as a sister taxon to the Hystricidae and Thryonomyidae (suborder Hystricognathi). Although this novel association was supported by two different methods of phylogenetic inference (neighbor-joining and maximum-likelihood), it is in conflict with paleontological, morphological, and developmental evidence (Luckett and Hartenberger 1985*a,* 1985*b*). Equally problematic was the diphyletic origin revealed for the Sciuridae which, while lacking phylogenetic support from nonmolecular data sets (Luckett and Hartenberger

Table 2
Base Composition at First, Second, and Third Codon Positions of Cytochrome *b* and the Stem and Loop Sections of the 12S rRNA Gene

	First Codon			Second Codon			Third Codon			Stems			Loops			Stems and Loops		
Species	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T	C
Ocu	22.7	28.6	26.5	22.3	13.9	20.2	40.3	25.6	1.7	35.7	23.5	39.1	25.8	24.2	26.3	23.7	9.8	48.5	20.4	21.3	17.8	36.4	23.3	22.5
Haf	22.3	28.2	27.7	21.8	14.3	20.2	39.9	25.6	3.8	40.8	23.5	31.9	24.6	26.5	27.3	21.6	10.3	49.5	21.9	18.3	17.5	38.0	24.6	19.9
Tsw	23.5	25.6	28.6	22.7	13.9	20.6	40.8	24.8	2.1	45.4	18.9	33.6	23.3	27.1	28.2	21.4	10.8	47.8	19.9	21.5	17.1	37.4	24.1	21.4
Pce	24.3	27.7	25.5	22.6	14.9	18.7	39.1	27.2	2.1	35.3	23.0	40.4	26.8	24.1	24.3	24.8	10.0	46.0	20.9	23.1	18.4	35.1	22.6	23.9
Sni	23.9	26.1	29.4	20.6	13.9	19.3	42.4	24.4	3.4	31.9	36.1	28.6	26.0	22.9	26.6	24.5	10.4	44.2	23.0	22.4	18.8	34.3	25.3	21.6
Mmu	23.9	29.0	27.3	19.7	13.9	19.7	41.6	24.8	2.5	43.7	20.2	33.6	21.6	28.5	27.7	22.2	8.9	48.7	24.3	18.1	15.3	38.6	26.0	20.1
Rno	23.9	26.4	26.9	22.7	13.9	19.7	41.2	25.2	2.1	42.4	14.3	41.2	23.5	26.8	26.8	22.9	9.1	47.2	22.9	20.8	16.3	37.0	24.8	21.9
Cca	22.7	27.3	29.8	20.2	15.1	21.0	40.3	23.5	2.1	45.0	21.0	31.9	24.2	26.8	26.3	22.7	11.3	50.3	20.3	18.1	17.7	38.6	23.3	20.4
Dor	23.8	27.8	26.9	21.6	15.4	22.0	37.9	24.7	2.6	33.9	29.5	33.5	26.2	24.4	23.9	25.5	9.2	49.1	24.9	16.8	17.7	36.7	24.4	21.2
Pca1	21.4	29.4	28.6	20.6	13.9	19.7	40.3	26.1	4.6	36.1	27.3	31.9	23.8	25.8	27.8	22.6	10.8	46.6	25.0	17.5	17.3	36.2	26.4	20.1
Pca2	21.8	29.8	29.0	19.3	13.9	19.7	40.3	26.1	5.9	34.5	26.5	33.2	24.0	25.7	27.8	22.5	10.3	46.9	25.8	17.0	17.1	36.3	26.8	19.8
Pca3	20.2	31.5	28.2	20.2	13.9	19.7	40.8	25.6	2.9	37.0	29.0	31.1	24.8	25.6	27.1	22.5	10.4	47.1	24.4	18.1	17.6	36.3	25.8	20.3
Mean	22.9	28.1	27.8	21.2	14.2	20.1	40.4	25.3	3.0	38.5	24.4	34.1	24.6	25.7	26.7	23.0	10.1	47.7	22.8	19.4	17.4	36.7	24.8	21.1
∂	1.3	1.7	1.3	1.2	0.6	0.8	1.2	1.0	1.3	4.7	5.7	3.9	1.5	1.6	1.4	1.3	0.7	1.7	2.1	2.2	0.9	1.3	1.3	1.2
Bias	0.079				0.210				0.301				0.032				0.303				0.156			

Note.—The bias in base composition was calculated using: $C = (\frac{3}{4}) \Sigma |c_i - 0.25|$, where C is the compositional bias and c_i is the frequency of the ith base (Irwin, Kocher, and Wilson 1991). Abbreviations are as follows: *Ocu, Oryctolagus cuniculus*; *Haf, Hystrix africaeaustralis*; *Tsw, Thryonomys swinderianus*; *Pce, Paraxerus cepapi*; *Sni, Sciurus niger*; *Mmu, Mus musculus*; *Rno, Rattus norvegicus*; *Cca, Cratogeomys castanops*; *Dor, Dipodomys ordii*; *Pca1, Pedetes capensis*; *Pca2, Pedetes capensis*; *Pca3, Pedetes capensis*.

1985*a*), was nonetheless suggested by all three methods of phylogeny reconstruction.

Cytochrome *b* mitochondrial DNA gene sequences have previously been used to assess relationships among mammalian families (Irwin, Kocher, and Wilson 1991; Irwin and Arnason 1994; Vrana et al. 1994; Honeycutt et al. 1995). Moreover, they have been particularly useful in resolving rodent evolutionary affinities within families (De Walt et al. 1993; Nazareth, Da Silva, and Patton 1993; Wettstein et al. 1995). It is evident from our study, however, that while analysis of cytochrome *b* sequences resulted in strongly supported associations within the Pedetidae, it failed to give robust resolution at the family level. This may be due, in part, to the rapid radiation of the Rodentia during the Eocene (over half of the extant families were present by the end of the Oligocene; Vaughan 1986) making the retrieval of shared derived characters problematic. Given that most rodent families last shared common ancestry 55–30 MYA, reverse mutations may have effectively randomized the character states with respect to phylogenetic history. Evidence of homoplasy in our cytochrome *b* data set is reflected by the low CI values (Swofford and Olson 1990) obtained from the various analyses (not shown), as well as by the saturated TI : TV values (see Hillis, Allard, and Miyamoto 1993). Unfortunately, excluding many of the sources of homoplasy (analyzing protein codons, excluding transitions at third positions, using first- and second-position changes, and using transversions only) reduced the number of potential informative characters to the point of precluding phylogenetic inference.

Given the gene's poor performance in resolving deeper phylogenies, it is not surprising that in instances where cytochrome *b* sequences have been used to infer rodent monophyly (Li et al. 1990; Graur, Hide, and Li 1991; Ma et al. 1993), the outcomes have been controversial. In these studies the gene invariably revealed a polyphyletic origin for rodents, a conclusion which has been criticized by several workers (Allard, Miyamoto, and Honeycutt 1991; Honeycutt and Adkins 1993; Luckett and Hartenberger 1993; Frye and Hedges 1995). In the light of the strong support for rodent monophyly suggested by other data sets (Luckett and Hartenberger 1993), and given the outcome of the present study, it seems likely that cytochrome *b* should not be the mitochondrial gene of choice when attempting to infer rodent familial and higher level relationships.

Characteristics of the 12S rRNA Data Set

The 12S rRNA data set consisted of 816 characters including alignment gaps (GenBank accession numbers are presented in table 1). Of these, 422 were found to be in the loop sections (comprising 184 parsimony sites), whereas 394 were in the stems (107 parsimony sites) allowing analysis of 291 informative characters.

The substitution rates of transitions relative to transversions for the 12S rRNA gene are consistent with other reports (Funk et al. 1995; Springer, Hollar, and Burk 1995); the numbers of transitions are approximately equal in the loops (TI = 227–264) and stems (TI

Table 3
Log Likelihood Ratio Significance Values for the Comparisons Between Rodent Taxa Included in this Study

Species	*Haf*	*Tsw*	*Pce*	*Sni*	*Mmu*	*Rno*	*Cca*	*Dor*	*Pca1*	*Pca3*
Haf.....		6.07*	0.27	1.94	2.67	3.74	4.00	1.12	1.01	2.53
Tsw	2.70		4.44	8.26*	10.1*	6.05*	0.35	3.27	2.56	1.02
Pce.....	0.15	0.99		1.39	1.43	1.50	2.53	0.07	0.91	2.13
Sni.....	1.62	0.42	3.10		0.52	0.76	5.28	1.42	3.66	5.80
Mmu....	3.43	0.28	2.89	0.45		0.84	6.31*	2.35	6.03*	7.59*
Rno	6.13*	1.30	4.37	0.42	1.99		2.79	1.00	2.79	3.85
Cca	9.37**	12.2**	14.7**	16.5**	8.22*	13.5**		1.69	0.84	0.26
Dor	6.22**	6.66*	4.79	6.98*	6.09*	10.9**	3.59		0.37	1.13
Pca1....	0.86	1.47	1.27	2.55	5.82	4.10	19.5**	7.19*		1.46
Pca3....	1.08	1.30	1.55	2.72	5.84	4.99	19.3**	7.01*	0.18	

NOTE.—The rabbit (*Oryctolagus cuniculus*) was used as reference taxon. Cytochrome *b* values are presented above the diagonal, while those below are from 12S rRNA. Abbreviations are as in table 2.

* 0.05% confidence $P < 5.992$.

** 0.001% confidence $P < 9.21$.

= 219–278). Strikingly, there is an approximate threefold increase in transversions within the loops (TV = 363–436) compared to the stems (TV = 122–147) and there are significantly more parsimony sites within loops than within stems ($\chi^2 = 21.08$, $P < 0.001$). The mean TI : TV value within stems was estimated at 1.90 while the loops are characterized by a higher saturation score of 0.61. The empirical rodent TI : TV score calculated for all 816 12S rRNA characters was 0.93.

Loop sections within the 12S rRNA gene showed a similar A-T bias to that reported for mammals (Springer, Hollar, and Burk 1995; table 2). This bias is probably due to the hydrophobic interactions with ribosomal proteins (Gutell et al. 1985). In comparison with the analysis of cytochrome *b*, the nucleotide frequencies for the 12S rRNA gene did not reflect differences between taxa and showed good correspondence with the general mammalian pattern (Springer, Hollar, and Burk 1995).

The g_1 statistic based on the ribosomal gene sequences was significant irrespective of whether closely related taxa were included ($g_1 = -0.97$; $P = 0.01$, Hillis and Huelsenbeck 1992) or excluded (one representative of each family; $g_1 = -0.99$; $P = 0.01$). These values indicate that the data are significantly more structured than random (Hillis and Huelsenbeck 1992) and contain meaningful phylogenetic signal.

12S rRNA Phylogeny

The 12S rRNA analyses consistently clustered Pedetidae/Geomyidae/Heteromyidae and Muridae in one clade to the exclusion of the Hystricidae/Thryonomyidae and Sciuridae (fig. 1*c*). Although <50% bootstrap support was obtained for the familial clusterings when transitions and transversions were weighted equally, the application of differential weighting (1:2; 1:3) noticeably improved the bootstrap support for the familial clusterings. An identical topology was obtained irrespective of whether stem characters were down-weighted or analyzed separately, or whether the analysis was based only on transversions (fig. 1*c*). An unweighted branch-and-bound parsimony analysis of the 12S rRNA sequences resulted in a single most parsimonious tree of 911 steps (CI = 0.67; CI excluding uninformative characters = 0.60; RI = 0.55; tree not shown). This tree is consistent with the one presented in figure 1*c* in that the Pedetidae/Geomyidae/Heteromyidae/Muridae clade was recovered, except in this instance, the Geomyoidae clustered as sister taxon to Pedetidae instead of Muridae. The maximum-likelihood analysis using a 1:2 weighting scheme resulted in the same tree as figure 1*c* with the Pedetidae/Geomyidae/Heteromyidae/Muridae clade being supported in 76 of 100 bootstrap iterations (cf. the 69% for the 1:2 weighted parsimony tree; fig. 1*c*). In contrast, the neighbor-joining analysis using the Kimura two-parameter correction resulted in several spurious associations. These included the failure of the Muridae to group with the Geomyoidae and the basal placement of the Muridae, which are not consistent with the morphological and paleontological data (Luckett and Hartenberger 1985*a*). However, by excluding all transitions, the neighbor-joining analysis differed only in the placement of the Hystricognathi, a node that is weakly supported by bootstrap in all analyses (fig. 1*d*). The neighbor-joining topologies were invariably retained irrespective of the order of taxa in the data set.

Combined Phylogeny

Both weighting schemes used for the combined data set resulted in the same tree topology. The unweighted tree including all characters was 1,991 steps long (CI = 0.59; CI excluding uninformative characters = 0.53; RI = 0.46; tree not shown). The combined tree was identical to that obtained from the unweighted parsimony analysis of the 12S rRNA gene (see above). Although all the analyses of the combined approach support the Pedetidae/Geomyidae/Heteromyidae/Muridae association, combining the two data sets generally resulted in low bootstrap support for this node (including all data = <50%, exclusion of third codons, TI : TV 1:2 = 54%).

The Phylogeny of the Pedetidae—a Summary of Conflicting Data Sets

Although springhares show both hystricomorphous and sciurognathous morphological characters, our phylogenetic analyses of the mitochondrial 12S rRNA sequences, as well as those based on both 12S rRNA and cytochrome *b* data, invariably resulted in the grouping

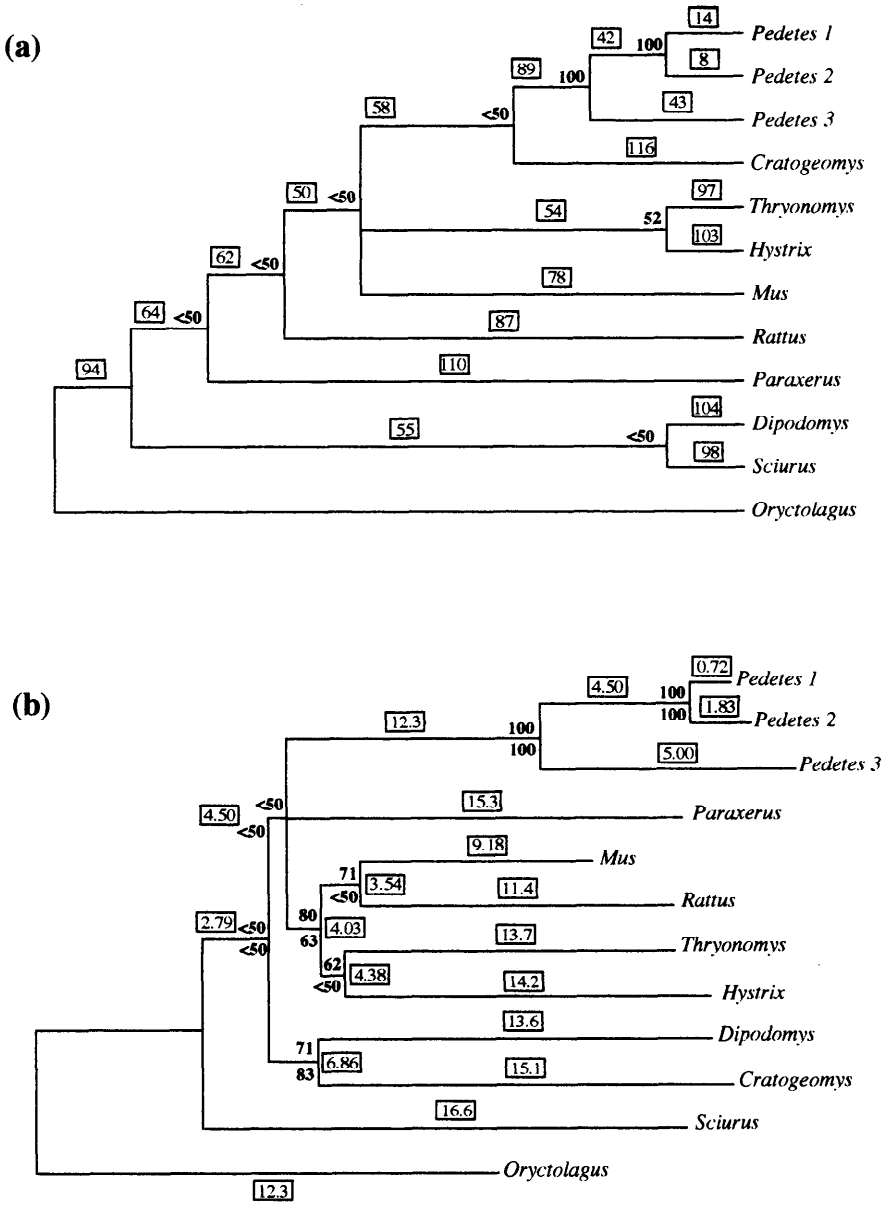

FIG. 1.—Phylogenetic relationships among seven rodent families based on mtDNA sequences. The numbers in bold on the branches reflect the bootstrap support for the nodes. *a,* Strict consensus of two equally parsimonious trees based on 714 unordered cytochrome *b* characters. Values in boxes represent the average number of changes along each branch. *b,* Neighbor-joining tree of the same data set based on Kimura two-way corrected values; branch lengths were drawn proportionally. The topology obtained from maximum-likelihood was identical and branch lengths are indicated in the boxes above or next to the branches. Bootstrap support is shown above (neighbor-joining) and below (maximum-likelihood) the various nodes. *c,* The single most parsimonious tree based on 816 bp of the 12S rRNA gene using a TI : TV weighting of 1:2. The maximum-likelihood topology for the same data set was identical. Bootstrap support is shown above (parsimony) and below (maximum-likelihood) the various nodes. Values in the boxes above the branches indicate the average number of changes leading to each node whereas underlined values represent the maximum-likelihood branch lengths. *d,* Neighbor-joining tree based on 12S rRNA transversions; branch lengths were drawn proportionally.

of the Pedetidae, Heteromyidae, Geomyidae, and Muridae (all Sciurognathi) into a single clade to the exclusion of the Hystricidae and Thryonomyidae (included in the Hystricognathi). Studies of rodent fetal membranes, reproductive features, and other morphological characteristics support this association (Luckett 1985). The alternative hypothesis (clustering *Pedetes* with the Hystricognathi), which is weakly supported by α-crystallin sequence data (De Jong 1985), is five steps longer than the most parsimonious solution using equally weighted mtDNA characters. Additionally, the Pedetidae/Geomyoidae/Muridae clade is supported by all methods of phylogenetic inference, although with varying bootstrap support. This strengthens previous arguments (see Luckett and Hartenberger 1985*a,* 1985*b*) that hystricomorphous characteristics present in *Pedetes* (for example, the masseter muscle passing through an enlarged infraorbital foramen as well as the incisor enamel) are due to parallelisms and are therefore not useful for phylogenetic inference.

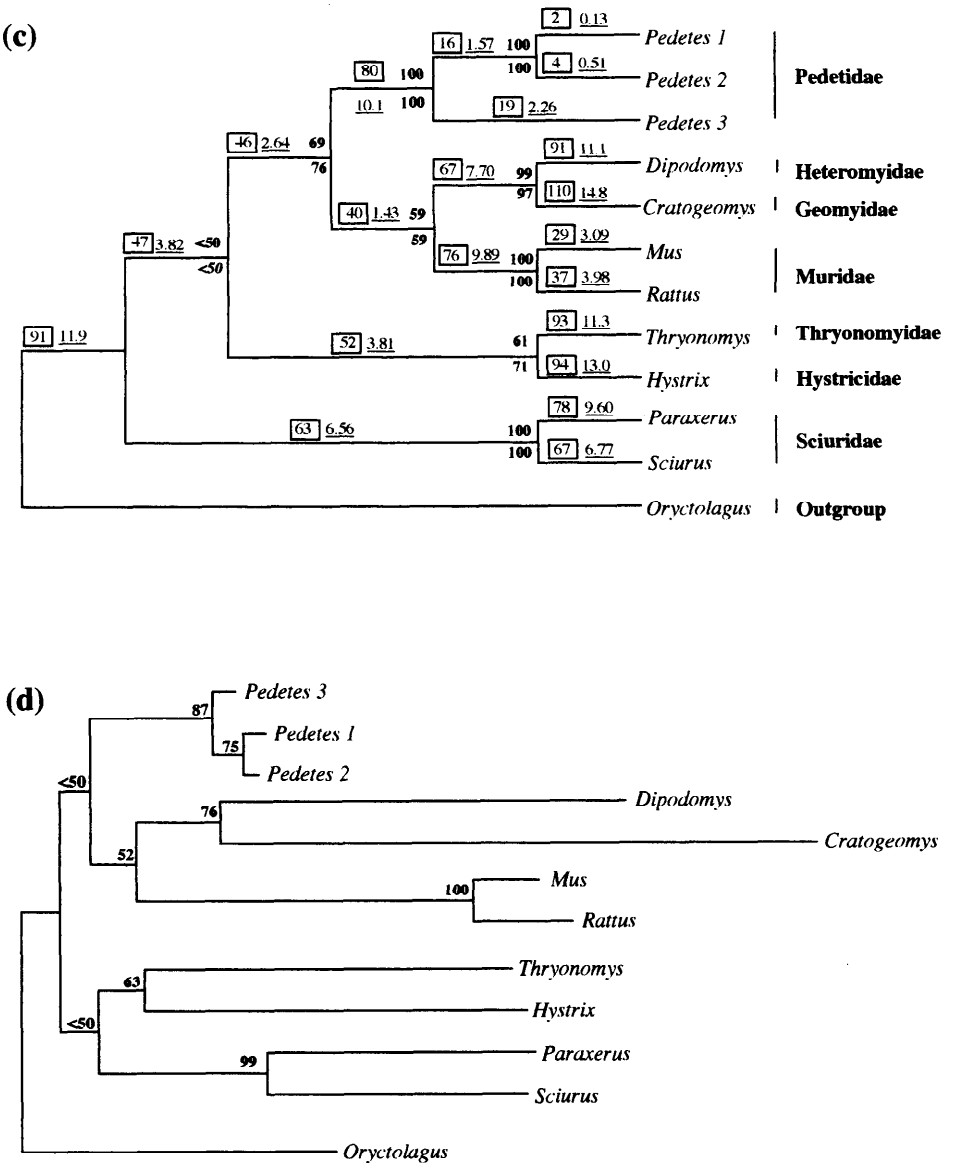

FIG. 1. *(Continued)*

Clearly, applying a single functional system (such as the masticatory apparatus or the inflections of the lower mandible) to define subordinal associations for rodents is questionable. Moreover, despite the relatively small number of rodent families encompassed by our study (7 out of 32), our results nonetheless underscore a weakness of the subordinal classification system adopted, by, among others, Carleton (1984). For example, in the present investigation the Sciuridae (placed in the Sciurognathi—Tullberg 1899; Carleton 1984) was basal in our phylogenetic reconstructions and quite separate from the other families comprising the Sciurognathi, a finding contrary to Carleton (1984). If this holds, it would imply a paraphyletic origin for the suborder Sciurognathi. In conclusion, therefore, it seems likely that only through the analysis of multiple, unrelated data sets will a clearer picture of rodent interfamilial evolutionary relationships emerge.

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