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Higher-Level Systematics of Rodents (Mammalia, Rodentia): Evidence from the Mitochondrial 12S rRNA Gene

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Phylogenetic relationships among major rodent superfamilies traditionally have been difficult to establish because of the apparent high level of convergence and parallelism seen among morphological characters and/or rapid differentiation of rodent groups in the Paleocene/Eocene. Nucleotide sequence data from the mitochondrial 12S rRNA gene were used to clarify phylogenetic relationships among the major groups of rodents as defined by Brandt (1855) and Tullberg (1899). Based on the approximately 800 bp analyzed for the 12S rRNA gene in 59 mammalian species, including 25 of the 32 extant rodent families, the major rodent groups that could be defined as monophyletic clades were the Hystricognathi, the Muroidea, and the Geomyoidea. In addition, support for superfamilial sister-group relationships was found for Aplodontoidea with Sciuroidea and Dipodoidea with Muroidea.

KEY WORDS: Rodentia; mitochondrial; phylogeny; 12S rRNA; Sciurognathi; Hystricognathi.

INTRODUCTION

The mammalian order Rodentia is divided into approximately 32 extant families and contains almost half of all living species of mammals (Hartenberger, 1985). Rodents show considerable diversity in morphology, habitat utilization, behavior, life history strategies, and biogeographic distribution. Although rodent monophyly has been questioned (Graur *et al.*, 1991; Li *et al.*, 1992; Ma *et al.*, 1993; D'Erchia *et al.*, 1996), a large number of shared-derived morphological characters found in both fossil and extant species are diagnostic for the order (Woods, 1972; Sarich and Cronin, 1980; George, 1985; Lavocat and Parent, 1985; Luckett, 1985; Sahni, 1985; Sarich, 1985; Shoshani *et al.* 1985; Woods and Hermanson, 1985; Luckett and Hartenberger, 1993; Martin, 1993). The primary characters supporting monophyly of Rodentia involve specializations of the masticatory apparatus (incisors, cheek teeth, and musculoskeletal features

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of the jaw and skull). Presumed natural groups within Rodentia have been identified using characters associated with size and shape of the infraorbital foramen, attachments and development of the masseter muscles, and position of the angular process relative to the plane of the incisor (Wood, 1955). Although most existing classifications have used combinations of these characters (Korth, 1994), rodents exhibit high levels of convergent and parallel evolution with respect to many morphological features, and the success of various classifications in identifying natural groups has been mixed (Patterson and Wood, 1982; Hartenberger, 1985; Jaeger, 1988).

One of the earliest detailed rodent classifications was that of Brandt (1855), who recognized, on the basis of the origin and insertion of masseter muscles relative to the infraorbital foramen, zygomatic arch, and rostrum, three major suborders: Sciuromorpha, Myomorpha, and Hystricomorpha. Tullberg's (1899) classification was somewhat simpler than Brandt's (1855) in that two major divisions, Sciurognathi and Hystricognathi, were identified using the angle of the jaw relative to the plane of the incisors. Most rodent systematists accept Tullberg's Hystricognathi as a monophyletic group. Nevertheless, there has been considerable disagreement as to the details of rodent relationships within the Hystricognathi, among sciurognathid families, and between the Hystricognathi and other rodent families (Fig. 1). As a result of this disagreement, many subordinal classifications have been proposed (Schlosser, 1884; Miller and Gidley, 1918; Winge, 1924; Wood, 1937, 1955, 1965; Ellerman, 1940; Simpson, 1945; Lavocat, 1951; Stehlin and Schaub, 1951; Landry, 1957; Chaline and Mein, 1979; Meng, 1990).

Both Brandt's (1855) and Tullberg's (1899) classifications provide good examples as to why interpretation of morphological features can be difficult. From a comparative morphological and paleontological viewpoint, sciurognathy of the lower jaw is presumably primitive, because it is recorded in the earliest known rodents and most other mammals (Jaeger, 1988). A protrogomorphous condition, seen in most mammals as well as the living Aplodontidae (mountain beavers) and extant mole-rats of the family Bathyergidae [but see Lavocat (1973, 1988) and Maier and Schrenk (1987) for ontogenetic and fossil evidence supporting the secondary derivation of protrogomorphy in this family], is presumably the primitive condition for the zygomasseteric system in rodents (Korth, 1994). The problem arises in how one interprets these morphological features throughout rodent evolution. For instance, the suborder Hystricognathi, as currently recognized, contains rodent families that are both hystricomorphous and hystricognathous, suggesting that both these suites of morphological features are characteristic of the suborder. In Tullberg's (1899) suborder Sciurognathi all rodent families are sciurognathous, yet differ with respect to being either protrogomorphous, sciuromorphous, hystricomorphous, or myomorphous (reflecting the divisions identified by Brandt, 1855). Therefore, in terms of the condition of the lower jaw, all families within Sciurognathi share the primitive condition, yet several arrangements of the zygomasseteric structure may represent a derived condition. This led Hartenberger (1985) to suggest that Sciurognathi is paraphyletic. If hystricomorphy is derived, then several families (Anomaluridae, scaly-tailed squirrels; Ctenodactylidae, gundis; Dipodidae, jerboas; and Pedetidae, springhare) currently within Tullberg's (1899) Sciurognathi may actually represent a monophyletic group sister to the suborder Hystricognathi because they all share a hystricomorphous condition with the Hystricognathi. A multiserial Schmelzmuster associated with incisor enamel microstructure is presumably a synapomorphy for the Hystricognathi and families

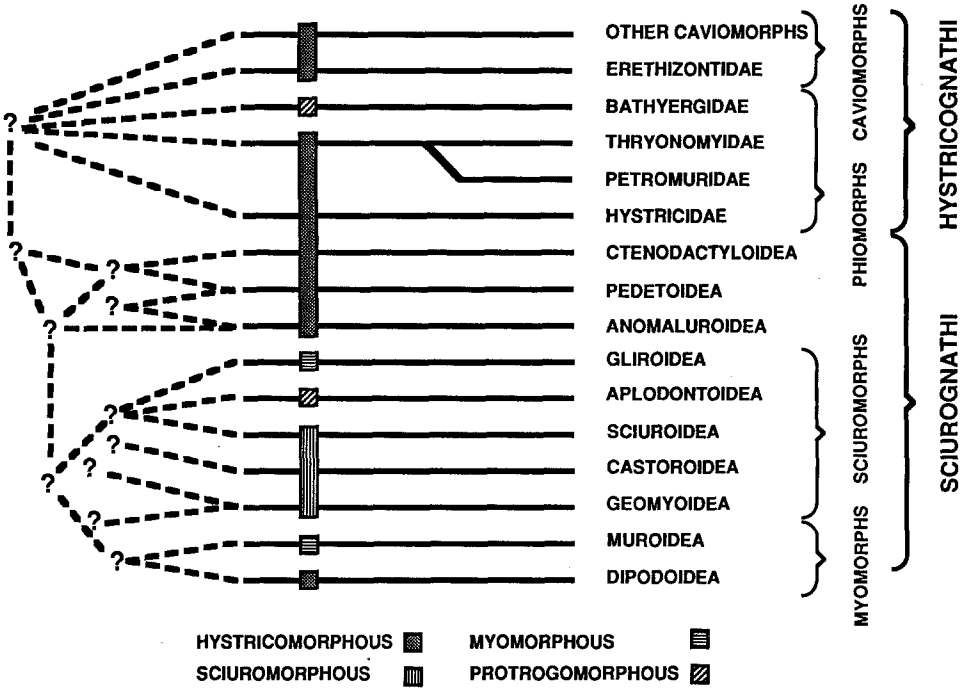

Fig. 1. Major division of Recent rodents within the suborders Hystricognathi and Sciurognathi. Hystricognathi is divided into two main groups, the African phiomorphs and the South American caviomorphs. The families Ctenodactylidae, Pedetidae, and Anomaluridae represent intermediate forms that are sciurognathous and hystricomorphous. The bars across the lineages represent the four zygomasseteric conditions (see text). Question marks and dashed lines represent various phylogenetic hypotheses.

Ctenodactylidae and Pedetidae but excluding the families Dipodidae and Anomaluridae (Martin, 1993). If the enamel microstructure data are correct, then hystricomorphy may have arisen independently two or more times throughout rodent evolution as suggested by Patterson and Wood (1982). Even with an examination of other characters, placement of one or more of these families as sister to the Hystricognathi has not been resolved (Simpson, 1945; Wood, 1955; Fischer and Mossman, 1969; Chaline and Mein, 1979; Meng, 1990; Beintema *et al.*, 1991; Otiang'a-Owiti *et al.*, 1992; Martin, 1993).

Identification of monophyletic groups within the Sciurognathi becomes more complicated when one considers other rodent families within this group. For instance, the family Aplodontidae is plesiomorphic in terms of its lower jaw and zygomasseteric structure, making placement using either of these features impossible. Other families or groups of families have a combination of primitive and derived features. Muroid rodents (rats and mice) share a sciurognathous lower jaw yet share a derived zygomasseteric condition [myomorphy (Flynn *et al.*, 1985; Vianey-Liaud, 1985; Catzeflis *et al.*, 1992]. Although muroid monophyly is supported using this feature, the relationships of muroids relative to other sciurognath rodents is unknown. For instance, some authors suggest that the family Dipodidae is sister to the superfamily Muroidea, even though dipodids are hystricomorphous rather than myomorphous, suggesting that the primitive condition of

Muroidea may have been hystricomorphy (Wilson, 1949; Wood, 1955; Klingener, 1964; Bugge, 1971; Luckett, 1985; Flynn *et al.* 1985). There is considerable disagreement regarding the affinities of other sciurognathous rodents, especially with respect to the placement of the families Castoridae [beavers (Simpson, 1945; Wilson, 1949; Bugge, 1974; Lavocat and Parent, 1985)] and geomyoid families Heteromyidae and Geomyidae [pocket mice and pocket gophers (McLaughlin, 1984; Fahlbusch, 1985; Wahlert, 1985, 1993)].

The purpose of the present study is to address, using nucleotide sequence data from the mitochondrial 12S rRNA gene, phylogenetic relationships among sciurognathous rodent superfamilies and their relationships with the suborder Hystricognathi. Molecular phylogenetic hypotheses were used to examine trends of morphological evolution in rodents, such as the modification of the jaw mechanism and jaw muscles that have been used to classify the major rodent lineages. This study is the first to utilize nucleotide sequence data in questions pertaining to the higher-level phylogeny of Rodentia.

MATERIALS AND METHODS

Nucleotide Sequences

Nucleotide sequence variation in the mitochondrial 12S ribosomal RNA (rRNA) gene was examined for 59 species of mammals, including taxa representing 25 of the 32 extant rodent families (Appendix). In all cases new sequences were obtained by PCR (polymerase chain reaction) amplification of 12S rRNA fragments from purified mitochondrial DNA. The DNA was isolated from liver, spleen, kidney, or brain using cesium chloride/propidium iodide gradient centrifugation (Brown, 1980). Although interrelationships of the Hystricognathi are not discussed in this paper (see Nedbal *et al.*, 1994), 37 hystricognaths were included in the analyses in order to reduce taxonomic sampling bias. Wheeler (1992) observed that within an analysis of a clade chances of finding the correct tree increased with increasing numbers of taxa. These taxa are represented as "Caviomorpha" and "Phiomorpha" in the trees illustrated, and the detailed results of relationships within the Hystricognathi will be published separately.

Domains I–III (approximately 900 bp) of the 12S rRNA mitochondrial gene were amplified with primers L82–5′-CATAGACACAGAGGTTTGGTCC and H900–5′-TGACTGCAGAGGGTGACGGGCGGTGTGT (Allard and Honeycutt, 1992). The names of the oligonucleotides indicate the mitochondrial heavy (H) or light (L) strands and the position of the 3′ end of the oligonucleotide according to the mouse mtDNA sequence (Bibb *et al.*, 1981). PCR (polymerase chain reaction) was performed using the following parameters: 95% C denaturation (1 min), 50% C annealing (1 min), and 72% C extension (1.25 min) for 30 cycles. The amplified portion of the 12S rRNA gene was cloned and sequenced according to the methods described elsewhere (Nedbal *et al.*, 1994). As a result of the observed error rate of *Taq* polymerase (Saiki *et al.* 1988; Tindall and Kunkel, 1988; Keohavang and Thilly, 1989), at least two clones were sequenced per species. In the five cases (*Castor, Dasypus, Jaculus, Pedetes*, and *Perognathus*) where the two clones differed, a third clone was sequenced and a consensus sequence was derived. In each of these cases clonal differences did not involve more than 1 bp.

Sequence Alignment

Sequences were aligned using both the Clustal V program (Higgins *et al.*, 1992) and visual inspection aided by the colored alignment program SeqPup (Gilbert, 1994). In addition, the secondary structure model for *Rattus* and *Bos* (Gutell *et al.* 1985) was used as a further guideline for alignment. Highly variable regions, containing nucleotide positions that could not be unambiguously aligned due to multiple insertion/deletion events (indels), were excluded from the analyses. These ambiguous regions included positions 50–62, 95–109, 212–228, 309–330, 420–428, 787–795, and 803–815. The approximate locations of these regions are described by Nedbal *et al.* (1994). The total number of sites removed was 98, leaving 794 sites available for phylogenetic analysis. The alignment was submitted to the EMBL database (No. DS26901).

Data Analyses

Patterns of Sequence Variation

The relative rate test was used to investigate rate heterogeneity among taxa (Tajima, 1993). Tests were performed using the 1D method of Tajima (1993), and significance ($P = 0.05$) was determined using the binomial distribution of Mindell and Honeycutt (1990) and the sequential Bonferroni correction for multiple tests (Holm, 1979; Rice, 1989). Separate tests were performed for all substitutions, loop substitutions, and stem substitutions. Two separate outgroups were used for the rodent relative rate tests, which included *Sylvilagus* and *Dasypus*. Nonrodent tests also were repeated with two separate outgroups, *Dasypus* and *Didelphis* (Janke *et al.*, 1994). The lagomorph *Sylvilagus* was chosen as an outgroup based on strong morphological evidence in support of a sister-group relationship between rabbits and rodents (summarized by Luckett and Hartenberger, 1985). The armadillo *Dasypus* was chosen as an outgroup for both rodent and nonrodent tests based on morphological arguments in support of an early split of edentates from other eutherians (McKenna, 1975). The marsupial *Didelphis* was chosen for its indisputable status as a eutherian outgroup (Novacek, 1992). Base composition bias of 12S rRNA genes was analyzed by the method of Irwin *et al.* (1991). Nucleotide composition was assessed for the data as a whole and for stem and loop regions separately using MEGA (Kumar *et al.*, 1993). In addition, GC and AT skews were calculated as defined by Perna and Kocher (1995).

Phylogenetic Analyses

Phylogeny reconstruction was performed using maximum parsimony as implemented by PAUP 3.1.1 (Swofford, 1993) and MacClade (Maddison and Maddison, 1992). All characters were unordered and indels were coded as nonadditive binary characters appended to the end of the aligned sequences. An exact search for the most parsimonious topology was too computationally expensive as a result of the large number of taxa (59). Therefore, all parsimony analyses were performed using at least 20 heuristic searches, employing the "tree-bisection-reconnection" search option and a randomized input order of taxa. The degree of sample error for particular nodes was evaluated with bootstrap replication (Felsenstein, 1985). Given the recent implications concerning the

accuracy of bootstrap values (Zharkikh and Li, 1992; Hillis and Bull, 1993), the Bremer support index or decay index (the number of extra steps required beyond those in the most parsimonious tree for a clade not to be unequivocally supported) also was used (Bremer, 1988, 1994; Donoghue *et al.*, 1992; Kallersjo *et al.*, 1992). In an effort to assess whether or not the outgroups were phylogenetically informative with respect to the ingroup, "Lundberg rooting" (Lundberg, 1972) was used, whereby 100 randomized sequences were formed by randomly selecting a base at each of the nucleotide positions among the 59 taxa. With outgroups pruned from the topology derived using equal weighting, each of the random sequences was joined to the tree a posteriori using the Lundberg rooting option in PAUP.

Recently, several authors, using a limited number of taxa, have suggested a polyphyletic origin of rodents (Graur *et al.*, 1991; Li *et al.*, 1992; Ma *et al.*, 1993; D'Erchia *et al.*, 1996). These studies suggest that hystricognath rodents (represented only by *Cavia porcellus*) are an early descendant of the eutherian mammalian radiation, with sciurognath rodents (specifically the myomorphs or murine genera *Mus* and *Rattus*), artiodactyls, and primates sharing a more common ancestry. A more recent study (D'Erchia *et al.*, 1996), using complete mitochondrial sequences of 15 protein encoding genes, suggested a somewhat different arrangement in that the guinea pig was sister to a clade containing six mammalian orders (Artiodactyla, Carnivora, Cetacea, Lagomorpha, Perrisodactyla, and Primates) with *Mus* and *Rattus* being divergent and basal to the clade containing guinea pig and the other orders. Our paper assumes rodent monophyly for three reasons. First, as substantiated in a recent cladistic analysis of morphological traits (Luckett and Hartenberger, 1993), rodent monophyly is unequivocal from a morphological and paleontological standpoint (Martin, 1993; Wyss *et al.*, 1993). Second, several recent molecular studies did not find support for rodent polyphyly (Allard *et al.*, 1991; Honeycutt and Adkins, 1993; Cao *et al.*, 1994; Phillipe and Douzery, 1994; Frye and Hedges, 1995; Porter *et al.*, 1996). Third, the studies proclaiming rodent monophyly are limited in taxonomic scope, and as suggested by Honeycutt and Adkins (1993) and Phillipe and Douzery (1994), this creates considerable bias in a group as taxonomically diverse as rodents.

RESULTS

Patterns of Sequence Variation

Nucleotide sequence variation was examined for 53 rodent taxa (Appendix). Six outgroup taxa (*Bos taurus*, *Balaenoptera physalus*, *Dasypus novemcinctus*, *Homo sapiens*, *Phoca vitulina*, and *Sylvilagus audubonii*), representing several divergent mammalian orders, were chosen. A comparison of the 12S rRNA rodent sequences to the secondary structure model for *Bos* and *Rattus* (Gutell *et al.*, 1985) revealed differences among rodents with respect to the presence or absence of a stem region at base positions 315–318 and 425–428 [positions refer to the submitted EMBL alignment and those shown by Nedbal *et al.* (1994)]. This stem region was most pronounced in muroid rodents (represented by *Rattus* and *Mus*), whereas this same region was less apparent or even absent (defined by Watson–Crick base pairing) in other rodent (Geomyoidea and Caviomorpha) and nonrodent taxa (*Bos* and *Balaenoptera*). Several regions within the

rodent 12S rRNA gene revealed a high frequency of insertion/deletion events (indels), making accurate alignment difficult. These ambiguous regions encompassed a total of 98 sites and were excluded from the phylogenetic analyses. The 794 nucleotide positions remaining after the exclusion of the ambiguous regions were subdivided into 354 sites within stems and 440 sites within loops, and 40% (320) of these sites were invariant among the taxa examined, with the proportion of invariant sites being similar in loops and stems.

Base Composition

Base composition for either all positions or positions partitioned separately among stems and loops was estimated (Fig. 2), and both the rodent ingroup taxa and the non-rodent outgroup taxa revealed the same pattern. Loops demonstrated a significant bias toward A at the expense of G, and this bias contributed to the overall composition bias seen for the entire 12S rRNA gene as stems did not show a comparable bias. The composition bias observed for the loop regions is similar to that seen for third codon positions of mitochondrial protein encoding genes (Irwin *et al.*, 1991; Adkins and Honeycutt, 1994; Tanaka and Ozawa, 1994; Honeycutt *et al.*, 1995), and the index of compositional bias, a measure of deviation from an equal (25%) frequency of each nucleotide (Irwin *et al.*, 1991), was twice that seen for stems (0.284 and 0.131, respectively). GC/AT skewness, a measure of strand specific compositional bias estimated for the light strand (Perna and Kocher, 1995), revealed a similar pattern of compositional bias in that stems had a positive value for both GC and AT skew, whereas loops had a positive AT skew and negative GC skew (Fig. 3).

Substitution Classes

The ratio of transitions to transversions (TS/TV) was lower (mean of 1.37 ± 0.02) among ingroup pairwise comparisons than among pairwise comparisons involving only the outgroups (mean of 1.91 ± 0.12), especially among taxa that differed by more than 25% sequence divergence. This result is unexpected if it is assumed that rodents are monophyletic and the substitution rate and transition/transversion bias are similar between rodent and non-rodent taxa. The distribution and frequency of substitution classes (TS, TV, and indels) differed among rodent taxa and between rodents and nonrodent taxa (Figs. 4 and 5). All taxa demonstrated a decrease in the rate of stem transversions and indels (Figs. 4C and D) compared to transitions (Figs. 4A and B) and loop tranversions (Fig. 4C). Comparing the average number of stem transversions among representatives of divergent rodent lineages (*Aplodontia*, *Mus*, *Castor*, *Geomys*, *Ctenodactylus*, *Pedetes*, *Hystrix*, and *Erethizon*) with the average number of stem transversions among the outgroup taxa, rodents demonstrated twice as many substitutions as nonrodents, averaging 21.5 ± 0.8% stem transversions per comparison relative to 11.5 ± 0.7% for nonrodents, including Lagomorpha (rabbits), with a mean of 13.2% (Fig. 5). Loop transversions also differed between rodent and nonrodents (48.5 ± 1.5 and 31.0 ± 1.9, respectively) but the overall difference (ratio 1.6) was somewhat less than that seen for stem transversions. Both rodents and nonrodents showed a similar frequency of loop and stem transitions (ratio, 0.9 and 1.2, respectively).

The difference among rodents and nonrodents in terms of the frequency of substi-

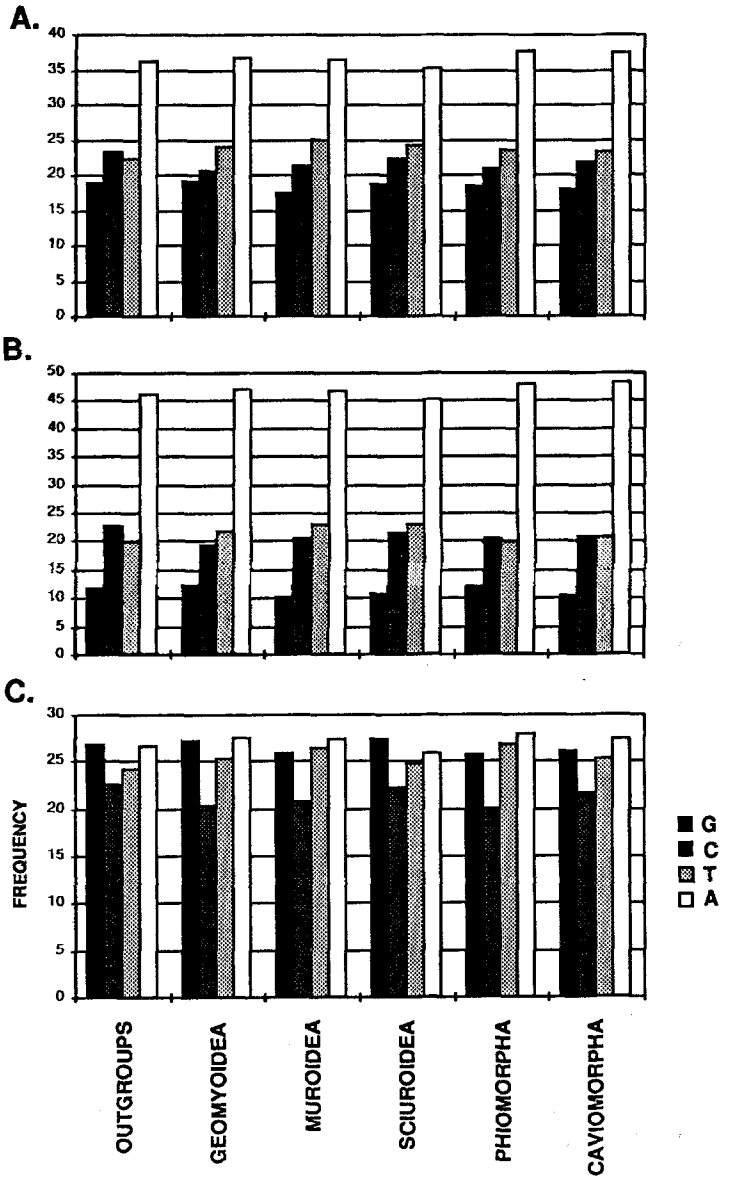

Fig. 2. Nucleotide composition bias among major rodent groups. Both variant and invariant sites were included. Base composition bias was calculated using (A) all sites, (B) sites within loop regions, and (C) sites within stem regions.

tutions also was examined using a relative rate test (Table I) (Tajima, 1993). Three separate data partitions (all sites, sites within loops, sites within stems) were used to test for rate heterogeneity among taxa. The only significant nonrodent pairwise comparison involved an "all-site" rate increase in *Homo* compared to the harbor seal (*Phoca*; $P = 0.002$). Among the comparisons between rodents and nonrodents using *Didelphis* as the

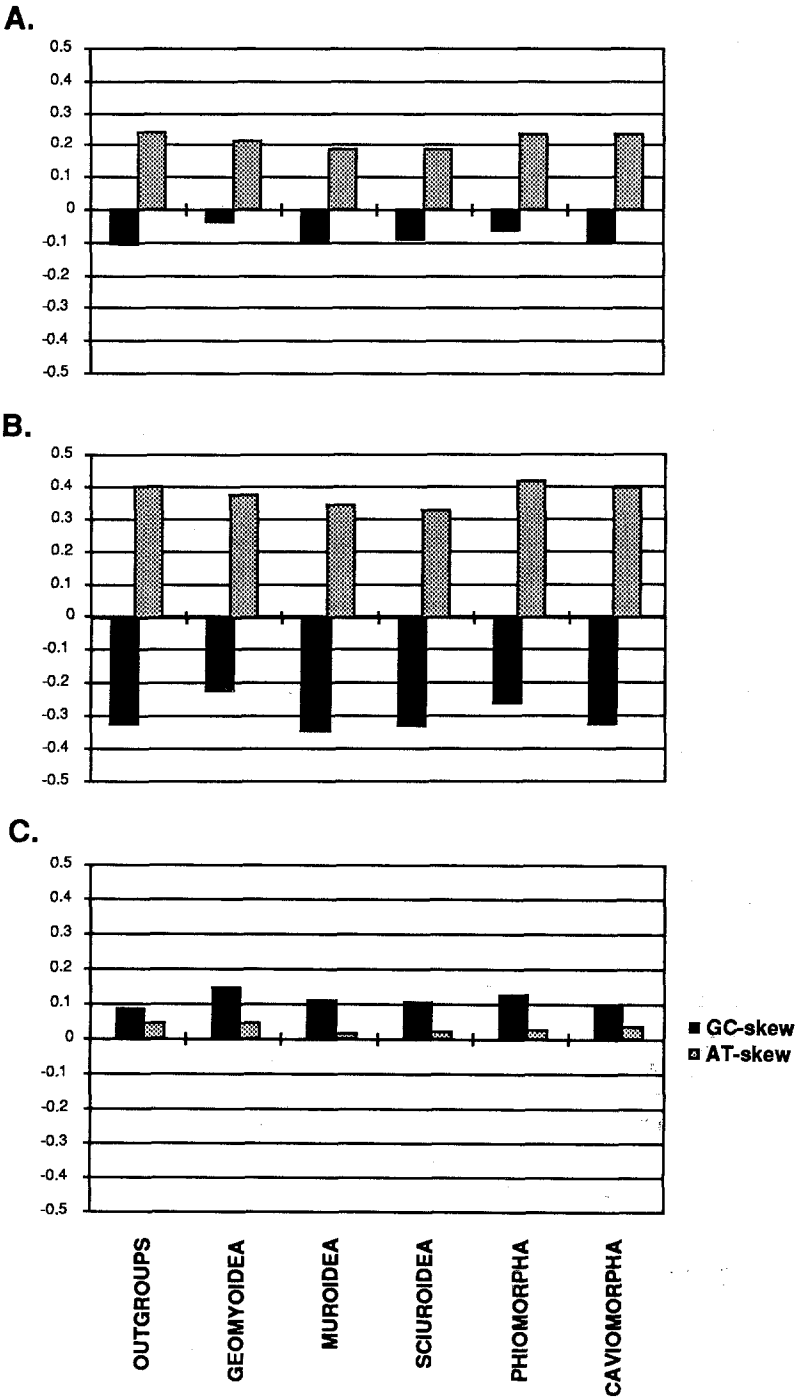

Fig. 3. Illustration of GC skew and AT skew among taxonomic groups as defined by Perna and Kocher (1995). Skew was calculated using (A) all sites, (B) sites within loop regions, and (C) sites within stem regions.

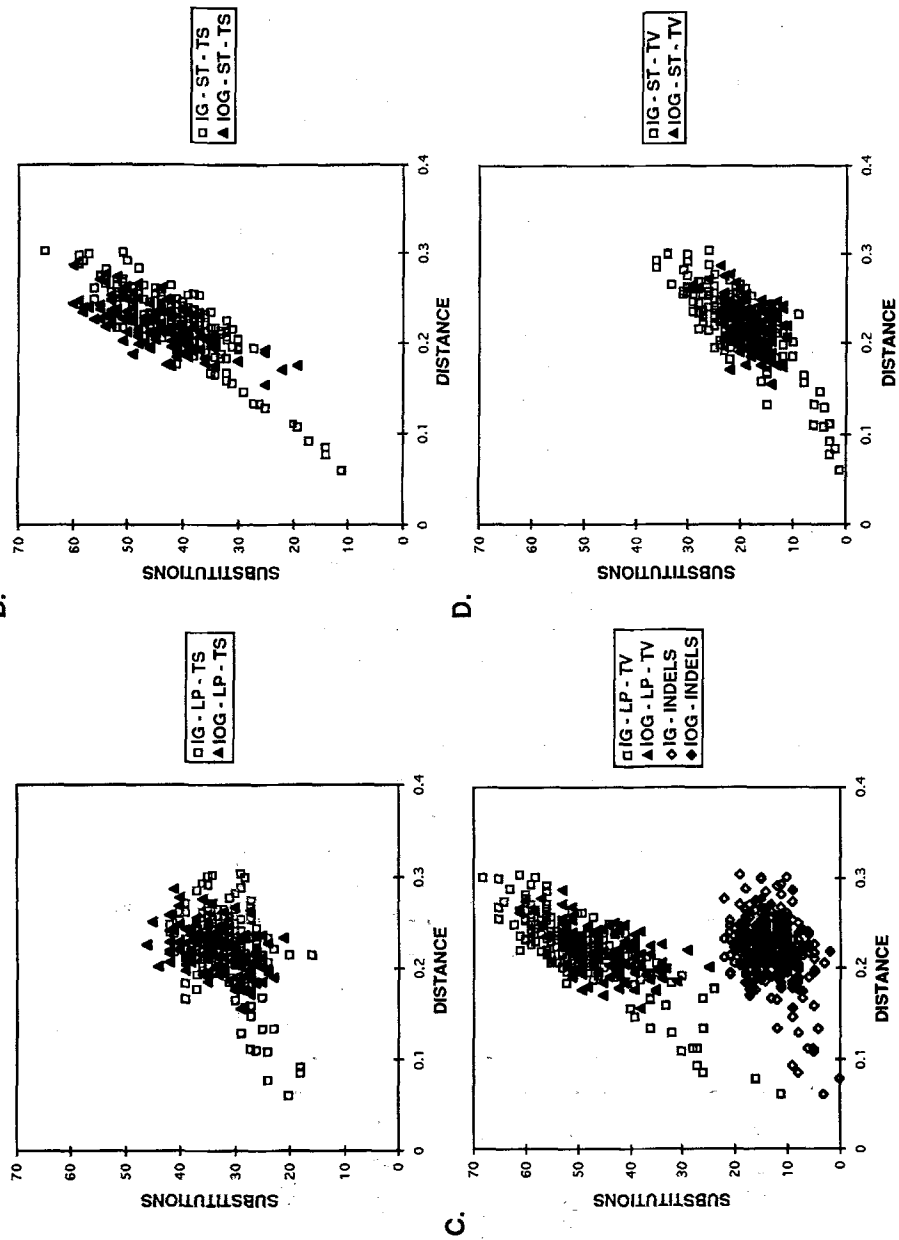

Fig. 4. Plots of substitution classes versus genetic distances among stem and loop regions and insertion/deletion events (indels) within the 12S rRNA gene: (A) loop transitions, LP–TS; (B) stem transitions, ST–TS; (C) loop transversions, LP–TV, and indels; (D) stem transversions, ST–TV. Two sets of calculations were performed. One excluded outgroup taxa (IG) and the other involved comparisons between the rodent ingroup and the nonrodent outgroup taxa (IOG). The numbers of differences are plotted versus the pairwise genetic distance values calculated by the method of Jukes and Cantor (1969) as implemented by the program MEGA (Kumar *et al.* 1993).

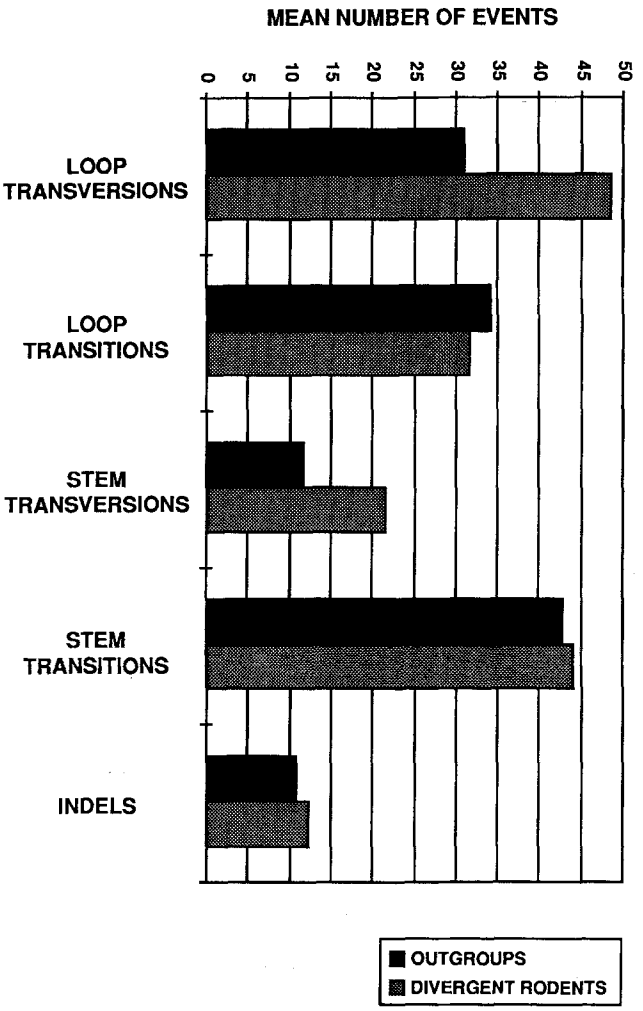

Fig. 5. Graph illustrating the mean frequency of substitutional classes compared between the outgroups and divergent rodents (*Aplodontia, Mus, Castor, Geomys, Ctenodactylus, Pedetes, Hystrix*, and *Erethizon*). Substitution and indel frequencies were derived using pairwise comparisons in the program MEGA (Kumar *et al.*, 1993).

outgroup, 9 of 108 comparisons were significant and 5 of the 9 involved the pocket mouse (*Perognathus*). The number of significant comparisons increased to 26 when *Dasypus* was used as the outgroup. In fact, at least two significant increases in the rate of rodent substitutions were observed among comparisons involving each nonrodent taxon. Rodent comparisons to *Phoca* yielded the greatest number of significant rate increases (10 of the 18), which was followed by the rabbit (*Sylvilagus*; 6), the cow (*Bos*; 5), the human (*Homo*; 3), and finally, the whale (*Balaenoptera*; 2). Among rodent rel-

Table I. Relative Rate Tests[a]

	Dasypus	Phoca	Balaenoptera	Bos	Homo	Sylvilagus	Graphiurus	Aplodontia	Sciurus	Spermophilus	Gerbillurus	Mus
Dasypus	—											
Phoca		—			A			A				
Balaenoptera			—									
Bos				—								
Homo					—							
Sylvilagus						—						
Graphiurus							—					
Aplodontia								—				
Sciurus							S	A-S	—		L	
Spermophilus										—		
Gerbillurus		A-L		A-L		A					—	
Mus		A					S					—
Rattus											S	
Lophuromys												
Osgoodomys		A										
Jaculus		A										
Castor												
Perognathus		A-L-S	A	A-L	A-S	A-S	A	A	A-S	A-S	A	A-S
Cratogeomys		A-L		A-L		A			A			
Geomys		A-L		A-L		A			S			
Ctenodactylus		A-S			S	S			A-S			
Pedetes		A										
Hystrix												
Erethizon		A-S	S	A	S	S			A			

[a] Relative rate tests were performed using the 1D method of Tajima (1993), and significance ($P = 0.05$) was determined using the binomial distribution of Mindell and Honeycutt (1990) and the sequential Bonferroni test (Rice, 1989) whereby a family of tests was defined as all pairwise comparisons of a taxon. In the case of *Perognathus* with respect to other rodents (using *Sylvilagus* as the outgroup), there was a total of 17 tests that were corrected for by the sequential Bonferroni test. This was done for each rodent taxon independently for a total of 17 sets of tests, each having 17 pairwise

ative rate tests, the most obvious rate deviation was that of the pocket mouse (*Perognathus*), which demonstrated a significant rate increase in the majority of comparisons (Table I). When *Sylvilagus* was used as the outgroup, *Sciurus* showed rate deviation in 7 pairwise comparisons, whereas *Rattus* demonstrated significant "stem-site" rate differences in 6 of the 17 comparisons. Some comparisons (*Ctenodactylus* and *Erethizon*) demonstrated significance only in the "stem-site" data partition, suggesting differences in secondary structure, although no obvious pattern was evident.

Phylogenetic Analyses

Equally Weighted Parsimony

A maximum-parsimony analysis was performed using equal weighting (including indels), 100 heuristic searches (tree bisection and reconnection), and the input order of the taxa randomized. This analysis yielded eight most-parsimonious trees (length = 3244 excluding uninformative characters, consistency index = 0.246, retention index = 0.470). Differences among these eight trees involved the lineages within the phio-

Table I. Continued

Rattus	Lophuromys	Osgoodomys	Jaculus	Castor	Perognathus	Cratogeomys	Geomys	Ctenodactylus	Pedetes	Hystrix	Erethizon
					A						
					A-S			A		A	
					A-S						
					A-S						
					A-S					A	
					A	A-L					
					A-L	A-L	L				
					A	A					S
	A										
—											
	—				A						
		—									
			—								
				—							
A-S	A-S	A-S	A-L-S	A	—						
S	A					—					
S					A		—				
S					A			—			
					A				—		
					A					—	
S					A						—

comparisons. The nonrodent and rodent relative rate tests above the diagonal used the outgroups *Didelphis* and *Dasypus* respectively. The nonrodent and rodent relative rate tests below the diagonal used the outgroups *Dasypus* and *Sylvilagus*, respectively. Significant relative rate tests are depicted by as follows: A, all substitutions; L, loop substitutions; S, stem substitutions. An empty cell denotes no significant rate difference.

morph and cavimorph rodent clades. Several observations can be made regarding the strict consensus tree (Fig. 6). First, the suborder Hystricognathi was monophyletic, with a bootstrap value of 71 and a Bremer support index of 4. Although the bootstrap and Bremer support values were low, a sister-group relationship between the Hystricognathi and a clade containing the Sciuroidea and several other families was indicated. An extra three steps was required for a sister-group relationship between Hystricognathi and Ctenodactylidae. Second, the suborder Sciurognathi was paraphyletic, forming two groups. A tree that constrained sciurognath monophyly was found to be four steps longer than the most-parsimonious tree. Third, the monophyly of several recognized superfamilies of rodents, including Sciuroidea, Geomyoidea, and Muroidea, was supported. Fourth, the family Aplodontidae formed a monophyletic group with Sciuroidea. Fifth, although the Bremer support indices and bootstrap values were low, the superfamilies Gliroidea, Castoidea, and Ctenodactyloidea represented part of a clade containing Sciuroidea/Aplodontoidea. In addition, a sister-group relationship between Pedetoidea and Geomyoidea was observed, and this clade grouped with Dipodoidea/Muroidea. Finally, the monophyly for Rodentia was weakly supported with a boostrap value of 22 and a decay index of 2. Successive approximations (using the rescaled consistency index) resulted in the same relationships among the rodents.

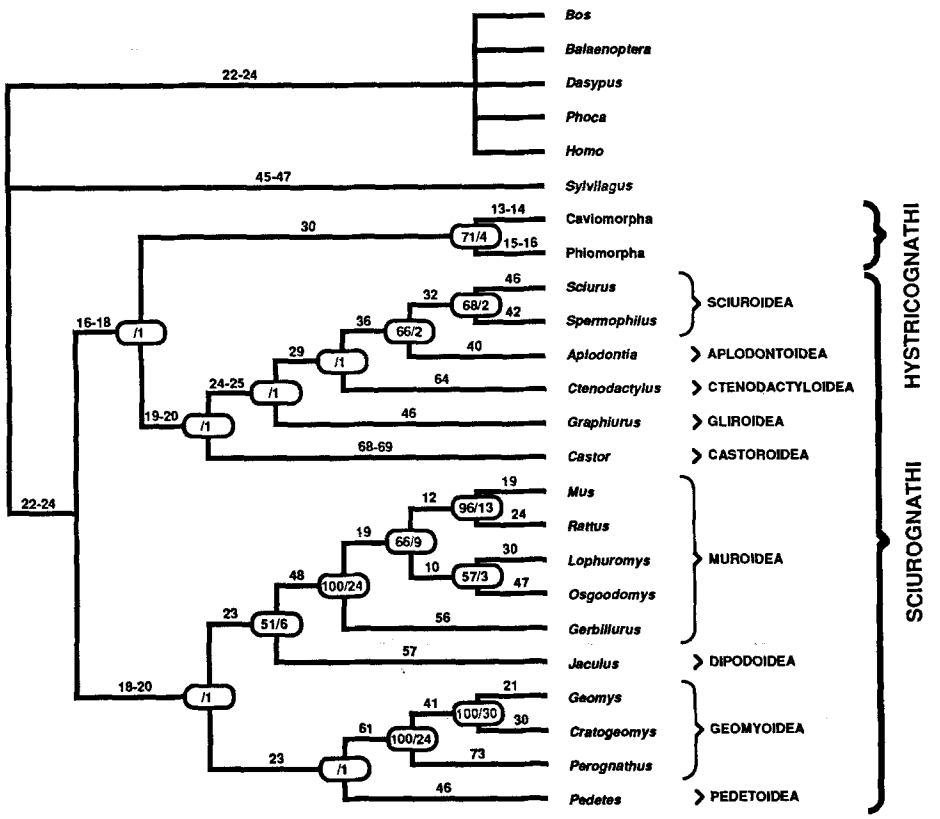

Fig. 6. Strict consensus tree of the phylogenetic relationships among rodents using maximum parsimony and equal weighting of mitochondrial 12S rRNA sequences. Eight most-parsimonious trees (length, 3244; CI = 0.246; RI = 0.470) resulted from 100 heuristic (tree bisection and reconnection) searches in which the input order of the taxa was randomized. Bootstrap values (only those greater than 50%) at nodes are to the left of the slash, and Bremer support indices are to the right. Values along the lineages represent the minimum possible branch length optimized using the ACCTRAN option in PAUP 3.1.1 (Swofford, 1993). Analyses included all available rodents (Appendix). All New and Old World hystricognath rodents were synonymized under the taxonomic labels Caviomorpha and Phiomorpha, respectively.

Lundberg Rooting

How much confidence can be placed on the position of the root in the phylogenetic analyses? Observations of the 12S rRNA data that could affect the nonrodent outgroup placement include heterogeneity in rate between rodents and nonrodents (Table I) and the distribution of pairwise substitutions between the ingroup and the outgroup (Fig. 4). The plots (Fig. 4) illustrate that in many cases the pairwise comparisons between rodents and nonrodents involve fewer changes than pairwise comparisons between rodent taxa. Assuming rodent monophyly and similar rates of evolution (which we know not to be the case in some comparisons; see Table I), the plots may be interpreted as demonstrating saturation within regions of the 12S rRNA gene. If saturation were not occurring, one would expect to see an increasing distance and increasing number of changes between rodent/outgroup (IOG) comparisons and rodent/rodent (IG) comparisons. It should be

noted, however, that these plots were derived from pairwise distances and not from a phylogenetic framework. Arguments of saturation (flattening of the curve) based on these plots assume that all sites are evolving at approximately the same rate. The likely existence of among-site rate variation (Sullivan *et al.*, 1995) weakens conclusions based on these plots. Substitutions that occur in slowly evolving sites will likely be less homoplastic than substitutions that occur in more rapidly evolving sites. Nevertheless, the plots indicate that there is no difference in terms of the amount of divergence for intrarodent comparisons (IG) versus rodent and outgroup comparisons (IOG), making careful interpretation of the root necessary.

Global parsimony, where ingroups and outgroups are analyzed together, is the preferred method of tree rooting (Maddison *et al.*, 1984; Nixon and Carpenter, 1993). In the case of highly divergent molecular sequence data, however, the outgroups may be essentially random (Wheeler, 1990), necessitating the need for alternative approaches to rooting. In order to assess whether the outgroups were random with respect to the ingroup, Lundberg (1972) rooting of 100 randomized sequences (formed by selecting a base at each of the nucleotide positions among the 59 taxa) was performed. Random sequences tend to root trees along the longest branches (Wheeler, 1990). Only 1 of the 100 random sequences rooted the ingroup topology at the same location as the outgroups, suggesting that the outgroups are not random with respect to the ingroup.

In addition to the outgroup randomization test, a second approach to rooting was performed, whereby a hypothetical ancestor was used to root the rodent topology (Lundberg, 1972; Nixon and Carpenter, 1993). Using a "known" topology among the outgroup taxa (Fig. 7), a "hypothetical ancestor" was estimated for the basal lagomorph node. Although molecular support for a sister-group relationship between Rodentia and Lagomorpha is not strong (Honeycutt and Adkins, 1993; Graur *et al.*, 1996), the orders Lagomorpha and Rodentia have traditionally been considered to be part of the superorder

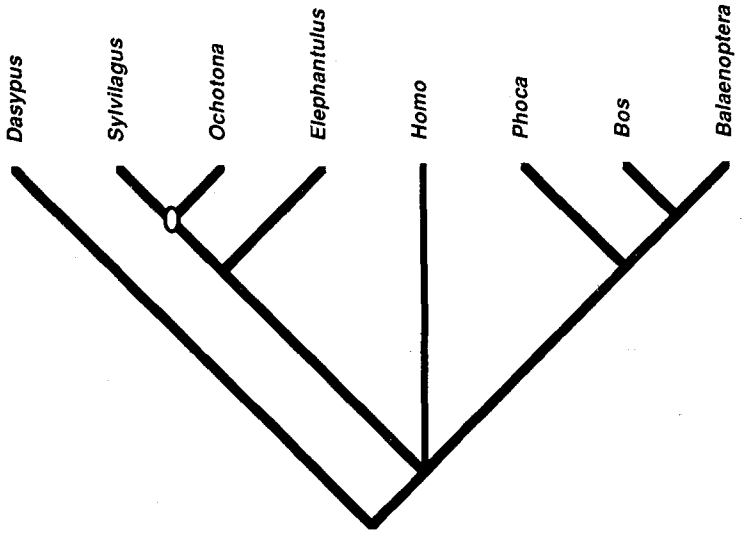

Fig. 7. Assumed phylogeny used to estimate a hypothetical lagomorph ancestor in Lundberg (1972) rooting. See text for details.

Glires on the basis of morphological evidence (Hartenberger, 1980; Li and Ting, 1985; Luckett and Hartenberger, 1985, 1993; Novacek, 1985, 1990). Therefore, the order Lagomorpha is the logical choice for an outgroup to rodents. Justification for other relationships shown in Fig. 7 include the following. (1) Embryological, morphological, paleontological, and molecular evidence supports the sister-group relationship between artiodactyls (*Bos*) and cetaceans (*Balaenoptera*) (Czelusniak *et al.*, 1990; Gingerich *et al.*, 1990; Arnason *et al.*, 1991; Novacek, 1992; Adachi *et al.*, 1993; Milinkovitch *et al.*, 1993; Graur and Higgins, 1994). (2) Recent molecular evidence suggests a sister-group relationship between the order Carnivora and the Artiodactyla/Cetacea clade (Arnason and Johnsson, 1992; Honeycutt and Adkins, 1993; Graur and Higgins, 1994; Honeycutt *et al.*, 1995; D'Erchia *et al.*, 1996). (3) A sister-group relationship between Macroscelidea and Rodentia/Lagomorpha is suggested on the basis of morphological synapomorphies (Novacek *et al.*, 1988). (4) Amino acid sequences (Miyamoto and Goodman, 1986) and, to some extent, morphology (McKenna, 1975; Novacek, 1992) support Edentata as representing an early branch in the eutherian tree (for an alternate opinion see Gaudin *et al.*, 1996). The hypothetical sequence was joined to the unrooted most parsimonious ingroup topology a posteriori and a maximum-parismony analysis performed. The hypothetical lagomorph ancestor rooted the rodent tree at the same branch as the previous globally parsimonious ingroup/outgroup analysis (Fig. 6), thus further corroborating placement of the root.

Generalized Parsimony

Generalized parsimony, as defined by Swofford *et al.* (1996), assigns a cost for the transformation at each character state to other possible states (Sankoff, 1975). While this has the obvious advantage of preferentially weighting particular classes of transformations that are more likely to be phylogenetically informative, the decision-making process of assigning transformation costs is less clear. The most obvious conclusion that can be drawn from the "saturation plots" are that loop transitions (Fig. 4A) may be interpreted as showing signs of saturation, especially with pairwise distances greater than 20% sequence divergence. The plot of indels (Fig. 4C) may represent either saturation or a slow rate of evolution. When indel and nonindel characters were mapped onto the phylogeny derived using equal weights (Fig. 6; excluding the outgroups), the retention indices were 0.73 and 0.47, respectively. Nonindel character partitioned among stem and loop sites had retention indices of 0.51 and 0.44, respectively. Therefore, the level of homoplasy among indel events appears to be less than that observed among nonindels [for a discussion of homoplasy index measures calculated from multistate versus binary characters see Naylor and Kraus (1995)]. A comparison of retention indices among the different transformations (indels versus loop transitions) was not performed because the calculations are time-consuming and neither PAUP nor MacClade provides homoplasy index measures for transformations weighted by means of a step matrix. In an attempt to increase the phylogenetic signal in all transformations (except loop transitions), two user-type stepmatrices were produced in PAUP, one for stem characters and the other for characters within loops. All stem substitutions and loop transversions received a weight of two, and loop transitions a weight of one. Rather than including indels in the step matrices, boosting the phylogenetic signal in indels was performed by increasing

the weight of indel sites to two (the weight of stem and loop sites was maintained at one).

A maximum parsimony analysis with unequal weighting was performed using 20 heuristic searches (tree bisection and reconnection) and the input order of the taxa randomized. This analysis yielded four most parsimonious trees (length = 5499 excluding uninformative characters). The unrooted strict consensus tree (Fig. 8) illustrates a paraphyletic Rodentia with one rodent clade (Sciuroidea, Aplodontoidea, Gliroidea, Castoroidea, Ctenodactyloidea, and Hystricognathi) depicted as a sister-group to a clade containing the whale (*Balaenoptera*), cow (*Bos*), and seal (*Phoca*) and a second rodent clade (Muroidea, Dipodoidea, Geomyoidea, and Pedetoidea) depicted as a sister-group to the rabbit (*Sylvilagus*). Relationships within the clade containing Muroidea, Dipodoidea, Geomyoidea, and Pedetoidea did not change from that seen in Fig. 6. The other rodent clade was different from that in Fig. 6 in that Aplodontoidea/Sciuroidea was basal and Gliroidea and Castoroidea formed a clade that grouped with a clade containing Ctenodactyloidea and Hystricognathi. This result is not too surprising given the follow-

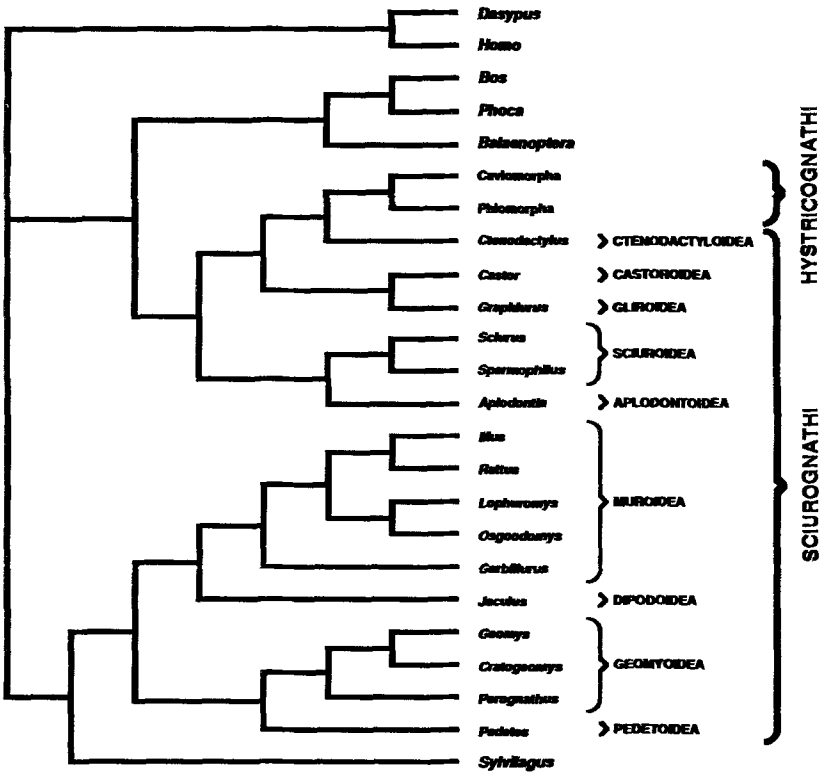

Fig. 8. Strict consensus tree of the phylogenetic relationships among major rodent groups using global maximum parsimony and unequal weighting of mitochondrial 12S rRNA sequences, whereby loop transitions were down-weighted. Four most-parsimonious trees (length, 5499) resulted from 20 heuristic (tree bisection and reconnection) searches in which the input order of the taxa was randomized. Analyses included all available rodents (Appendix).

ing observations: (1) the plots in Fig. 4 demonstrate some rodent/outgroup comparisons being less divergent than some seen among rodents; (2) rodent and outgroup taxa show apparent differences in the frequency of particular classes of substitutions, especially stem and loop transversions (Fig. 5); and (3) some taxa demonstrate rate heterogeneity, especially in comparisons between rodent and outgroup taxa (Table I).

In order to minimize potential conflicts between the phylogenetic reconstruction within Rodentia and the placement of the outgroups, a two-step Lundberg (1972) rooting procedure was performed in the following manner. First, an unrooted phylogeny was obtained for the ingroup taxa only (excluding outgroups), and a constraint tree was constructed from the most-parsimonious tree(s). Second, all taxa were used in a second phylogenetic reconstruction constrained to the backbone (ingroup) tree produced in the previous step. Backbone constraints force a relative pattern of relationships, and taxa may be added at any point on the constraint tree as long as the backbone is not violated (Swofford, 1993). This procedure provides a method of rooting the tree without introducing molecular biases from outgroup taxa that may influence the phylogenetic relationships among the ingroup.

This procedure was performed for both equal-weighting and generalized (unequally weighted) parsimony. The equal-weighting unrooted analysis resulted in the collapse of all nodes supported by a Bremer support index of one (see Fig. 6), except the nodes supporting the sister-group relationship of Gliroidea and Ctenodactyloidea/Aplodontoidea/Sciuroidea and Ctenodactyloidea and Aplodontoidea/Sciuroidea. When this tree was rooted a posteriori with the nonrodent taxa, the resultant rodent topology was identical to that of global parsimony (Fig. 6). The generalized (weighted) parsimony unrooted analysis resulted in a tree identical to that of the equal-weighted global parsimony analysis (Fig. 6). When this tree was used in Lundberg rooting, the root was positioned along the same branch as the equal-weighted parsimony analysis, and the Bremer support values were higher for some nodes (Fig. 9).

DISCUSSION

Patterns of Sequence Variation

While there does not appear to be any obvious taxonomic bias within the 12S rRNA gene, loops and stems differed with respect to base composition (Fig. 2). Loops showed a base composition bias similar to that seen for fourfold degenerate sites in protein coding genes (Gutell *et al.*, 1985; Irwin *et al.*, 1991; Adkins and Honeycutt, 1994; Honeycutt *et al.*, 1995; Springer *et al.*, 1995), with an excess of adenine at the expense of guanine. This suggests that loops and fourfold degenerate sites are evolving in a similar fashion, and the observed bias may be a result of the underlying mutational pressure of the mitochondrial genome (Tanaka and Ozawa, 1994). In contrast, stems do not show a significant bias in nucleotide composition, with the exception of a small decrease in cytosine. The logical assumption is that, similar to first and second codon positions, there exists some form of selective constraint acting on sites located within stems. This constraint may be associated with the maintenance of a free energy window (Noller, 1984; Zuker, 1989), whereby the stability of the stem structure increases with the pro-

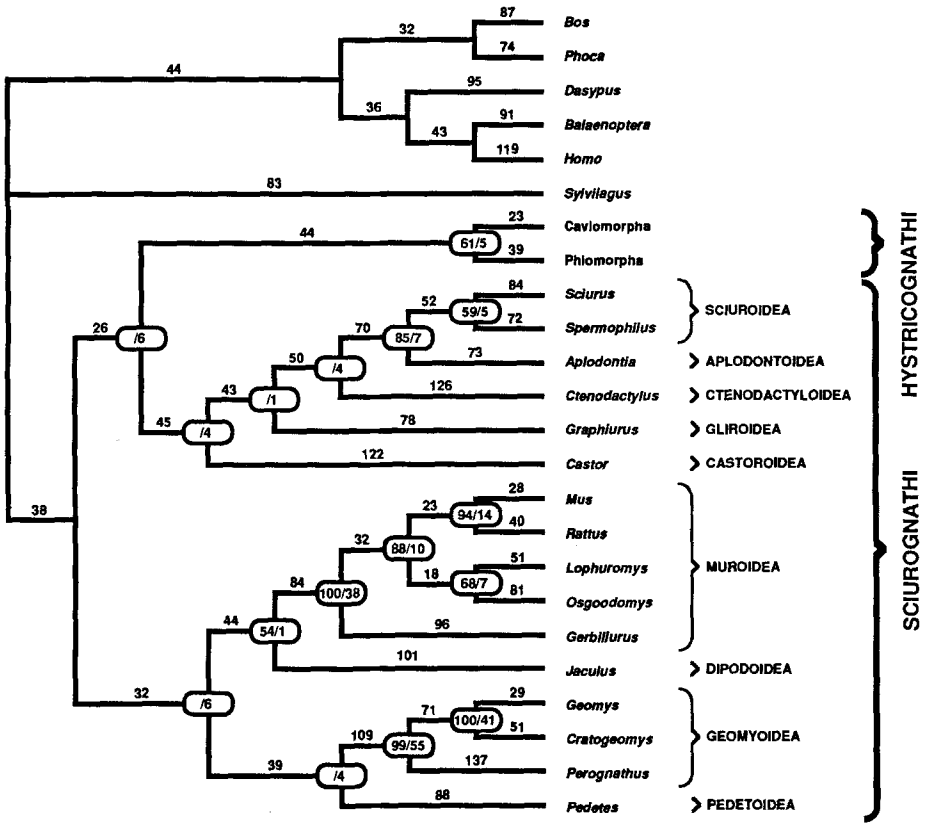

Fig. 9. Strict consensus tree of the phylogenetic relationships among major rodent groups derived using maximum parsimony and the two-step weighted Lundberg rooting procedure outlined under Results. One most-parsimonious tree resulted from the unrooted analyses (length, 4718) and one most-parsimonious tree resulted after the addition of the outgroup taxa (length, 5515). Analyses used 20 heuristic (tree bisection and reconnection) searches in which the input order of the taxa was randomized to estimate the most parsimonious trees. Bootstrap values (only those greater than 50%) at nodes are to the left of the slash, and Bremer support indices are to the right. Analyses included all available rodents (Appendix). Values along the lineages represent the minimum possible branch length optimized using the ACCTRAN option in PAUP 3.1.1 (Swofford, 1993).

portion of G–C pairs. This prediction is consistent with the observed increase in G at the expense of A in stem regions (Fig. 2).

Relatively few transversions were observed within stems (Figs. 4 and 5). This observation may relate to compensatory mutations (Wheeler and Honeycutt, 1988; Kraus *et al.*, 1992; Dixon and Hillis, 1993; Gatesy *et al.* 1994) and the transition/transversion bias seen in the mitochondrial genome (Brown *et al.*, 1982). Springer *et al.* (1995) suggested that, given transitions are more common than transversions among sites within loops, bias should increase so that base pair complementarity in stems can be maintained. Compensatory mutations consist of either two transitions or two transversions (i.e., there is no mixing of the two substitution classes). Therefore, mitochondrial transition bias should increase among stem regions.

Phylogenetic Observations

Systematics of Sciuroidea

Sciuroids are sciurognathous and sciuromorphous, and previous classifications have placed this superfamily in its own suborder (Wood, 1955) or infraorder (Chaline and Mein, 1979). The sister-group to Sciuridae is the family Aplodontidae, supported by many shared features of the auditory region (Wahlert, 1972; Lavocat and Parent, 1985) and by serum immunological analysis (Sarich, 1985). Unlike the family Sciuridae, however, Aplodontidae is highly specialized for a fossorial habitat and differs from Sciuridae with respect to skeletal and dental specializations, including the zygomasseteric system (Vianey-Liaud, 1985). When fossil aplodontids are included in comparisons of the families Aplodontidae and Sciuridae, distinction between the two families becomes more obscure as a result of the retention of primitive dental characters. Therefore, the main characteristic differentiating the families Aplodontidae and Sciuridae relate to the zygomasseteric system, with the former being protrogomorphous and the latter sciuromorphous. Assuming that sciurids evolved from a protrogomorphous ancestor (Korth, 1994), one might expect some extinct sciurids to be protrogomorphous. As suggested by Vianey-Liaud (1985), if the primary distinguishing feature between Tertiary aplodontids and sciurids is the infraorbital region of the skull, any protrogomorphous sciurids would be difficult to distinguish from an aplodontid based on morphology alone. All analyses conducted on the 12S rRNA gene sequences supported monophyly of Sciuroidea and demonstrated equal or greater support for an association between the families Sciuridae and Aplodontidae (Figs. 6 and 9). This observation together with the morphological evidence suggests that the Aplodontidae should be included in the superfamily Sciuroidea (*sensu* Hartenberger, 1985; but not Simpson, 1945).

Systematics of Castoroidea

Castoroids are sciurognathous and sciuromorphous, and early classifications placed this superfamily within the suborder Sciuromorpha on the basis of mandible and skull characteristics (Brandt, 1855; Miller and Gidley, 1918; Simpson, 1945; Wilson, 1949). Wood (1955) placed Castoroidea in a separate suborder because sciuromorphy was the only derived character linking it to the Sciruomorpha. Nevertheless, Chaline and Mein (1979) maintained Castoroidea within Sciuromorpha. The only known and well-supported sister-group of castorids is the extinct family Eutypomyidae (based on derived characters of the sphenopalatine, interorbital, and dorsal palatine foramina; Wahlert, 1972, 1977). This fossil family has been placed with the family Castoridae into the superfamily Castoroidea by some authors (Stirton, 1935; Wilson, 1949; Wahlert, 1977). The lack of pre-Oligocene castorids (Vianey-Liaud, 1985; Korth, 1994) makes it difficult to determine both the ancestry of the castorids and their affinity with other extant families. Maximum parsimony analyses of the 12S rRNA data provided limited support (Bremer decay values of 1 and 4 for equal weighting and unequal weighting, respectively; Figs. 6 and 9) for a sister-group relationship between *Castor* and a clade containing *Graphiurus*, *Ctenodactylus*, *Aplodontia*, and the family Sciuridae.

Systematics of Gliroidea

Gliroids are sciurognathous and myomorphous, and classifications have placed this superfamily within the suborder or infraorder Myomorpha (Simpson, 1945; Wood, 1965;

Chaine and Mein, 1979; Wahlert, 1978, 1983; Wahlert *et al.*, 1993) together with the Muroidea. However, according to several authors (Hartenberger, 1971; Wood, 1980; Dawson and Krishtalka, 1984; Flynn *et al.*, 1985), the condition of myomorphy found in gliroids is a result of homoplasy, and evidence from both fossils (Vianey-Liaud, 1985), middle ear features (Lavocat and Parent, 1985; Meng, 1990) and internal carotid arterial patterns (Bugge, 1985) supports an association with Sciuridae. Maximum-parsimony analyses of 12S rRNA characters provided limited support (one step) for a sister-group relationship between Gliroidea and a clade containing the superfamilies Ctenodactyloidea and Sciuroidea (including Aplodontidae). No analyses grouped Gliroidea within or near the clade containing muroid rodents. Therefore, based upon all available evidence, except that of Sarich (1985), whose albumin data demonstrated a relationship between Gliridae and Muridae, Gliroidea evolved the myomorphic condition in parallel with muroid rodents (as suggested by Vianey-Liaud, 1985) and shares affinity with the superfamilies Sciuroidea (including Aplodontidae) and Ctenodactyloidea (as suggested by the 12S rRNA data).

Systematics of Geomyoidea

The superfamily Geomyoidea (Geomyidae and Heteromyidae) is sciurognathous and sciuromorphous, and some classifications have placed geomyoids within the suborder Sciuromorpha (Simpson, 1945; Fahlbusch, 1985). Other authors have proposed an association between geomyoids and myomorphs based upon the cranial foramina (Wahlert, 1983) and other morphological similarities (Wood 1955, 1965; Chaline and Mein, 1979; Wahlert, 1985). Data from the 12S rRNA gene strongly supported geomyoid monophyly, with an equal-weighting bootstrap value of 100 and a Bremer support index of 24. With regard to placement of Geomyoidea relative to other rodents, the 12S rRNA sequence data are less robust. Equal-weighted (Fig. 6) and generalized (Fig. 9) maximum parsimony weakly placed Geomyoidea as a sister-group to Pedetoidea, followed by an association with a clade containing Muroidea and Dipodoidea. A maximum-parsimony analysis using characters from the auditory region did not support a relationship between Muroidea and Geomyoidea but instead placed Geomyoidea (Heteromyidae) sister to a clade containing Reithroparamyinae, Gliridae, Aplodontidae, and Sciuridae (Meng, 1990). From a molecular evolutionary viewpoint, the superfamily Geomyoidea is an interesting group in that one of the three species examined demonstrated a significant rate increase with respect to other rodent taxa examined. This is important because rate heterogeneity can adversely affect phylogenetic reconstruction using some methods (Felsenstein, 1985; Swofford and Olsen, 1990). In an effort to remove this potential bias, the taxon that demonstrated a significant rate increase (*Perognathus*) was removed from the data set and analyses were rerun. The exclusion of this taxon did not result in any changes in the topologies. It can be concluded that based on the 12S data, Geomyoidea demonstrate an association with Pedetoidea and the Dipodoidea/Muroidea clade. This result is in contrast with the mitochondrial cytochrome *b* (cyt *b*) gene, which suggests rodent paraphyly by placing the Geomyidae as a basal eutherian lineage relative to other orders, including a clade containing muroids (*Mus* and *Rattus*) and the hystricognath genus *Hystrix* (Philippe and Douzery, 1994). In the case of cyt *b*, the divergent nature of the geomyids relative to the other rodent lineages may be the result of a rate increase associated with the geomyoid lineage (DeWalt *et al.*, 1993; Honeycutt *et al.*, 1995).

Systematics of Muroidea and Dipodoidea

The superfamily Muroidea represents approximately 27% of all extant mammalian species (Catzeflis *et al.*, 1992). These rodents are sciurognathous and myomorphous, and classifications have placed them within the suborder or infraorder Myomorpha together with Dipodoidea, Gliroidea, and sometimes Geomyoidea (Simpson, 1945; Wood, 1955, 1965; Chaline and Mein 1979). Characteristics associated with myology (Klingener, 1964), cephalic arteries (Bugge, 1971), and fetal membranes (Luckett, 1985) support a sister-group relationship between the muroids and the superfamily Dipodoidea. Based on dental similarity the sciuravids have been suggested to be the ancestor of the muroids, dipodoids, and geomyoids (Matthew, 1910; Wilson, 1949; Wood, 1959; Black, 1965; Fahlbusch, 1979). Flynn *et al.* (1986), however, have suggested a hystricomorphous ctenodactyloid ancestor because primitive muroids were hystricomorphous (Lindsay, 1977). The 12S rRNA gene sequences provided strong support for the monophyly of Muroidea, with an equal-weighting bootstrap value of 100 and a Bremer support index of 24. In addition, all analyses of the 12S rRNA data support a sister-group relationship between the Muroidea and the Dipodoidea (equally and unequally-weighted bootstrap and Bremer support index of 51/6 and 54/1, respectively). Although support was minimal, all analyses supported a sister-group relationship between a clade containing Pedetoidea and Geomyoidea and the muroid/dipodid clade.

Systematics of Pedetoidea

Pedetoids are sciurognathous and hystricomorphous and have been placed as *incertae sedis* within the suborder Sciurognathi or Sciuromorpha (Simpson, 1945; Wood, 1955; Chaline and Mein, 1979). The sister-group of Pedetoidea is uncertain but characters of the middle ear (Lavocat and Parent, 1985), the pattern of carotid arterial branches (Bugge, 1985), characters of the auditory region (Meng, 1990), and a cluster analysis of a wide variety of characters (Bugge, 1985; George, 1993) indicate a possible relationship with the family Anomaluridae (not included in this study). The affinities of Pedetidae and Anomaluridae to other rodent families is even less clear (Luckett and Hartenberger, 1985). Although some studies based on fetal membranes and placental characters revealed a closer relationship of *Pedetes* to sciuromorphs (Fischer and Mossman, 1969; Otiang'a-Owiti *et al.*, 1992), Luckett (1985) suggested that these similarities are the result of symplesiomorphies. As indicated by Sarich (1985) and George (1985), Pedetidae does not share a close affinity to the Hystricognathi, even though the family has a hystricomorphous zygomasseteric system. Jaeger (1988) suggested that the possible ancestor of *Pedetes* may be a member of the Baluchimyinae, an old and morphologically primitive southern Asiatic group of hystricomorphous and sciurognathous rodents. Recently, Martin (1993) indicated that the families Pedetidae and Ctenodactylidae shared with the Hystricognathi a derived condition (multiserial Schmelzmuster) associated with incisor enamel. None of the analyses based on the 12S rRNA sequence supported a relationship of *Pedetes* to either the Hystricognathi or Ctenodactyloidea, two groups with a similar hystricomorphous zygomasseteric structure and incisor enamel. The retention of the primitive sciurognathous condition and a presumably derived hystricomorphous condition in *Pedetes, Ctenodactylus*, and *Anomalurus* suggest an intermediate position between rodent groups that exhibit hystricomorphy

and hystricognathy and groups that exhibit sciuromorphy and sciurognathy. Although maximum parsimony analyses of the 12S rRNA data (Figs. 6 and 9) provided limited support (equal weighting = one step, unequal weighting = four steps) for a sister-group relationship between *Pedetes* and Geomyoidea, no most parsimonious trees suggested a relationship with either the Ctenodactyloidea or Hystricognathi. The sister-group relationships between either Pedetoidea and Hystricognathi or Pedetoidea and Ctenodactyloidea required an additional seven steps for the 12S tree (equal weighting). Thus, results from the 12S rRNA data suggest that either the primitive condition for rodents is hystricomorphy or hystricomorphy arose three times independently (discussed under Systematics of Rodent Suborders).

Systematics of Ctenodactyloidea

Similar to the family Pedetidae, ctenodactyloids also are sciurognathous and hystricomorphous. Ctenodactyloidea has been placed either in the suborder Sciuromorpha (Simpson, 1945; Wood, 1955) or in a separate infraorder (Chaline and Mein, 1979). Some authors have suggested that ctendodactyloids represent the sister-group of all hystricognaths (George, 1985; Flynn *et al.*, 1986). Jaeger (1988) also supported an affinity of the ctenodactyloids with the hystricognaths, based upon the character of a large hypocone on the upper molars. Further characters uniting the two groups include fetal membranes and reproductive and musculoskeletal features (Luckett, 1980, 1985; George, 1985), multiserial incisor enamel (Sahni, 1985; Martin, 1992, 1993), middle ear features (Lavocat and Parent, 1985), fusion of the malleus and incus (Wood, 1985), and myoglobin sequences (Beintema *et al.*, 1991). In contrast, maximum-parsimony analyses of the 12S rRNA data (except for the weighted parsimony results in Fig. 8) supported a sister-group relationship between Ctenodactylidae and a clade containing Aplodontidae and Sciuridae. Support was minimal, however, in that one step (four steps for unequally weighted parsimony) collapsed the branch.

The phylogenetic reconstructions from the 12S rRNA data are not in agreement with current interpretations of the fossil record. The earliest fossil record of rodents indicates an ancient diversity that included at least two superfamilies (Hartenberger, 1980; Luckett and Hartenberger, 1985). The Ctenodactyloidea are represented by *Cocomys* from the Eocene of Asia (Dawson *et al.*, 1984; Wang, 1994; but see Flynn *et al.*, 1986), the morphologically most primitive rodent known (Li *et al.*, 1989). The Ischyromyoidea are represented by *Paramys* (Hartenberger, 1980; Luckett and Hartenberger, 1985), the oldest rodent known (Korth, 1994), from the late Paleocene of North America and Eocene of North America and Europe. The maximum parsimony topologies for the 12S rRNA data do not support the simplistic hypothesis that these early taxa indicate a basal bifurcation of all rodents into "hystricognaths" (Ctenodactyloidea and Hystricognathi) and paraphyletic "sciurognaths"; this would require an additional four steps for equal weighting and six steps for unequal weighting. The hypothesis that modern Ctenodactylidae are remnants of a once-diverse ctenodactyloid radiation that also gave rise to the Hystricognathi (George, 1985) is not supported by the 12S rRNA data, because *Ctenodactylus* does not fall sister to the hystricognath taxa. *Ctenodactylus* and *Cocomys* may be members of the same monophyletic superfamily Ctenodactyloidea, but its phylogenetic position may have been misinterpreted. Conversely, *Ctenodactylus* may belong

to a different lineage from the fossil "ctenodactyloids"; however, the ancient ctenodactyloid *Cocomys* still may be the sister-taxon to hystricognaths.

Systematics of Hystricognathi

Hystricognaths consist of families distributed in either the Americas, Africa, or Asia. All are hystricognathous and hystricomorphous, except for the African family Bathyergidae, which is considered to have secondarily derived protrogomorphy based on ontogenetic and paleontological evidence (Lavocat, 1973; 1988; Luckett and Hartenberger, 1985; Maier and Schrenk, 1987). Classification schemes either place all hystricognath members in a single suborder based on the skull and mandible (Simpson, 1945; Chaline and Main, 1979) or separate the African and South American members into several suborders, Caviomorpha, Hystricomorpha, and Bathyergomorpha (Wood, 1955). All analyses of the 12S data supported monophyly of the hystricognaths, with an equal weighting bootstrap value of 71 and Bremer support index of 4. Therefore, collectively the 12S data and a large number of derived characteristics associated with a lower jaw, origin and insertion of the masticatory muscles, fetal membranes, dental characteristics, features of the middle ear, the circulatory system, and albumin immunology (Bugge, 1971; Woods, 1972; Lavocat and Parent, 1985; Luckett, 1985; Luckett and Hartenberger, 1985; Sarich, 1985; Jaeger, 1988; Wyss *et al.*, 1993) provide strong support for a monophyletic Hystricognathi. As discussed previously, potential sister-groups include Ctenodactyloidea, Anomaluroidea, and Pedetoidea, with the greatest level of morphological support for Ctenodactyloidea. Strong support for any one closest sister-group was not found, and only the unequal-weighting results in Fig. 8 demonstrated a sister-group relationship between ctenodactyloids and Hystricognathi. Equal-weighted and generalized maximum-parsimony analyses weakly supported a sister-group relationship with the clade containing the Castoroidea, the Gliroidea, the Ctenodactyloidea, and the Sciuroidea (including Aplodontidae).

Systematics of Rodent Suborders

How do results from the 12S rRNA sequences compare to the traditional classifications based upon characteristics of the zygomasseteric musculature (Brandt, 1855) and lower jaw (Tullberg, 1899)? While Tullberg's classification suggests a dichotomous relationship among families of rodents depending upon hystricognathy or sciurognathy of the lower jaw, results from 12S rRNA sequences clearly do not support such a phylogenetic separation. Although the 12S data supported a monophyletic Hystricognathi, the internal position of hystricognathous rodents with respect to sciurognathous rodents supported a paraphyletic Sciurognathi (Figs. 6 and 9; extra steps required for a monophyletic Sciurognathi were four for equal-weighted parsimony). Therefore, based upon 12S rRNA sequences, sciurognathous rodents do not constitute a natural group. It should be noted, however, that sciurognathy is the plesiomorphic condition for all eutherian mammals, and as such the condition does not represent a shared-derived feature uniting some rodent lineages.

The classification of Brandt (1855) consists of three groups: myomorphs, hystricomorphs, and sciuromorphs (protrogomorphy represents a hypothetical ancestral anatomical condition and not a fourth group). Assuming that the myomorphous condition

of Gliroidea is an example of homoplasy [based on the absence of myomorphy in at least two protrogomorphous lower Oligocene glirid lineages and a seemingly hystricomorphous condition in *Graphiurus* (Vianey-Liaud, 1985; Wahlert *et al.*, 1993)], then other extant rodents having the myomorphous zygomasseteric condition (superfamily Muroidea) represent a natural rodent group based on phylogenetic reconstructions using the 12S rRNA data (Fig. 10). The hystricomorphous and sciuromorphous conditions, however, are not as easily interpreted. Extant sciuromorphous superfamilies include Sciuroidea, Castoroidea, and Geomyoidea. None of the 12S rRNA analyses support monophyly of this group. Maximum-parsimony analyses supported a relationship in which the geomyoids (plus *Pedetes*) were more closely related to the muroids than to the superfamily Sciuroidea (Fig. 6 and 9), and this finding is in agreement with the views of several authors (Wahlert, 1978, 1985; Dawson and Krishtalka, 1984; Luckett, 1985). Therefore, the sciuromorphous condition in geomyoids appears to be independently derived. Although the 12S rRNA results do not support monophyly of the remaining lineages demonstrating sciuromorphy, this condition of the zygomasseteric system is restricted to a larger clade containing the remaining lineages that are sciuromorphous (Fig. 10). Extant rodents that exhibit hystricomorphy include members of the Hystricognathi [Caviomorpha, and Phiomorpha (excluding the family Bathyergidae)] and the

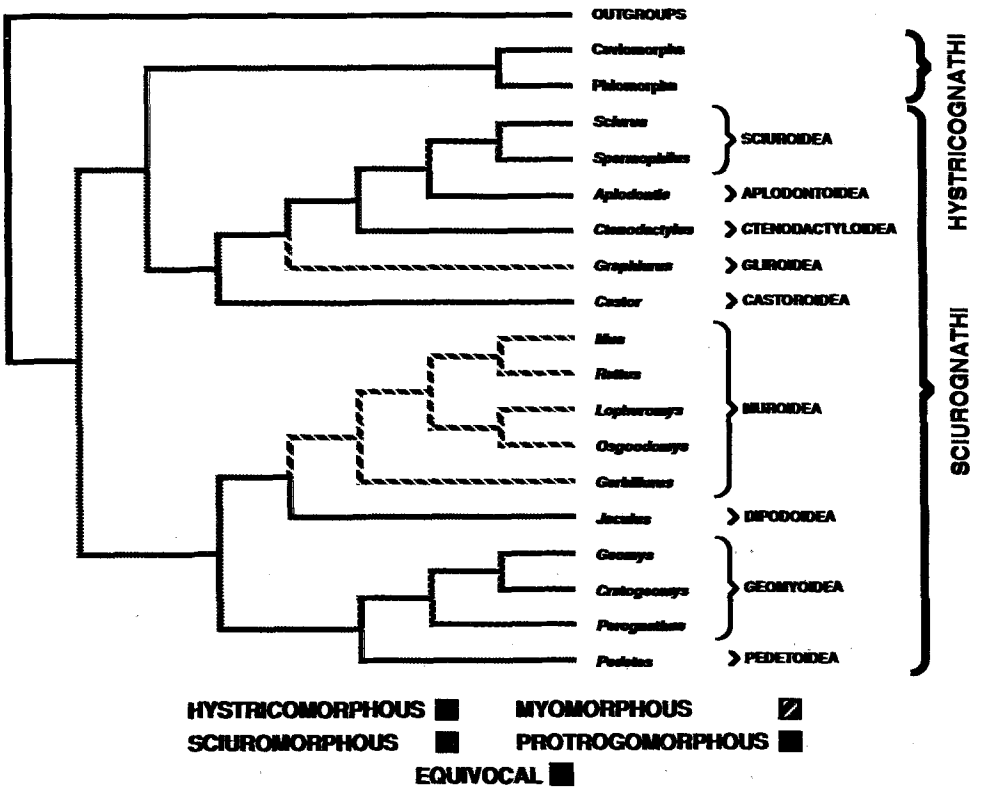

Fig. 10. Characteristics of lower jaw and zygomasseteric musculature plotted on rodent phylogenies depicted in Figs. 6 and 9.

superfamilies Ctenodactyloidea, Dipodoidea, Anomaluridae, and Pedetoidea. Results based on the 12S rRNA gene do not support a monophyletic "hystricomorphous" rodent assemblage (Fig. 10). Maximum parsimony placed Ctenodactyloidea within a weakly supported clade (largely "sciuromorph") composed of Castoroidea, Gliroidea, Aplodontoidea, and Sciuroidea (Figs. 6, 9, and 10). In agreement with morphological analyses, the superfamily Dipodoidea was placed as a sister-group to the myomorphous Muroidea, and the hystricomorphous superfamily Pedetoidea was placed as a sister-group to the clade containing the "pseudo-sciuromorphous" Geomyoidea (Figs. 6, 9, and 10). Thus, with respect to the 12S rRNA-derived topology, hystricomorphy either represents the ancestral condition for Rodentia or arose four times independently (assuming no reversals; Fig. 10).

In light of the 12S rRNA results, what is the most parsimonious ancestral zygomasseteric structure with reference to extant taxa? The primitive mammalian condition, in which the origins of the lateral and medial masseter muscles are restricted to the zygomatic arch (and no substantial part of the masseteric musculature is transmitted by the infraorbital foramen), is shared by protrogomorphous rodents. Therefore, when zygomasseteric conditions were mapped onto the 12S rRNA phylogeny, the sister-group to rodents was assigned the protrogomorphous condition (Fig. 10). Regardless of what is assumed about the zygomasseteric conditions of Gliroidea and Geomyoidea, the designation of either protrogomorphy or hystricomorphy as the rodent ancestral condition required seven steps, whereas either sciuromorphy or myomorphy as the ancestral condition required eight steps. The inclusion of fossil taxa, however, can influence the reconstruction of the ancestral zygomasseteric condition for rodents. Therefore, the protrogomorphous family Ischyromyidae, which has been suggested by several authors to have been the ancestral lineage for the extant families Aplodontidae, Sciuridae, Castoridae, and possibly Gliridae (Hartenberger, 1980; Dawson *et al.*, 1984; Flynn *et al.*, 1986; Korth, 1994), was included in the reconstructions. Ischyromyidae was placed as a sister-group to the clade containing the Aplodontidae, Sciuridae, Ctenodactylidae, Gliridae, and Castoridae and, alternatively, as a sister-group to the clade containing the Aplodontidae and Sciuridae. Again, both hystricomorphy and protrogomorphy were the most-parsimonious ancestral conditions, with sciuromorphy and myomorphy each requiring an additional step.

CONCLUSIONS

Diagnosing relationships among major rodent lineages has been of interest to both neontologists and paleontologists for well over a century (Brandt, 1855; Tullberg, 1899; Hartenberger, 1985; Luckett and Hartenberger, 1985; Jaeger, 1988), yet many questions regarding the phylogeny of rodents are still poorly resolved. More recently, molecular characters have been used to address rodent relationships (Sarich, 1985; Beintema *et al.*, 1991; Catzeflis *et al.*, 1992; DeWalt *et al.*, 1993; Nedbal *et al.*, 1994), and in some cases molecular results have been congruent with many previous morphological hypotheses, whereas in other cases molecular and morphological results are highly incongruent. Two of the most surprising differences between morphological (from both a neontological and a paleontological perspective) and some molecular studies are associated with the sister-group to and monophyly of Rodentia.

Although a source of controversy in the past (Wood, 1957), more recent morphological and paleontological evidence provides support for the monophyly of Glires, a superorder containing the orders Rodentia and Lagomorpha (Novacek, 1992; Luckett and Hartenberger, 1993). In contrast to the morphological data, support for the monophyly of Glires based on molecular data is not strong, with many recent studies suggesting a closer relationship among Lagomorpha and other eutherian orders (e.g., Primates) and Rodentia occupying a more basal position on the eutherian tree (Li *et al.*, 1990; Honeycutt and Adkins, 1993; Graur *et al.*, 1996). From a morphological and paleontological standpoint, the monophyly of Rodentia is well supported (Luckett and Hartenberger, 1993), yet several recent molecular papers have challenged the idea of a monophyletic Rodentia by suggesting that the guinea pig (*Cavia procellus*) and presumably all hystricognaths represent a separate lineage from the remaining rodent families (Graur *et al.*, 1991; Li *et al.*, 1992; Ma *et al.*, 1993; D'Erchia *et al.*, 1996).

There are several explanations for the lack of congruence between morphology and some molecular studies with respect to the monophyly of both Rodentia and Glires and the difficulties associated with finding strong molecular support for particular groupings of divergent rodent lineages. These explanations are not mutually exclusive in that several of these factors are associated with rate heterogeneity among lineages and sites that can influence the ability of tree building methods to retrieve consistent phylogenies. First, heterogeneity in branch lengths as a result of either differential rates of molecular evolution or old and divergent lineages can result in a "long-branch effect," whereby inconsistent results are obtained by long branches grouping together in a phylogenetic analysis (Felsenstein, 1978; Hardy and Penny, 1989; Huelsenbeck, 1995; Swofford *et al.*, 1996). Second, long-branch effects can also be enhanced by limited taxonomic sampling (Wheeler, 1992). Third, among-site rate variation can influence phylogeny reconstruction (Yang, 1994; Sullivan *et al.*, 1995), and either maximum-likelihood or distance-based models following a gamma distribution can be used to accommodate such heterogeneity (Tamura and Nei, 1993; Yang, 1993; Swofford *et al.*, 1996). Fourth, heterogeneity in base composition among taxa can be a source of error in phylogeny reconstruction (Lockhart *et al.*, 1994). Finally, the choice of an appropriate outgroup can influence the placement of the tree root and support for ingroup monophyly, especially when considerable branch length heterogeneity exists (Wheeler, 1990; Swofford, *et al.*, 1996).

How do the 12S rRNA results presented here relate to ongoing debates regarding rodent monophyly, the sister-group to Rodentia, and hypotheses of relationships among rodent families? Most molecular studies that address the monophyly of Rodentia and Glires are limited in taxonomic breadth. For instance, *Cavia porcellus* and maybe one other hystricognath plus *Mus* and *Rattus* (both myomorph rodents and members of the same subfamily) represent the taxa most often used to address questions of rodent monophyly. Molecular studies that address the placement of Glires also are limited to one representative of the order Lagomorpha, and none have included the divergent lagomorph family Ochotonidae. If many rodent lineages differentiated rapidly in the Paleocene/Eocene (Jaeger, 1988), then such sparse sampling can influence phylogenetic results. The 12S results are exceptional with respect to the diversity of ingroup and outgroup taxa used. Equally weighted parsimony with multiple outgroup taxa did reveal a monophyletic Rodentia (Fig. 6), yet support in terms of bootstrap and decay values

was weak. Frye and Hedges (1995), using a larger amount of mitochondrial rRNA sequences and fewer rodent taxa (*Cavia*, *Mus*, and *Rattus*), found strong support for rodent monophyly. This suggests that the inclusion of more rodent taxa in the study by Frye and Hedges (1995) has the potential of complicating the clear support for rodent monophyly shown by these authors. The 12S data also suggest that the dichotomy identified for rodent lineages is considerably more complicated than that suggested by advocates of a polyphyletic Rodentia. Monophyly of the suborder Hystricognathi is supported by all analyses (equally and unequally weighted parsimony, maximum likelihood, and neighbor joining), and this clade contains 16 families of rodents. Within this clade the guinea pig (*Cavia porcellus*) groups with other members of the family Caviidae and is part of a monophyletic caviomorph clade. As can be seen in Fig. 6, several other divergent lineages (Sciuroidea/Aplodontoidea, Ctenodactyloidea, Gliroidea, and Castoroidea) group separately from the divergent *Mus/Rattus* lineage identified by D'Erchia *et al.* (1996) in their analysis of the entire mitochondrial genomes of selected eutherian orders. In the case of the 12S data, *Mus/Rattus* are part of a large clade containing other members of Muroidea as well as Dipodoidea, Geomyoidea, and Pedetoidea.

Although rodents do not show any appreciable base composition differences from those seen in outgroup taxa (Figs. 2 and 3), some rodent lineages do show rate heterogeneity (Table I), and the distribution and frequency of substitution classes (TS, TV, and indels) differed among rodent taxa and between rodent and outgroup taxa (Figs. 4 and 5). The difference between rodents and nonrodents is especially strong for stem transversions. Attempts to correct for substitutional heterogeneity involved generalized parsimony (the assignment of differential weights to substitutional classes), maximum likelihood (Felsenstein, 1993), and neighbor joining (Saitou and Nei, 1987). In the case of maximum-likelihood, four analyses were conducted using default parameters with TS/TV varying within one standard deviation of the calculated mean over all ingroup taxa (range, 0.5–2.2), and neighbor-joining analyses used Tajima and Nei (1984) and Jukes and Cantor (1969) distances incorporating a gamma distribution as well as Kimura (1980) distances without a gamma distribution. Because it was difficult to know which maximum-likelihood and distance-based models fit the data, in each case a strict consensus tree was produced for topologies obtained from the respective maximum-likelihood and neighbor-joining analyses (data not shown).

Several observations can be made regarding corrections for substitutional heterogeneity. First, although generalized parsimony failed to support rodent monophyly, the resultant phylogeny (Fig. 8) was consistent with equally weighted parsimony in that two major groups of rodent lineages could be identified, with at least two divergent clades within each group (Figs. 6, 8, and 9). Second, generalized parsimony provided support for a sister-group relationship between Lagomorpha (represented by *Sylvilagus*) and the rodent clade containing Muroidea/Dipodoidea and Geomyoidea/Pedetoidea. Honeycutt *et al.* (1995), using two protein-encoding genes, also found support for lagomorphs grouping with a subset of rodents. Third, generalized parsimony (Fig. 8) supported a sister-group relationship between hystricognaths and *Ctenodactylus*, a finding congruent with morphology. All the neighbor-joining analyses (not shown) supported this arrangement with a bootstrap value of 46, whereas maximum likelihood supported the relationships shown by equally weighted parsimony (Fig. 6). Therefore, the sister-group relationship between hystricognaths and ctenodactyloids should be investigated in more

detail by the inclusion of additional ctenodactyloid taxa and perhaps some consideration of among-site and branch-length heterogeneity. Finally, although correction for heterogeneity using different models did differ in the placement of some rodent lineages, both maximum-likelihood and neighbor-joining supported monophyly of Hystricognathi, a sister-group relationship between Sciuroidea and Aplodontoidea, monophyly of Muroidea, and monophyly of Geomyoidea. Maximum likelihood also supported monophyly of both Muroidea/Dipodoidea and a clade containing a polytomy involving Muroidea/Dipoidea, Geomyoidea, and Pedetoidea. Both maximum-likelihood and neighbor-joining were equivocal relative to the placement of gliroids and castorids. Thus, the placement of these two lineages may be enhanced by the inclusion of more gliroid and castorid taxa.

The ability to find molecular support for rodent monophyly and possibly Glires may be compromised by the heterogeneity observed among some rodent lineages and between rodent and nonrodent taxa. Corrections using either generalized parismony or other models do not increase support for the monophyly of either Rodentia or Glires. This suggests that the inclusion of nonrodent outgroups with rodents may be influencing the attraction of particular branches, and as such, molecular phylogenies that suggest rodent polyphyly and the inclusion of lagomorphs as closer to other eutherians may be an artifact of the heterogeneity observed for rodents relative to that seen for other eutherians. One interesting observation from the studies of Graur *et al.* (1991) and D'Erchia *et al.* (1996) is that the two divergent lineages of rodents, hystricognaths and myomorphs, do not group together but both do fall basal to most eutherian lineages. This suggests that rodents in general are quite divergent from most eutherians examined, and this may simply be a consequence of process differences associated with how rodent genes are varying. If this is true, a detailed analysis that includes a large number of rodent taxa and genes may provide important information regarding the process of molecular evolution. Regardless of the differences seen by equally weighted and generalized parsimony, the inclusion or exclusion of outgroups with the ingroup analysis, and models of evolution incorporating either maximum-likelihood or neighbor-joining approaches, the overall patterns of lineage relationships and diversity shown by the 12S data (Figs. 6, 8, and 9) are consistent in many respects. Therefore, these results should provide a meaningful framework for further studies that include more rodent taxa and nucleotide sequences.

APPENDIX. SPECIMENS EXAMINED

Taxon	Museum or Genbank No.[a]
Order Artiodactyla	
Bos taurus	V00654
Order Cetacea	
Balaenoptera physalus	X61145
Order Carnivora	
Phoca vitulina	X63726
Order Lagomorpha	
Sylvilagus audubonii	H2311
Order Primates	
Homo sapiens	V00662

APPENDIX. Continued

Taxon	Museum or Genbank No.[a]
Order Xenarthra	
Dasypus novemcinctus	H2317
Order Rodentia (sensu Chaline and Mein, 1979)	
Suborder Sciurognathi	
Infraorder Protrogomorpha	
Superfamily Aplodontoidea	
Family Aplodontidae	
Aplodontia rufa	H2370
Infraorder Sciuromorpha	
Superfamily Sciuroidea	
Family Sciuridae	
Sciurus niger	H2376
Spermophilus tridecemlineatus	H2147
Superfamily Castoroidea	
Family Castoridae	
Castor canadensis	H2205
Infraorder Ctenodactylomorpha	
Superfamily Ctenodactyloidea	
Family Ctenodactylidae	
Ctenodactylus gundi	H2206
Incretae sedis	
Superfamily Pedetoidea	
Family Pedetidae	
Pedetes capensis	SP6352
Infraorder Myomorpha	
Superfamily Gliroidea	
Family Gliridae	
Graphiurus murinus	SP5577
Superfamily Geomyoidea	
Family Geomyidae	
Cratogeomys castanops	H110
Geomys bursarius	TK30723
Family Heteromyidae	
Perognathus flavus	AK10368
Superfamily Dipodoidea	
Family Dipodidae	
Jaculus jaculus	SP10206
Superfamily Muroidea	
Family Muridae	
Gerbillurus vallianus	SP4232
Lophuromys flavopunctatus	SP5301
Mus musculus	V00711
Osgoodomys banderanus	TK19663
Rattus norvegicus	V00680
Suborder Hystricognathi	
Infraorder Phiomorpha	
Superfamily Thryonomyoidea	
Family Thryonomyidae	
Thryonomys swinderianus	M63570
Family Petromuridae (sensu Wood, 1965)	
Petromus typicus	M63571
Superfamily Bathyergoidea	
Family Bathyergidae	
Bathyergus janetta	M63565
Bathyergus suillus	M63564
Cryptomys damarensis	M63569
Cryptomys hottentotus hottentotus	M63567

APPENDIX. Continued

Taxon	Museum or Genbank No.[a]
Cryptomys hottentotus natalensis	M63568
Georychus capensis	M63566
Heterocephalus glaber	M63563
Heliophobius argenteocinereus	M63562
Superfamily Hystricoidea	
Family Hystricidae	
Atherurus macrourus	U12451
Hystrix africaeaustralis	U12448
Infraorder Caviomorpha	
Superfamily Octodontoidea	
Family Ctenomyidae (sensu Wood 1965)	
Ctenomys boliviensis	U12446
Ctenomys mendocinus	NK13192
Family Octodontidae	
Aconaemys fuscus	K38
Octodon degus	U12452
Octodontomys gliroides	AK15685
Octomys mimax	AK13474
Spalacopus cyanus	K50
Tympanoctomys barrerae	AK13811
Family Echimyidae	
Proechimys longicaudatus	U12447
Family Abrocomidae	
Abrocoma cinerea	NK30665
Superfamily Erethizontoidea	
Family Erethizontidae	
Coendou bicolor	K5
Erethizon dorsatum	U12450
Superfamily Chinchilloidea	
Family Dinomyidae	
Dinomys branickii	K8
Family Chinchillidae	
Chinchilla laniger	U12445
Family Dasyproctidae	
Dasyprocta punctata	U12453
Myoprocta acouchy	K13
Family Agoutidae	
Agouti paca	K7
Family Myocastoridae	
Capromys pilorides	U12443
Myocastor coypus	U12444
Superfamily Cavioidea	
Family Caviidae	
Cavia aperea	TK17830
Cavia porcellus	U12449
Dolichotis salinicola	AK14046
Galea musteloides	AK13818
Microcavia australis	AK13309
Family Hydrochaeridae	
Hydrochaeris hydrochaeris	U12454

[a] M, U, V, or X—Genbank accession numbers; H—research collection of R. L. Honeycutt; K—Zadock Thompson Natural History Collections, University of Vermont; AK—Texas Cooperative Wildlife Collection, Texas A&M University; NK—Museum of Southwestern Biology, University of New Mexico; SP—The Carnegie Museum of Natural History, Pittsburgh; TK—The Museum, Texas Tech University.

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