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Molecular Phylogeny of European Muroid Rodents Based on Complete Cytochrome *b* Sequences

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Phylogenetic relationships among 18 species of mainly European muroid rodents that belong to three subfamilies were estimated using complete sequences of the mitochondrial cytochrome *b* gene. The inferred monophyly of the subfamilies Murinae (mice and rats) and Arvicolinae (voles, lemmings, and muskrats) is in agreement with previous studies. Within the Murinae, the morphology-based division of the genus *Apodemus* into three subgenera is supported by these DNA sequence data. The relationships among the different genera of the Murinae were generally poorly resolved, and the relationships of *Micromys* and *Acomys* to the other murine genera remained unresolved. Within the subfamily Arvicolinae, the relations of the genera *Arvicola, Clethrionomys,* and *Microtus* remained tentative with our data. However, within the *Microtus* group, there is a good molecular support for the phylogenetic relationships. These findings suggest that the origin of the different murine and arvicoline lineages was rapid, indicating an adaptive radiation with fast speciation. © 2000 Academic Press

Key Words: **evolution; phylogeny; cytochrome *b;* muroid rodents; rapid radiation.**

INTRODUCTION

Rodents are often used as model systems in a wide range of biological disciplines. While *Mus* and *Rattus* are the classical laboratory animals, behavioral studies were performed on many different rodent species within the past decades (Crowcroft, 1966; Robitaille and Bovet, 1976; Berry, 1981; McClintock, 1983; Elwood, 1985; Brown, 1986; Hurst, 1986; König and Markl, 1987; Gerlach, 1996; Gerlach, 1998). To explain the evolution of social behavior and other traits in this order of mammals, exact knowledge of their phylogenetic relationships is tantamount. Through the comparative method, one can address questions such as whether mating systems, social behavior such as cooperative behavior, or particular dispersal patterns have evolved independently. Currently, phylogenetic relationships within the family Muridae, to which many of the most commonly studied rodents belong, remain uncertain.

The family Muridae is the largest mammalian family and accounts for approximately one-quarter of all mammalian species. It contains 1326 species, which are divided into 17 subfamilies (Musser and Carleton, 1993). Some of these subfamilies, such as Murinae, Sigmodontinae, Arvicolinae, and Gerbillinae, comprise large clades that contain approximately 225 genera worldwide with a broad geographic distribution. Three of these subfamilies are included in this study and we place special emphasis on the subfamilies Murinae and Arvicolinae.

In Europe, five genera of the subfamily Murinae (mice and rats) are found. They range in body size from 5 to 50 cm and are camouflaged in coloration. In general, their tails are long, and their eyes and ears are relatively large. There are many specializations, for example in habitat preference and social structures, and different mating systems are found within this subfamily. Representatives of all five genera of the Murinae are included in this study.

In comparison with the Murinae, the species of the subfamily Arvicolinae (voles, lemmings, and muskrats) have relatively small eyes and ears, and their tails are shorter than their bodies. The voles are the dominant rodent group in open grassland and cultivated land. In Europe, there are 11 genera found within the Arvicolinae. In our study, we examined 3 of the European genera.

The classification and evolution of the muroid rodent subfamilies Arvicolinae and Murinae have been studied by a variety of methods. Morphological, karyological, biochemical, and molecular data have all been used (Brownell, 1983; Gill *et al.*, 1987; Catzeflis *et al.*, 1987; Chaline and Graf, 1988; Robinson *et al.*, 1997). Initial attempts to classify rodent families were based on dental structures of fossil and extant rodents. The phylogenetic relationships among the Murinae (Misonne, 1969) and Arvicolinae (Niethammer and Krapp, 1982) were based largely on differences in their molar mor-

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phology. In some cases, the results of morphological studies contradict results that are obtained with biochemical and molecular characters. For example, controversy persists about the relationships among the genera *Arvicola, Microtus,* and *Clethrionomys.* According to Rabeder (1980), the genera *Microtus* and *Clethrionomys* are more closely related to each other than they are to *Arvicola* (Niethammer and Krapp, 1982). In contrast, studies of allozymes (Graf and Scholl, 1975) and DNA/DNA hybridization data (Catzeflis *et al.,* 1987) suggest that the genera *Microtus* and *Arvicola* are more closely related. No DNA sequence data relevant to this question were available before this study.

Also, the phylogenetic relationships among members of the subfamily Murinae are still somewhat unresolved. Based on morphological features, *Mus* and *Rattus* are believed to be more closely related to each other than *Mus* is to *Apodemus* (Misonne, 1969). However, Catzeflis *et al.* (1987) using DNA/DNA hybridization data and Nikoletopoulos *et al.* (1992) using immunological data postulated that *Mus* and *Apodemus* form the sister group to *Rattus.*

Even more contentious have been the phylogenetic relationships among the genera *Acomys* and *Micromys* relative to the other murine genera. Both share a unique molar tooth pattern with the other murine genera, but, particularly in morphological characters, they differ strongly from other genera within the subfamily Murinae. *Acomys* has a different coat with rigid bristles and *Micromys* has differently shaped sperm and a different skull with a relatively larger and elongated cerebral region and a rather shortened rostral region.

Robinson *et al.* (1997) were the first to use DNA sequences of the nuclear LCAT gene for the determination of the phylogenetic relations among the Muroidea. However, their phylogenetic analysis included only a few genera from the different subfamilies, and important genera of the Murinae and Arvicolinae, such as *Apodemus* or *Arvicola,* were not included. Different genes were sequenced for species of the Murinae and Arvicolinae, but no large sets of homologous sequences were available to compare these European rodent species. For a multitude of comparative studies in many biological disciplines, it is important to know exactly the phylogenetic relationships between the different rodent model systems.

To investigate the phylogenetic relationships among European muroid rodents, we determined the complete sequences of the mitochondrial cytochrome *b* gene. The widespread successful use of the cytochrome *b* gene (cyt *b*) made this gene the obvious choice as a phylogenetic marker for this study (Meyer, 1994). The wide availability of cytochrome *b* sequences further permits comparison of our data with some previously published sequences, such as that of *Mus domesticus* (Bibb *et al.,* 1981), *Rattus norvegicus* (Gadaleta *et al.,* 1989), and *Acomys cahirinus* (Barome *et al.,* 1998), and choice among several available outgroup taxa.

MATERIALS AND METHODS

Specimens Examined

Our analysis included 18 muroid rodent species, which belong to three subfamilies: 12 species belong to the subfamily Murinae (mice and rats), 5 species belong to the Arvicolinae (voles and lemmings), and 1 species belongs to the Gerbillinae (gerbils) (Table 1). Four outgroup taxa were included from previously published sources; these are *Chelemys macronyx* (Accession No. U03533; Smith and Patton, 1999), *Geoxus valdivianus* (Accession No. U03531; Smith and Patton, 1999), *Kunsia tomentosus* (Accession No. AF108670; Smith and Patton, 1999), and *Lenoxus apicalis* (Accession No. U03541; Smith and Patton, 1999). These outgroup taxa all belong to the subfamily Sigmodontinae (New World cricetids).

DNA Extraction

Total genomic DNA was extracted from small (4-mm^2) pieces of muscle or ear tissue. The tissue was digested overnight at 47°C in a total volume of 400 μl, including 53 μl proteinase K (14 mg/ml) (Boehringer Mannheim) and 347 μl ATL Tissue Lysis Buffer (Qiagen), and were subjected to standard phenol/chloroform extraction and ethanol precipitation (Sambrook *et al.,* 1989).

DNA Amplification and Sequencing

Mitochondrial sequences containing the cytochrome *b* gene were isolated via the polymerase chain reaction (PCR). The entire cyt *b* gene was amplified using the primers L14115 (5′-AAT GAC ATG AAA CAT CGT TG-3′) and H15288 (5′-ACA AGA CCA GAG TAA TGT TTA TAC TAT C-3′) in the flanking region of the glutamin and threonin tRNA genes. Additional internal primers used only for sequencing were L14648 (5′-TGA ATY TGA GGR GGC TTC TCA GTA-3′) and H14742 (5′-GGG TTG TTD GAT CCW GTT TC-3′). Primer names indicate the DNA strand (H, heavy or L, light) and the position of the most 3′ end of the oligonucleotide with reference to the *Mus domesticus* mtDNA sequence (Bibb *et al.,* 1981).

Double-stranded PCR amplifications were performed in 40-μl reaction volumes using primers L14115 and H15288. Each reaction included 2 μl primer (20 μM), 4 μl of deoxynucleoside–triphosphate mixture, 4 μl 10× reaction buffer (Pharmacia), and 0.2 μl *Taq* DNA polymerase (5 U/μl, Pharmacia). All PCRs used the following thermal cycling parameters: 2 min at 94°C, 40 cycles (1 min at 94°C, 1 min at 52°C, and 2 min at 72°C), plus 15 min at 72°C, in a PTC 100 thermal cycler (Biozym). PCR products were gel-purified in 0.8% agarose.

TABLE 1

Species of Muroid Rodents Included in the Phylogenetic Analysis

Subfamily	Species	Origin of sample or previous sequence (citation given)
Murinae	*Acomys cahirinus*	Z96053 (Barome *et al.*, 1998)
	Apodemus agrarius	MVZFC 5214
	Apodemus alpicola	Switzerland
	Apodemus flavicollis	Konstanz (Germany)
	Apodemus microps	T-850
	Apodemus mystacinus	T-851
	Apodemus sylvaticus	Konstanz (Germany)
	Micromys minutus	Konstanz (Germany)
	Mus domesticus	J01420 (Bibb *et al.*, 1981)
	Mus spicilegus	T-408
	Mus spretus	T-392
	Rattus norvegicus	X14848 (Gadaleta *et al.*, 1989)
Arvicolinae	*Arvicola terrestris*	MVZFC 4873
	Clethrionomys glareolus	Konstanz (Germany)
	Microtus agrestis	Lengwil (Switzerland)
	Microtus arvalis	Wolfach (Germany)
	Microtus epiroticus	MVZFC 5216
Gerbillinae	*Meriones unguiculatus*	Institut für Haustierkunde Kiel
Sigmodontinae	*Chelemys macronyx*	U03533 (Smith and Patton, 1999)
	Geoxus valdivianus	U03531 (Smith and Patton, 1999)
	Kunsia tomentosus	AF108670 (Smith and Patton, 1999)
	Lenoxus apicalis	U03541 (Smith and Patton, 1999)

Note. Listing is alphabetical and does not reflect phylogenetic relationship. T numbers refer to the catalogue of mammalian tissues curated at the University of Montpellier (Catzeflis, 1991). MVZ are catalogue numbers of specimens from the Museum of Vertebrate Zoology (University of California, Berkeley).

All taxa were sequenced directly from purified PCR products using the primers mentioned above. Approximately 100 ng of double-stranded PCR product was used in cycle sequencing reactions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). All sequencing reactions were performed according to the manufacturer's instructions. Sequencing was done on an ABI Prism Automated 377 HT DNA Sequencer (Applied Biosystems). Sequences for both strands were determined.

All sequences are available in GenBank/EMBL under Accession Nos. AF159390–AF159405.

Phylogenetic Analysis

Phylogenetic relationships were analyzed by maximum-parsimony (MP), neighbor-joining (NJ), and maximum-likelihood (ML) methods. The phylogenetic analyses were conducted using the program PAUP* test version 4.0d65 (Swofford, 1998). Critical values of skewness (g_1 statistics) of the tree length distribution were used to assess overall phylogenetic signal in the data set (Hillis and Huelsenbeck, 1992). The critical values were computed from a distribution of 10,000 randomly generated trees using PAUP. The maximum-parsimony analysis was done with a heuristic search using stepwise addition and performing tree–bisection–reconnection (TBR) branch swapping. We employed several weighting schemes to correct for the possible saturation for third position transitions and to accommodate the cytosine to thymine (C ↔ T) changes at first positions that specify different codons of leucine. In the first weighting scheme, the transitions at the third codon position and the C ↔ T substitutions for leucine at the first codon position were excluded. In the second, we used a 10:1 transversion to transition weight for third positions, a weight of 10 for all second positions, and a weight of 1 for all first position C ↔ T substitutions, with all others weighted 10. The 10:1 third position transition to transversion ratio was the empirically observed rate difference. Support for each clade in the most parsimonious reconstructions was assessed by bootstrap (BP) analysis from 500 bootstrap replicas. Neighbor-joining analyses were performed with distance matrices calculated with the Kimura two-parameter model, and the robustness of inferences was assessed through bootstrap resampling (1000 repetitions). Maximum-likelihood analyses were performed with the Hasegawa–Kishino–Yano model (Hasegawa *et al.*, 1985) and the empirically observed transition:transversion ratio of 1.98.

The maximum-likelihood method was used for statistically testing alternative topologies against the one with the highest likelihood. This test of Kishino and Hasegawa (1989) uses the mean and variance of log-likelihood differences between trees. If the mean is

more than 1.96 standard deviations different, then the trees are declared significantly different. The topology of the ML tree was constrained according to the alternative topologies obtained by the MP or NJ method.

RESULTS

Cytochrome b Gene Sequences

The complete cytochrome *b* gene was determined for 15 taxa and analyzed together with additional previously published muroid rodent sequences. The gene begins in all examined species with the conserved initiating methionine codon ATG. Within the Arvicolinae, the gene has the stop codon TAA. Within the Murinae, there is, except for *Rattus norvegicus*, no complete stop codon. There is a single T at the 5′ end of the following tRNA gene. The cytochrome *b* gene ends with a translational termination signal, which is presumably produced by the polyadenylation of the processed mRNA, as in many animal mitochondrial genomes (Anderson *et al.*, 1981). There are slight differences in gene length in different rodent groups—within the Arvicolinae, it is 1143 bp and in some Murinae the gene is 1144 bp long. In some hystricognath rodents, the gene is also 1143 bp long (Ma *et al.*, 1993), but in many other rodents, the gene is at the end one codon shorter (DeWalt *et al.*, 1993; Thomas and Martin, 1993; Lara *et al.*, 1996; Lessa and Cook, 1998).

Base Composition

Base composition of the cytochrome *b* gene in the examined muroid rodents was quite similar to that of previously reported mammalian sequences (Irwin *et al.*, 1991) and comparable to values found in some caviomorph rodents (Lara *et al.*, 1996; Lessa and Cook, 1998). As expected, the abundance of Gs was low (12.8%), whereas the percentages of A, T, and C were quite similar (27.7–30.8%). The frequency of guanine differs greatly among the three codon positions. At the first position, the content is 22.3%, at the second position, it is 12.9%, and at the third position, guanine is rare (3.1%). Second positions have more thymine (42.4%), whereas first and third positions are richer in adenine (29.8 and 42.2%). These values are similar to base compositional patterns previously found in various mammalian species, including some caviomorph rodents (Irwin *et al.*, 1991; Lara *et al.*, 1996; Lessa and Cook, 1998). The bias in base composition was calculated as in Irwin *et al.* (1991). Compositional bias is smaller at first and second positions (0.0633 and 0.2313) than at third positions (0.3607). As expected, the first and second codon positions show less variability than third codon positions (Irwin *et al.*, 1991).

Sequence Variation

For the phylogenetic analyses, a data set of 18 species and 1140 nucleotides was used and could be unambiguously aligned. Of these sites, 512 were variable and 416 were parsimony informative; 123 (24%) of the variable nucleotides were at first codon positions, 39 (8%) were at second codon positions, and 350 (68%) were at third codon positions. Of the phylogenetically informative sites, 86 (21%) were at first positions, 18 (4%) were at second positions, and 312 (75%) were at third positions. As expected for this phylogenetic "depth," most variable sites were in third codon positions.

Genetic Distances

Within genera the genetic distances (corrected with the Kimura two-parameter model) range from 6 to 15%. This is similar to genetic distances within other rodent genera, which are known to span a wide range of divergence. For example, within some genera of caviomorph rodents, between 9 and 20% sequence divergence have been reported (Lara *et al.*, 1996). The values can be about as great as those among some genera, which range in our study from 15.5 to 20.3%. The sequence divergence between genera of the different subfamilies is in a range from 21 to 32%. This is also comparable to those reported distances between 20 and even 40% of some caviomorph rodents of the family Echimyidae (Lara *et al.*, 1996).

Phylogenetic Analysis of the Muridae

Phylogenetic trees based on maximum-parsimony, neighbor-joining, and maximum-likelihood methods were calculated (Figs. 1, 2, and 3). The different weighting schemes in parsimony analysis produced similar trees with the same tree topologies. Consequently, only results with exclusion of the first position C $\leftrightarrow$ T changes and without third position transitions are presented (Fig. 1). The strength of phylogenetic signal in the data set was assessed from the length distribution of 10,000 random trees for both weighting schemes. Skewness was investigated for all sequences and subsets of taxa, because significant skewness can result from only one strongly supported clade. The analysis first contained all sequences, but clades were subsequently taken out to represent all hierarchical levels in the tree. When all sequences are considered, the g_1 statistic is highly significant ($g_1 = -0.654$; $P < 0.01$). Also, with subsets of taxa, there is a highly significant skew in the distribution, indicating strong phylogenetic signal under both step matrices.

Phylogenetic Relationships of the Muridae

With all three methods, the Arvicolinae form a strongly supported clade with bootstrap values of 98 and 100%; however, the monophyly of the subfamily Murinae was not quite as convincingly supported (bootstrap values between 65 and 99%). The only representative species of the Gerbillinae examined, *Meriones unguiculatus*, forms, together with the Murinae, a sister group to the Arvicolinae. Phylogenetic relation-

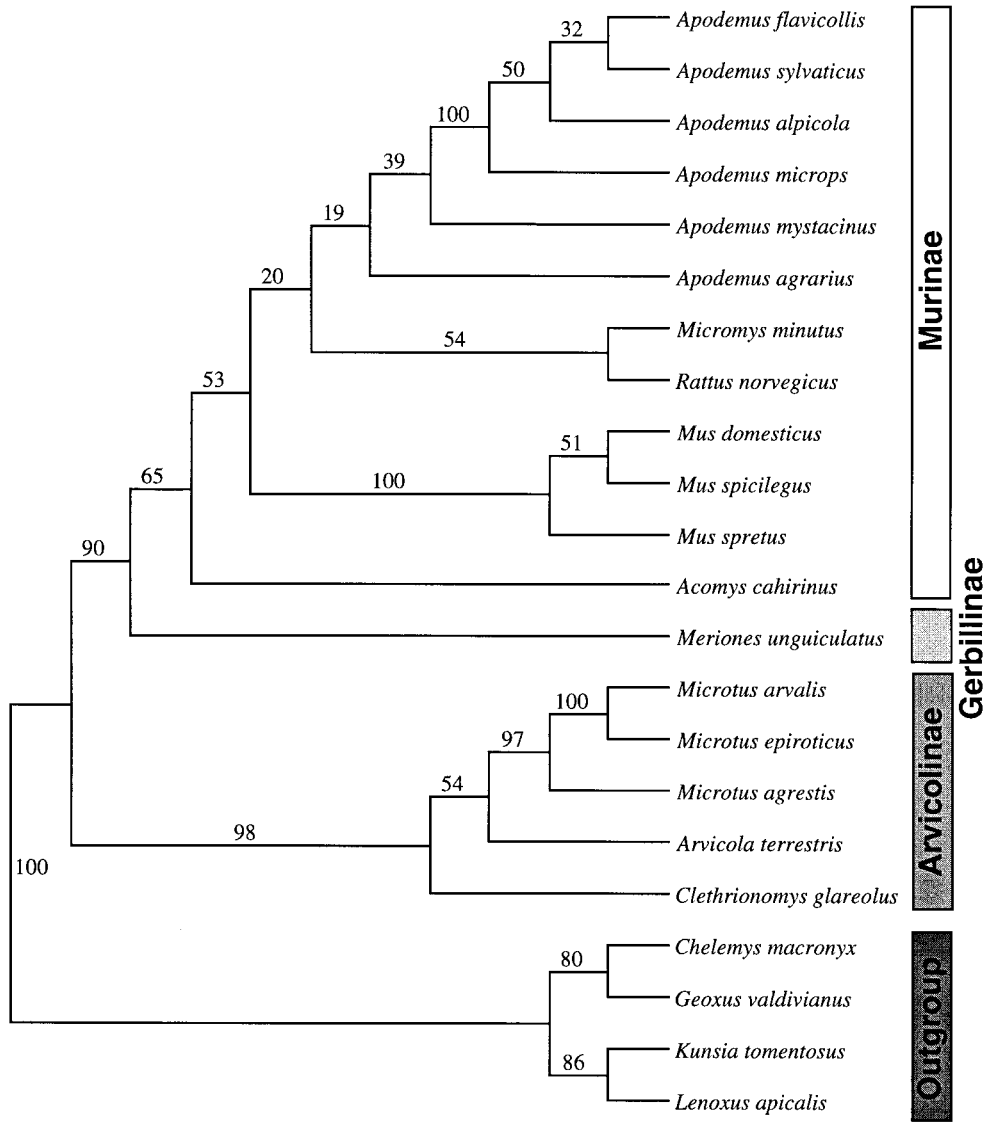

FIG. 1. Phylogenetic tree showing the relationships of the different species of the subfamilies Murinae, Arvicolinae, and Gerbillinae. It is based on a maximum-parsimony analysis of complete cytochrome *b* gene sequences. In the weighting scheme, the transitions at the third codon position and the C ↔ T substitutions for leucine at the first codon position were excluded. The numbers above branches are bootstrap values obtained in 500 replicates. The species *Chelemys macronyx, Geoxus valdivianus, Kunsia tomentosus,* and *Lenoxus apicalis* were used as outgroup.

ships among the different genera were not always clearly resolved by cytochrome *b* sequences. Among the arvicoline genera, the resolution is good, but the phylogenetic relationships of the murine genera remain partly unresolved, especially the relationships among the genera *Apodemus, Mus,* and *Rattus.* A NJ analysis based on 10 sequences of the Murinae, but only two species of the Arvicolinae as outgroup, was conducted (Fig. 4). Some of the bootstrap values within the Murinae were higher than those in the previous analyses, which included all species. The partial analysis showed that *Mus* and *Apodemus* together form the sister group to *Rattus.*

DISCUSSION

The results of our phylogenetic analysis are largely consistent with expectations based on previous studies. The distinction between the subfamily Murinae and the subfamily Arvicolinae is also supported by morphological characters, such as the different structure of their molars. Also, with DNA/DNA hybridization data and DNA sequence data of the nuclear LCAT gene, the monophyly of these subfamilies is supported (Catzeflis *et al.,* 1987; Robinson *et al.,* 1997). A close relationship of the subfamilies Murinae and Gerbillinae was also found in the study of Dubois *et al.* (1999), which is

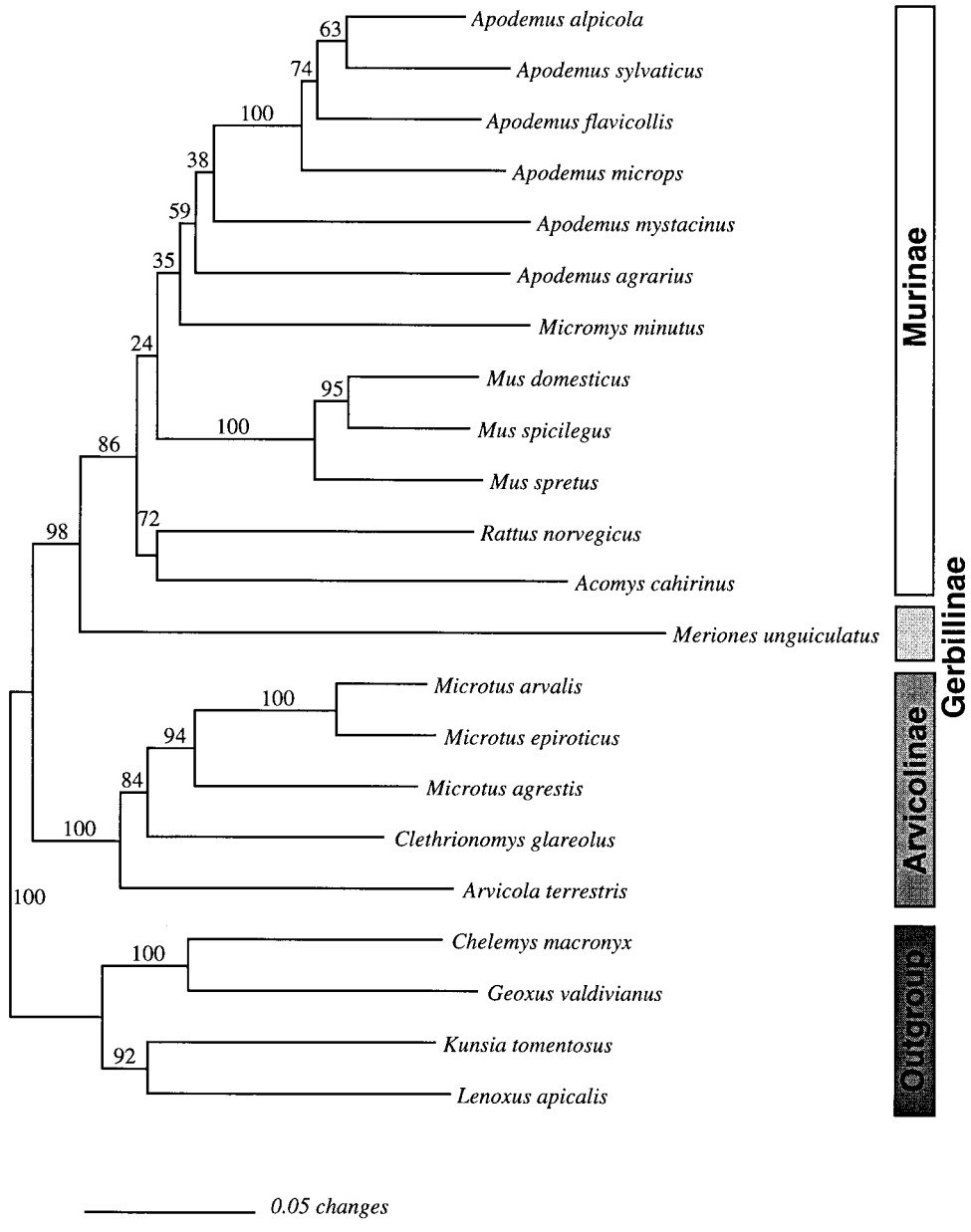

FIG. 2. The phylogenetic tree shown is a neighbor-joining reconstruction performed on a Kimura two-parameter corrected distance matrix. Numbers represent percentage bootstrap support values of 1000 replicates. Branch lengths are proportional to the genetic distance.

based on sequences of the nuclear pancreatic ribonuclease A gene.

The Subfamily Murinae

The phylogenetic relationships determined for the murine genera *Mus, Apodemus,* and *Rattus* in this study are in agreement with the results of Catzeflis *et al.* (1987) and Nikoletopoulos *et al.* (1992). Good resolution was obtained for the different *Mus* species. With all phylogenetic methods used in this study, the relationships are stable and in agreement with those inferred from previous published D-loop data (Prager *et al.,* 1996).

The genus Apodemus. With all methods used in this study (MP, NJ, and ML), the genus *Apodemus* is a monophyletic clade, but sometimes with quite low bootstrap values (e.g., MP analysis). The subgenus *Sylvaemus,* with the species *A. alpicola, A. sylvaticus, A. flavicollis,* and *A. microps,* forms a monophyletic group, which is strongly supported (bootstrap value is always 100%), and accordingly the species *A. agrarius* and *A. mystacinus* are always placed outside this group. With the NJ and ML methods, *A. alpicola* appears to be more closely related to *A. sylvaticus* than to *A. flavicollis.* With the MP method, *A. alpicola* is a sister taxon to *A. flavicollis* and *A. sylvaticus.* The KH test of these

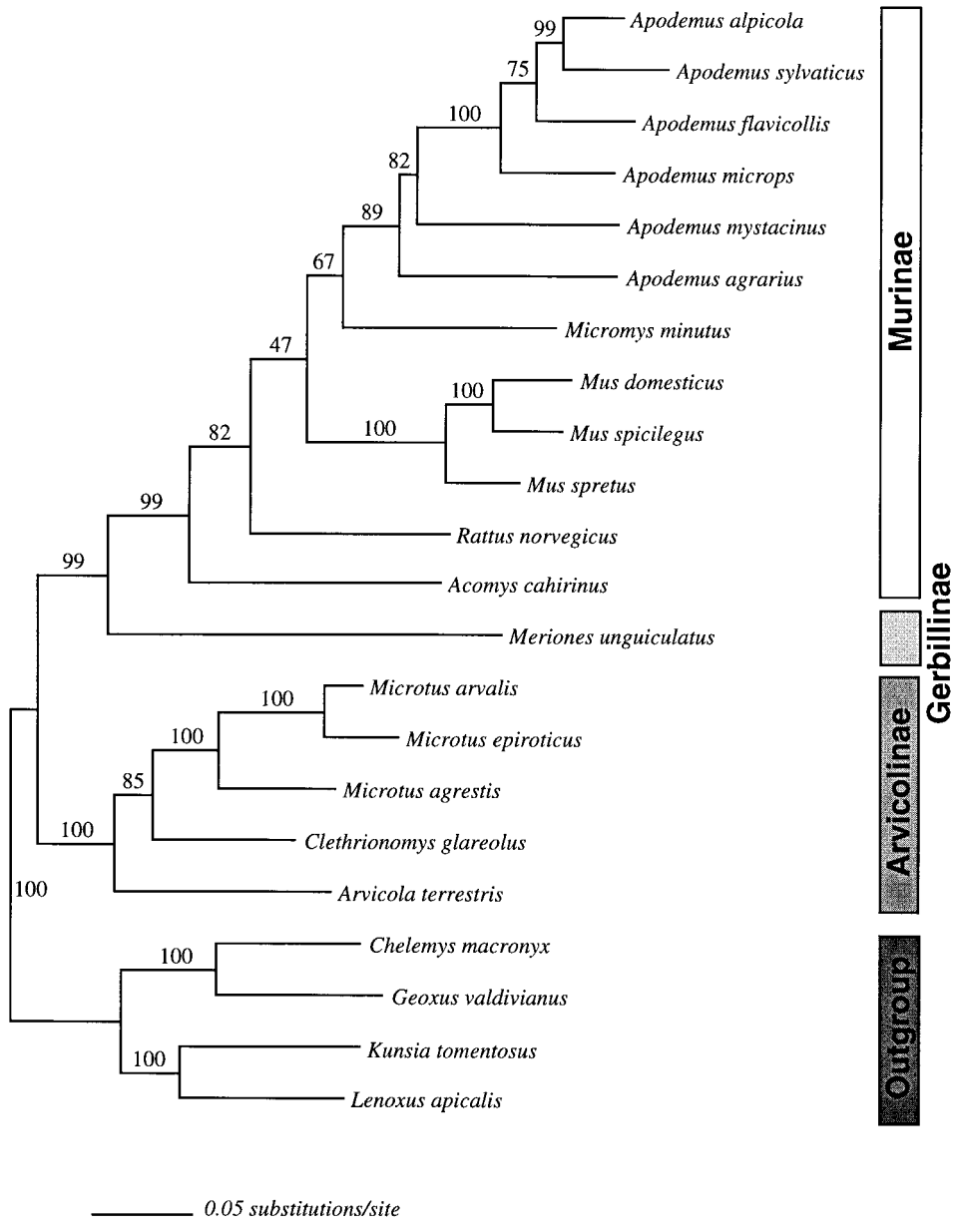

FIG. 3. Maximum-likelihood reconstruction assuming the HKY model of evolution (Hasegawa *et al.*, 1985) and a transition:transversion ratio of 1.98. The outgroup taxa are *Chelemys macronyx, Geoxus valdivianus, Kunsia tomentosus,* and *Lenoxus apicalis.* Values are quartet puzzling reliability percentages.

different alternative tree topologies was not significant (Table 2). In the allozyme study of Vogel *et al.* (1991), *A. alpicola* is also more closely related to *A. sylvaticus,* whereas in a previous study of Filipucci (1987), *A. alpicola* is placed together with *A. flavicollis.* The genetic distances among the species in the subgenus *Sylvaemus* are between 8 and 12%. The genetic distances of the subgenus *Sylvaemus* to the species *A. mystacinus* and *A. agrarius* are between 16 and 19%, which is as great as those of the *Sylvaemus* group to other murine genera. An allozyme study of Britton-Davidian *et al.* (1991) also shows a great genetic distance of *A. agrarius* to the *Sylvaemus* group, whereas *A. mystacinus* is not so extremely distant. Based on morphological characters, the genus *Apodemus* is divided into three subgenera: the subgenus *Apodemus* with *A. agrarius;* the subgenus *Sylvaemus* with *A. sylvaticus, A. flavicollis, A. alpicola,* and *A. microps;* and the subgenus *Karstomys* with *A. mystacinus* (Niethammer and Krapp, 1978). Our cytochrome *b* sequence data support the same subdivision.

The genus Acomys. For *Acomys,* different results are obtained with the MP, NJ, and ML methods. With

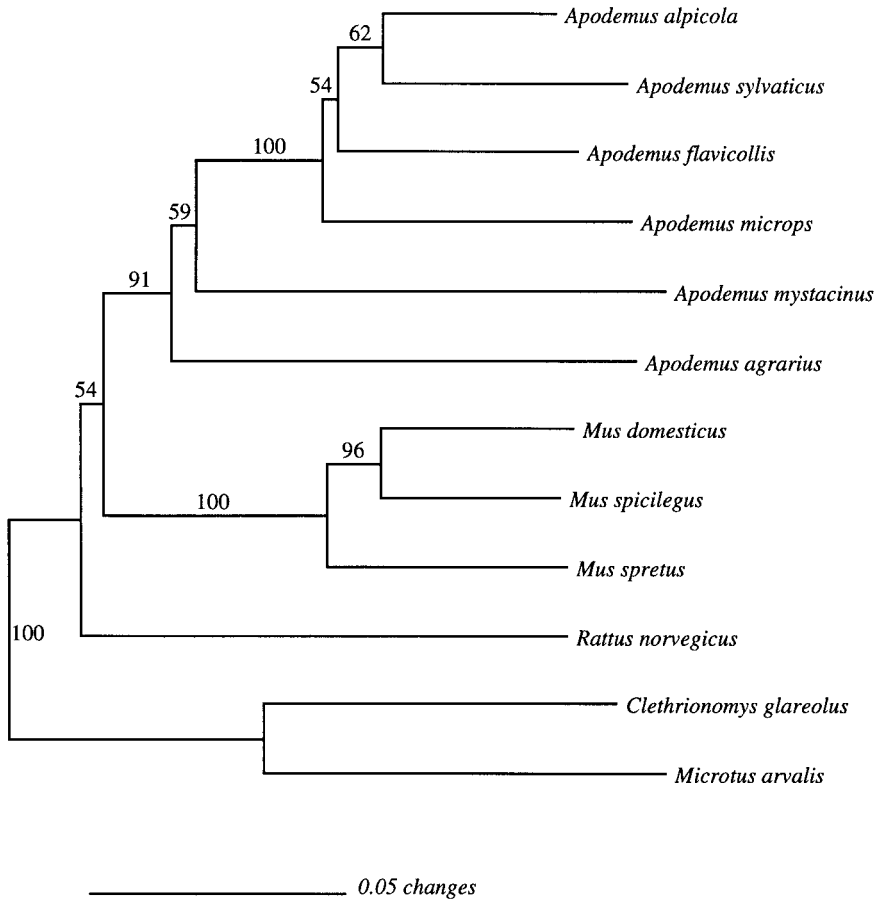

FIG. 4. The phylogenetic tree shown is a partial analysis of the Murinae with the neighbor-joining method (Kimura two-parameter model) and two species of the Arvicolinae as outgroup (*Clethrionomys glareolus* and *Microtus arvalis*). Numbers above branches are bootstrap values obtained in 1000 replicates.

maximum-parsimony and maximum-likelihood, *Acomys* is identified as sister taxon to the other genera of the Murinae (Figs. 1 and 3). In the neighbor-joining analyses, *Acomys* is placed together with *Rattus norvegicus* as a sister group to the other murine genera (Figs. 2 and 4). Unfortunately, the position of *Acomys* relative to the other examined species remains unresolved with the cytochrome *b* data. The log likelihood test shows that the difference between these alternatives is not significant at the $P < 0.05$ level (Table 2).

The determination of the phylogenetic position of the genus *Acomys* (spiny mice) has been difficult and its taxonomic position remains controversial. Based on morphological data, especially due to their similar molar structure, it was assumed that *Acomys* belongs to

TABLE 2

Log-Likelihood Ratio Tests for the Comparison of Unconstrained and Constrained Maximum-Likelihood Topologies Generated from Cytochrome *b* Sequence Data

Comparison	Log likelihood (LnL)	Difference LnL	Standard deviation	*P*
ML tree vs MP topology (*Clgl/Arte*)	−12069.31 −12072.85	−3.54	6.04	0.56
ML tree vs MP topology (*Apfl/Apal*)	−12069.31 −12082.67	−13.36	7.72	0.08
ML tree vs MP topology (*Acca/Rano*)	−12069.31 −12059.57	−9.73	10.96	0.37
ML tree vs MP topology (*Mimi/Rano*)	−12069.31 −12049.85	−19.46	12.45	0.12

the Murinae and was a close relative to *Mus* (Jacobs, 1978). However, immunological results of Sarich (1985) showed that *Acomys* is as distant from *Mus* as it is from other subfamilies of the Muridae. Chevret *et al.* (1993) examined the relationships of *Acomys* to other rodent species with DNA/DNA hybridization data and found a closer relationship of *Acomys* to the subfamily Gerbillinae (gerbils) than to the Murinae. Another indication that *Acomys* is not a member of the Murinae is the distribution of L1 repeated DNA elements. In many genera of the Murinae, there are Lx sequences, which are an ancient amplification of the L1 family, but these sequences are not found in other subfamilies, such as the Arvicolinae, Gerbillinae, and Cricetinae, and are not found in *Acomys* (Furano *et al.,* 1994). In a phylogenetic analysis based on DNA sequences of the fifth exon of the kallikrein-binding protein gene, a close relationship between *Acomys cahirinus* and *Meriones unguiculatus,* which belongs to the Gerbillinae, was found (Agulnik and Silver, 1996). In a recent study of Dubois *et al.* (1999), a subfamily level for *Acomys* and *Uranomys* (Acomyinae) was suggested based on sequences of the nuclear pancreatic ribonuclease A gene. The cytochrome *b* data also suggest an early separation of *Acomys* from the other murine genera.

The genus Micromys. The position of *Micromys* differs according to the method of analysis of our cytochrome *b* data. With the MP method, *Micromys* is placed as sister taxon to *Rattus,* but with a bootstrap value of only 54%, whereas with the NJ method, it is identified as sister taxon to *Apodemus* (BP 35%). The same result is obtained with the maximum-likelihood method, but with a higher bootstrap value of 67%. Similar to *Acomys,* the position of *Micromys* relative to the other murine genera remains unresolved with the cytochrome *b* data. A Log likelihood test showed no significant difference between the alternative tree topologies (Table 2).

Similar to *Acomys,* the phylogenetic position of *Micromys* also has been controversial. *Micromys* has the characteristic molar pattern of *Progonomys,* the presumed ancestor of the Murinae, and of the recent species of the Murinae, such as *Apodemus;* so a close relationship with this genus has been assumed (Misonne, 1969). However, other morphological features, such as the form of the skull and the sperm, show that *Micromys* differs from the other species of the subfamily Murinae. In the study of Furano *et al.* (1994), a relatively small expansion of the Lx family in *Micromys* compared to other recent murine species was found. Therefore, they assumed that this genus diverged early from the main murine lineage. With DNA sequence data of the nuclear gene LCAT, there was also no resolution regarding this matter (Robinson *et al.,* 1997).

The Subfamily Arvicolinae

With the cytochrome *b* data, different tree topologies regarding the question of the relationship of *Arvicola* and *Clethrionomys* relative to *Microtus* are recovered. With MP, *Arvicola* and *Microtus* are more closely related to each other than both are to *Clethrionomys.* With NJ and ML, *Clethrionomys* and *Microtus* are more closely related to each other and form a sister group to *Arvicola.* There is no significant difference between the alternative hypotheses (Table 2). The resolution within the genus *Microtus* is good, with high statistical values for all three analysis methods (Figs. 1–3). *M. arvalis* and *M. epiroticus* together form the sister group to *M. agrestis.*

Within the Arvicolinae, the morphology-based relationships among the genera are based on dentitional differences. Based on these structures, the genera *Microtus* and *Clethrionomys* are more closely related to each other than both are to *Arvicola* (Niethammer and Krapp, 1982). However, biochemical data (Chaline and Graf, 1988) and DNA/DNA hybridization data (Catzeflis *et al.,* 1987) suggested a closer relationship between *Microtus* and *Arvicola.* With our data, the alternative placement cannot be rejected.

Divergence Times

Based on a good fossil record, molecular clocks can be calibrated for genes. Such a molecular clock has been calibrated for cytochrome *b* (Smith and Patton, 1993). A rate of change can be calculated based on the transversions at the third codon position and the divergence of *Mus* and *Rattus.* Paleontological data suggest the split of mice and rats at 12–14 Myr (Jaeger *et al.,* 1986; Jacobs *et al.,* 1989, 1990). If a divergence time of 12 Myr is accepted, then the rate of change is 1.6% per Myr. The calculated divergence times for the murine genera are between 9 and 13 mya and for the arvicoline genera between 7 and 8 mya. This period of time is rather short and could be an indication that the origin of the different murine and arvicoline lineages was rapid and that a fast radiation of new lineages took place.

The degree of resolution for the reconstruction of phylogenetic relationships among the examined taxa was surprisingly variable. Among some species, such as *Mus* or *Microtus,* the relationships are stable and all phylogenetic methods support them with high bootstrap values. Generally, the resolution of relationships among different species within one genus was quite good. In other studies, the resolution among closely related species with cytochrome *b* data is good (Barome *et al.,* 1998; Ducroz *et al.,* 1998). On the other hand, there are species, such as *Acomys* and *Micromys,* which cannot be assigned a firm phylogenetic position with these data and the recovered topologies are different with different phylogenetic methods. Among the Murinae, the resolution is weak above the species level. In

other studies also, only weak resolution at the genus or family level was found (Lessa and Cook, 1998; Lara *et al.*, 1996; Verheyen *et al.*, 1995). One explanation is that not enough phylogenetic information is available due to saturation, but the tree length distributions are significantly skewed, indicating high phylogenetic information content in the data. Another possible explanation for the weak resolution above the species level is that it is caused by the rapid evolution of different lineages. Hence, an adaptive radiation may be the biological reason for the weak resolution among the genera of European muroid rodents.

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