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BASAL CLADES AND MOLECULAR SYSTEMATICS OF HETEROMYID RODENTS

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The New World rodent family Heteromyidae shows a marvelous array of ecomorphological types, from bipedal, arid-adapted forms to scansorial, tropical-adapted forms. Although recent studies have resolved most of the phylogenetic relationships among heteromyids at the shallower taxonomic levels, fundamental questions at the deeper taxonomic levels remain unresolved. This study relies on DNA sequence information from 3 relatively slowly evolving mitochondrial genes, cytochrome *c* oxidase subunit I, 12S, and 16S, to examine basal patterns of phylogenesis in the Heteromyidae. Because slowly evolving mitochondrial genes evolve and coalesce more rapidly than most nuclear genes, they may be superior to nuclear genes for resolving short, basal branches. Our molecular data (2,381 base pairs for the 3-gene data set) affirm the monophyly of the family and resolve the major basal clades in the family. Alternative phylogenetic hypotheses of subfamilial relationships are examined statistically and the Perognathinae and Heteromyinae are found to represent sister clades relative to the Dipodomyinae. The 3 traditional subfamilial groupings are supported; the controversial placement of *Microdipodops* as a sister clade to *Dipodomys* in the Dipodomyinae is affirmed, *Perognathus* and *Chaetodipus* are distinct sister clades within the Perognathinae, and species of *Liomys* and *Heteromys* form the resolved clade Heteromyinae. However, *Liomys* is found to be paraphyletic relative to *Heteromys* and, given that this finding corroborates earlier studies, we present a formal taxonomy of *Heteromys* wherein we place *Liomys* in synonymy. Semiparametric and parametric methods are used to estimate divergence times from our molecular data and a chronogram of the Heteromyidae, calibrated by the oldest known fossils of *Dipodomys* and *Perognathus*, is presented. Our time estimates reveal subfamilial differentiation in the early Miocene (22.3–21.8 million years ago) and pose testable times of divergence for the basal heteromyid nodes. With the basal heteromyid clades resolved and cladogenic events positioned in a time framework, we review the major geological and paleoecological events of the Oligocene and Miocene associated with the early historical biogeography of the family.

Key words: *Chaetodipus*, *Dipodomys*, Heteromyidae, *Heteromys*, historical biogeography, *Liomys*, *Microdipodops*, mitochondrial DNA, *Perognathus*, phylogenetics

The New World rodent family Heteromyidae shows a remarkable array of morphologically and ecologically diverse forms. The family includes the scansorial pocket mice (*Perognathus* and *Chaetodipus*) and spiny pocket mice (*Heteromys* and *Liomys*), which show the generalized rodent *Bauplan*. In addition to these scansorial body types, macroevolutionary diversification has produced novel morphologies: the quadrupedal, ricochetal kangaroo mice (*Microdipodops*) and the bipedal, ricochetal kangaroo rats (*Dipodomys*—Hafner 1993; Hafner and Hafner 1983). Ecologically, heteromyids inhabit a broad spectrum of terrestrial habitats ranging from tropical evergreen forests to stabilized sand dunes in the deserts. Not surprisingly, heteromyid rodents have captured the attention of biologists and paleontologists who view them as

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model organisms for studies of adaptive radiation. So attractive are the heteromyid rodents as study organisms that an entire edited volume (Genoways and Brown 1993) was devoted to a review and synthesis of information concerning their biology.

Inferences drawn from comparative and integrative biological studies rely on our understanding of the evolutionary history of that lineage. If we wish to study the morphological, ecological, physiological, or behavioral modifications that accompanied a group's adaptive radiation, it must be kept in mind that the strength of our interpretations rests on the accuracy of the phylogenetic hypothesis. This is particularly important in a lineage like the Heteromyidae that is rife with evolutionary parallelism (Hafner and Hafner 1983; Wood 1935). Despite the popularity of the Heteromyidae as a biological model, major uncertainties remain today concerning basal clades and their relationships within the family.

Extant genera of heteromyids are relatively ancient, with a fossil record extending back to the early Miocene (Wahlert 1993). Alexander and Riddle (2005) and Rogers and Vance (2005) have resolved in detail specific relationships among most terminal taxa of the family using DNA sequence data. Riddle et al. (2000a, 2000b) have correlated phyletic divergence of the arid-adapted genera *Chaetodipus* and *Dipodomys* with Pliocene–Pleistocene geological events associated with the North American aridlands. Resolution of the timing and patterns of deeper relationships within the family may allow placement of this earlier diversification in the context of older (i.e., Oligocene and Miocene) geological and paleoclimatic events. Our study uses mitochondrial DNA sequence information in an attempt to obtain phylogenetic resolution of the major lineages within the Heteromyidae and to make inferences regarding the timing of cladogenic events in the context of historical biogeography.

Monophyly of the Heteromyidae.—The evolutionary history of the Heteromyidae is intimately linked with that of the sister family Geomyidae (pocket gophers). Extant geomyoid rodents are characterized by bilophodont cheek teeth and fur-lined cheek pouches that are external to the buccal cavity. Although extant heteromyids and geomyids are distinguished readily using morphological characters, rodent systematists encounter difficulty assigning fossils of extinct geomyoids to family because of extensive parallelism in the group and a reliance on phenotypic similarity without consideration of character-state polarity (Wahlert 1985, 1993; Wood 1935).

Documentation of reciprocal monophyly of the Heteromyidae and the Geomyidae using phylogenetic systematic methods was 1st provided by the biochemical investigation of Hafner (1982). Soon after, evolutionary morphologists identified a series of synapomorphic traits that distinguish the Heteromyidae. In his study of cranial features, Wahlert (1985) recognized 7 synapomorphic characters for the Heteromyidae, including the unique rostral perforation (large perforation in the wall of the rostrum anterior to the infraorbital canal), a character noticed by early rodent systematists. To this list, Ryan (1989) contributed an additional 7 shared-derived characters for the family from his comparative myological study. There is now broad recognition that extant members of the 2 families represent monophyletic units within the superfamily Geomyoidea (Alexander and Riddle 2005; Hafner 1982, 1993; Hafner and Hafner 1983; Reeder 1956; Ryan 1989; Wahlert 1985, 1993; Wood 1935).

Despite near-universal agreement that the Heteromyidae is a monophyletic group, there are lingering challenges to this hypothesis that stem from 3 sources: paleontological studies that relied primarily on similarity in dental morphology (Lindsay 1972; Rensberger 1971; Shotwell 1967; Wilson 1936); a cladistic analysis of carotid circulation (Brylski 1990); and a molecular phylogenetic analysis of rodents using nuclear genes (DeBry 2003). However, more-recent paleontological studies of geomyoid rodents (e.g., Wahlert 1985, 1991, 1993; Wahlert and Souza 1988) emphasized the importance of recognizing shared-derived characters in studies of dental and cranial morphology and demonstrated that the Heteromyidae and Geomyidae are separate, monophyletic taxa. Brylski (1990) relied on a single character (stapedial artery absent in adults) to suggest a possible sister relationship between spiny pocket mice and pocket gophers, but, in consideration of the recent molecular and morphological data that argue against such an interpretation, it is more likely that the stapedial artery was lost independently in these groups (see Wahlert 1985). Finally, DeBry (2003) suggested that pocket mice are more closely related to pocket gophers than to kangaroo rats. However, only 3 geomyoid taxa (including 2 heteromyids) were used in this analysis and DeBry (2003:614) acknowledged much conflict existed in the separate analyses (e.g., some analyses supported heteromyid monophyly) and concluded ''Expanded taxonomic sampling is clearly needed to sort out the relationships within Geomyoidea.'' Although monophyly of the Heteromyidae seems to be documented well based on a wide variety of character sets, we reexamine this issue using data from relatively slowly evolving mitochondrial DNA sequences.

Supraspecific evolutionary relationships.—Although monophyly of the Heteromyidae is accepted by most authorities, ambiguities still persist regarding evolutionary and taxonomic relationships above the species level. The current taxonomic arrangement of the Heteromyidae includes 3 subfamilies and 6 genera (Hafner and Hafner 1983; Patton 2005; Williams et al. 1993): Perognathinae (*Perognathus* and *Chaetodipus*), Heteromyinae (*Heteromys* and *Liomys*), and Dipodomyinae (*Dipodomys* and *Microdipodops*). Taxonomic difficulties exist at all supraspecific levels, including recognition of genera, uncertainties regarding subfamilial composition, and ambiguous relationships among the subfamilies.

At the generic level, taxonomic interest has focused on the status of 2 genera: *Chaetodipus* and *Liomys*. *Chaetodipus*, although recognized as a subgenus of *Perognathus* for nearly 100 years, was elevated to full generic stature only 2 decades ago (Hafner and Hafner 1983). Additionally, accumulating evidence seems to indicate that the venerable genus *Liomys* (described over a century ago) may be paraphyletic relative to *Heteromys* (Anderson et al. 2006; Rogers 1990; Rogers and Vance 2005).

Subfamilial classification of the genera seems to be rather stable, with 2 exceptions. First, much controversy enveloped the subfamilial placement of *Microdipodops* during much of the 20th century (for review see Hafner 1978; Lindsay 1972;

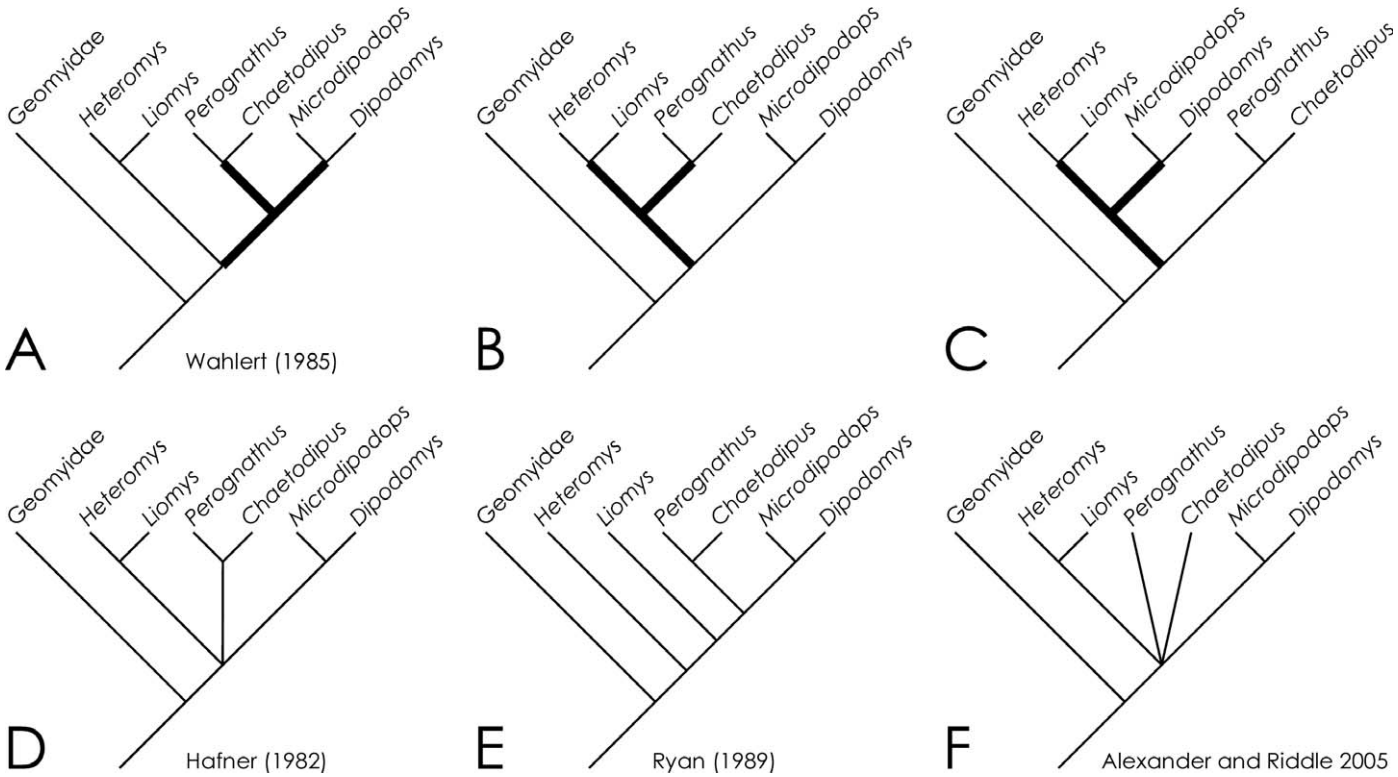

Fig. 1.—Alternative phylogenetic hypotheses depicting relationships among the 3 subfamilies in the Heteromyidae: Heteromyinae (includes *Heteromys* and *Liomys*), Perognathinae (includes *Perognathus* and *Chaetodipus*), and Dipodomyinae (includes *Microdipodops* and *Dipodomys*). A–C) The 3 possible topologies reflecting the pairwise permutations of the subfamilies. A, D–F) Dendrograms expressing the results of recent phylogenetic studies of the Heteromyidae (see text for discussion). All trees use the sister taxon Geomyidae as the outgroup.

Reeder 1956; Setzer 1949; Wood 1935) but more recent studies supported the placement by Hafner and Hafner (1983) of *Microdipodops* in the Dipodomyinae (Alexander and Riddle 2005; Hafner 1993; Ryan 1989; Wahlert 1985). A 2nd and more substantive challenge to the current allocation of genera to subfamilies by Alexander and Riddle (2005) used 2 rapidly evolving mitochondrial genes, cytochrome *c* oxidase subunit 3 and cytochrome *b*. Alexander and Riddle (2005:376) showed ''no evidence for a subfamily Perognathinae containing *Perognathus* and *Chaetodipus*.''

Lastly, uncertainty clouds our understanding of the relationships among the subfamilies (Fig. 1). Of the 3 possible pairwise permutations of the subfamilial arrangements (Figs. 1A–C), 1 topology, the alignment of the Perognathinae with the Dipodomyinae as sister taxa relative to the Heteromyinae (Fig. 1A), is supported by cladistic analyses (Wahlert 1985, 1991). Hafner and Hafner (1983) also suggested this subfamilial arrangement but, given that the heteromyines were used as the outgroup in their restricted cladistic study, their interpretations are moot on this question. Hafner (1982) found support for the recognition of the 3 subfamilies but was unable to resolve the interrelationships among them (Fig. 1D). Ryan's (1989) cladistic study of myological characters supported a clade uniting the Dipodomyinae with the Perognathinae but found no evidence for the currently recognized Heteromyinae (Fig. 1E). Finally, the molecular analysis of Alexander and Riddle (2005) recognized the Dipodomyinae and the Heteromyinae as separate clades, but did not support the currently recognized Perognathinae (discussed above) nor did it resolve basal relationships among the subfamilies (Fig. 1F).

Reeder's (1956:3) statement that ''Subfamily designations have been used by previous authors and at best are equivocal'' unfortunately still rings true one-half century later. It is the intent of our study to use molecular phylogenetic techniques to identify basal clades and hypothesize supraspecific taxonomic groupings within extant members of the Heteromyidae.

Why use slowly evolving mitochondrial genes?—Because nuclear-encoded genes are known to evolve more slowly than mitochondrial genes, it follows that nuclear gene sequences should be better for resolving deep (older) phylogenetic relationships. This is because rapidly evolving mitochondrial genes tend to saturate too quickly to provide resolution at deeper nodes. However, this advantage of nuclear genes over mitochondrial genes may become a disadvantage when attempting to resolve deep branches that are also short. In these circumstances, there may be too little time between cladogenic events to accumulate a sufficient number of nucleotide substitutions in a nuclear gene to resolve the intervening branch (Lanyon 1988). This phenomenon may explain, in part, why DeBry's (2003) study of rodent relationships based on 3 nuclear-encoded genes produced conflicting results from different partitions of the sequence data: 1 partition of the data linked kangaroo rats with pocket gophers (heteromyid paraphyly), 2 different partitions linked pocket

mice with pocket gophers (a different kind of paraphyly), 3 partitions linked kangaroo rats with pocket mice (monophyly), and 1 partition lacked sufficient variation for analysis.

Although the rapid rate of nucleotide substitution in mitochondrial genes may be an advantage in certain circumstances, an accurately resolved mitochondrial tree (gene tree) may not be congruent with the species tree because of lineage sorting of ancestral polymorphisms. However, because of their relatively rapid rate of coalescence, mitochondrial genes may have a substantially higher probability (compared to nuclear genes) of accurately tracking species trees when short branches are involved (Moore 1995). Thus, attempts to resolve basal branches in phylogenetic trees, which often involve branches that are short relative to time since bifurcation, require examination of sequences that change rapidly enough to provide resolution, slowly enough to avoid saturation, and coalesce quickly enough to track the species tree. Slowly evolving mitochondrial genes would seem to fulfill these requirements.

We propose to enhance the resolution of clades at deeper taxonomic levels in the Heteromyidae by examining 3 mitochondrial genes that evolve more slowly than most other mitochondrial genes yet have a coalescence time that is shorter than most nuclear-encoded genes: the cytochrome *c* oxidase subunit I (*COI*) gene and the 12S and 16S ribosomal RNA genes (Barros et al. 2003; Ducroz et al. 2001; Ferris et al. 1983; Frye and Hedges 1995; Johnson and O'Brien 1997; Pesole et al. 1999). Each of these genes, especially when used in combination with others, has proven phylogenetically informative for several mammalian groups at both higher and lower taxonomic levels (Delpero et al. 2001; Gates et al. 1992; Hafner et al. 2005, 2006; Kuznetsova et al. 2002; Ledje and Arnason 1996; Olson et al. 2005; Ruedas and Morales 2005; Spradling et al. 2004; Steppan et al. 2005). Given the relatively rapid coalescence rate of mitochondrial genes, it is unlikely that lineage sorting of ancestral polymorphisms will cause the mitochondrial tree to differ from the species tree for basal heteromyid relationships (Moore 1995). Nevertheless, any phylogeny based solely on mitochondrial genes should be corroborated by evidence from nuclear-encoded characters.

Materials and Methods

Taxon sampling and specimens examined.—The studies of Alexander and Riddle (2005) and Rogers (1990) were used as working templates for the selection of species sampled. To achieve a manageable number of taxa and yet survey the cladistic diversity of the family, we sampled species from all of the divergent clades and principal species groupings identified in those studies. As a result, 34 specimens representing 34 species and all 6 currently recognized genera of Heteromyidae were used in the mitochondrial DNA analyses (Appendix I). All specimens were captured in the wild using standard trapping methods and treated in a humane manner as approved by the Occidental College and Louisiana State University Institutional Animal Care and Use Committees and by following guidelines of the American Society of Mammalogists (Gannon et al. 2007). Outgroup taxa consisted of 4 pocket gopher species, *Cratogeomys perotensis*, *Orthogeomys grandis*, *Pappogeomys bulleri*, and *Zygogeomys trichopus* (Appendix I). Seven sequences were obtained from GenBank: the *COI* gene from *C. perotensis*, *O. grandis*, *P. bulleri*, and *Z. trichopus* (GenBank accession numbers AY649478, AY331082, AY331084, and AY331087, respectively) and the 16S gene from *Dipodomys microps* (DQ422887), *Microdipodops megacephalus* (DQ422891), and *M. pallidus* (DQ534288). All other sequences were generated in this study.

Mitochondrial DNA amplification and sequencing.—Genomic DNA was isolated from liver or kidney tissue using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California). The protocol recommended by QIAGEN was used; slight adjustments to this protocol are available upon request. Extracted DNA was stored at −70°C.

Amplification by polymerase chain reaction and sequencing of the mitochondrial *COI* (1,551 base pairs [bp]) were performed using the following primers: *COI*-5285f, *COI*-6929r (Spradling et al. 2004), *MCo*-173f (5′-TAT TAG GNG AYG AYC ARA T-3′), *MCo*-1480r (5′-GCT TCT CAR ATT ATR WAR ATT AT-3′), and *MCo*-1345r (5′-TGT TGW GGG AAR AAD GTT A-3′). Polymerase chain reaction amplifications using primers *COI*-5285f with *COI*-6929r were performed in 50-μl reaction volumes following the protocol of Hafner et al. (2005). If this primer pair failed to yield acceptable sequences, reactions were performed using primers *MCo*-173f with *MCo*-1480r. Amplification protocol using these primers followed Hafner et al. (2005), except for an initial annealing temperature of 50°C.

Polymerase chain reaction amplification and sequencing of a portion of the mitochondrial 12S (412 bp) and 16S (582 bp) rRNA genes were performed using the 12SaL and 12SbH and 16SarL and 16SbrH primers, respectively (Hillis et al. 1996). Polymerase chain reaction amplifications for 12S and 16S were completed in 50-μl reaction volumes as in Hafner et al. (2006). Amplification protocol for the 12S gene required an initial denaturation stage of 94°C for 2 min, followed by 30 polymerase chain reaction cycles of 94°C (1 min 30 s), 45°C (2 min), and 72°C (1 min 30 s). Amplification protocol for the 16S gene was performed as described in Hafner et al. (2006).

Before sequencing, amplified *COI*, 12S, and 16S products were purified using the QIAquick PCR Purification Kit protocol (QIAGEN). Amplified products were sequenced in both directions at the DNA Sequencing Facility at California State University, Northridge, and the Museum of Natural Science, Louisiana State University. Sequencing reactions were performed in volumes of 10 μl following the methods of Hafner et al. (2005), although an alternative 10-μl reaction, including 1.6 μl of BigDye (Applied Biosystems, Inc. [ABI], Perkin-Elmer Corporation, Foster City, California), 0.32 μl of 10-μM primer, 4.08 μl of double-distilled H_2O, 2 μl of 5X ABI sequencing buffer, and 2 μl of amplification product, also was performed. Samples were sequenced, purified, and electrophoresed as described in Hafner et al. (2005).

Mitochondrial sequences were edited using Sequencher Version 4.1 software (Gene Codes Corporation, Ann Arbor, Michigan) and the *COI* gene was then aligned using Se-Al

version 2.0a11 (http://evolve.zps.ox.ac.uk/Se-Al/Se-Al.html). Similar to what Spradling et al. (2004) reported for pocket gophers, there appears to have been several insertion–deletion (indel) events at the end of the *COI* gene. The stop codon and up to 21 bp of sequence upstream of the stop codon were eliminated before phylogenetic analysis so that each sequence terminated with the same set of conserved amino acids. For the rRNA genes, sequences were aligned by eye with reference to the secondary structure models of Springer and Douzery (1996) and Olson et al. (2005) for 12S, and Burk et al. (2002) for 16S. Regions that could not be aligned confidently (primarily regions in loops) were identified as ambiguously aligned sites. Only the results excluding ambiguously aligned sites are presented here because inclusion or exclusion of these sites yielded similar and nonconflicting results (data available upon request). All sequences were submitted to GenBank (GenBank accession numbers EF156834–EF156867 for *COI*, EF156761–EF156798 for 12S, and EF156799–EF156833 for 16S).

Data analysis.—Phylogenetic inference can be affected by base composition heterogeneity, especially for older divergences (Barker and Lanyon 2000; Galtier and Gouy 1998; Jansa and Weksler 2004; Lockhart et al. 1994). Therefore, base composition bias was evaluated for each gene (and codon positions for the *COI* gene) across all taxa (Jansa et al. 2006b; Jansa and Weksler 2004). Departures from average base composition were determined for each taxon using chi-square goodness-of-fit tests in PAUP* (Swofford 2002). Transitions and transversions (calculated using MEGA [Kumar et al. 2004]) were plotted against uncorrected p-distances (Barker and Lanyon 2000) to assess saturation in the *COI* gene. An incongruence length difference test (Farris et al. 1994) using a heuristic search with 100 random addition replicates (implemented as the partition homogeneity test in PAUP*—Swofford 2002) was used to determine if significant conflict existed between *COI* codon positions. Phylogenetic signal of each gene was assessed with the *g*-statistic according to the procedures of Hillis and Huelsenbeck (1992), and sequence divergence among taxa was calculated using uncorrected *p*-distances in PAUP*.

Phylogenetic congruence of all possible combinations of the 3 mitochondrial genes *COI*, 12S, and 16S was evaluated using the partition homogeneity test (Farris et al. 1994) in PAUP* (Swofford 2002). One thousand partition replicates were analyzed by maximum parsimony (heuristic search option and random addition replicates of tree-bisection-reconnection branch swapping). The partition homogeneity test did not detect significant heterogeneity among the 3 mitochondrial genes ($P = 0.7$). Therefore, analyses focused on the combined, 3-gene data set throughout this study.

Phylogenetic analyses were performed using maximum-parsimony, maximum-likelihood, and Bayesian approaches. Equally weighted maximum-parsimony searches were performed with 100 random taxon addition replicates and tree-bisection-reconnection branch swapping (PAUP*—Swofford 2002). Nonparametic bootstrap analyses (1,000 pseudoreplicates and 10 random sequence additions) were performed to assess nodal support (Felsenstein 1985).

To generate the best maximum-likelihood tree, Modeltest (version 3.6—Posada and Crandall 1998) was used to select the best model of evolution for the data according to hierarchical ratio tests and the Akaike information criterion (Huelsenbeck and Rannala 1997; Posada and Buckley 2004). Only the results of the hierarchical ratio tests are presented here because both approaches selected similar models and phylogenetic analysis using these models of evolution yielded the same topology. The general time-reversible model including among-site rate variation and invariable sites (GTR+I+Γ—Gu et al. 1995; Tavaré 1986; Yang 1994) was chosen as the best model of evolution according to hierarchical likelihood-ratio tests for the 3-gene data set. A full heuristic maximum-likelihood search was conducted by using the successive-approximations approach with the preferred model in PAUP* (Swofford 2002). A full heuristic bootstrap also was performed (200 pseudoreplicates) using the preferred model.

Partitioned and nonpartitioned Bayesian phylogenetic analyses were performed on the 3-gene data set using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Partitioned Bayesian analyses were performed to avoid potential systematic error, parameter mismodeling, and biased posterior probability estimates (Brandley et al. 2005; Castoe et al. 2004; Castoe and Parkinson 2006; Lemmon and Moriarty 2004; Mueller et al. 2004). Data partitions examined each gene separately, accounted for rDNA secondary structure (stems and loops were examined separately), and accounted for both *COI* codon positions in addition to rDNA secondary structure. Unique models, gamma shapes, and rates were allowed for each partition. The GTR+I+Γ model was used in all analyses and model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. Bayesian analyses were initiated with random starting trees, run for 10 million generations with 4 incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo—Huelsenbeck and Ronquist 2001), and sampled at intervals of 1,000 generations. Two independent Bayesian analyses were run to avoid entrapment on local optima, and log-likelihood scores were compared for convergence (Huelsenbeck and Bollback 2001; Leaché and Reeder 2002). Stationarity was assessed and all burn-in points were discarded as in Hafner et al. (2005). The retained equilibrium samples were used to generate a 50% majority-rule consensus tree with the percentage of samples recovering any particular clade representing that clade's posterior probability (Huelsenbeck and Ronquist 2001).

Alternative phylogenetic hypotheses of subfamily relationships (Figs. 1A–C) were compared statistically using the Kishino–Hasegawa, Shimodaira–Hasegawa, and likelihood-ratio tests as implemented in PAUP* (maximum-parsimony and maximum-likelihood analyses using RELL optimization and 1,000 bootstrap replicates—Goldman et al. 2000; Shimodaira and Hasegawa 1999). However, these methods have been criticized for not being appropriate for combined or partitioned analyses (Weins et al. 2005). Therefore, suboptimal trees from the Bayesian nonpartitioned and partitioned analyses also were

examined to assess alternative phylogenetic hypotheses. The frequency of the Markov chain Monte Carlo trees in agreement with an alternative hypothesis equals the probability of that alternative hypothesis being correct (Ihlen and Ekman 2002 and references therein). The probability of trees agreeing with alternative subfamily hypotheses was calculated by applying constraint-based filter trees implemented in PAUP* (Hoofer and Baker 2006; Ihlen and Ekman 2002).

Estimates of divergence dates and branch lengths.—Before estimating divergence times, we used likelihood-ratio tests to determine if our sequence data departed significantly from clocklike behavior. We tested the assumption of rate uniformity of DNA substitutions through time within the Heteromyidae by comparing the maximum-likelihood topology obtained with and without a molecular clock constraint using PAUP* (Swofford 2002).

Semiparametric and parametric methods were used to estimate divergence dates among lineages within the Heteromyidae (both methods relax the assumption of a molecular clock). The topology resulting from the maximum-likelihood analysis of the combined 3-gene data set was used in all dating analyses. The semiparametric penalized-likelihood method (Sanderson 2002) was implemented in the program r8s (Sanderson 2003). A cross-validation procedure was used in r8s to determine the optimal size of the penalty function using the truncated Newton algorithm (Sanderson 2002) with 5 random restarts and perturbations. Confidence intervals evaluating the sampling error of estimated divergence times were calculated by generating 100 bootstrap pseudoreplicates using the seqboot program in PHYLIP (Felsenstein 1993). For each pseudoreplicate, branch lengths were estimated on topologically identical trees. Penalized-likelihood analyses were then performed on each data set and the command ''profile'' was used to summarize the node statistics (mean and *SD*).

Unlike penalized-likelihood, the parametric Bayesian approach of Thorne and Kishino (2002) is able to accommodate multiple loci with variable evolutionary characteristics. For the parametric Bayesian analysis, the molecular data from the 3 genes (12S, 16S, and *COI*) were treated as separate data partitions. Model parameters for the F84+Γ model were estimated for each gene partition using the *baseml* program in PAML version 3.14 (Yang 1997) and these parameters were then used in the program *estbranches* (Kishino et al. 2001; Thorne and Kishino 2002) to estimate the maximum-likelihood and the variance–covariance matrix (also using the F84+Γ model) of the branch-length estimates for each gene. Lastly, the program *multidivtime* (Kishino et al. 2001; Thorne and Kishino 2002), utilizing the output files from *estbranches* and implementing Markov chain Monte Carlo sampling, was used to estimate prior and posterior distribution of both the substitution rates for each gene partition and the estimated divergence time for each node. The prior assumption for the mean and *SD* of the time of the ingroup root node (rttm) was set to 3.0 time units, where 1 time unit represents 10 million years. This time estimate (30 million years ago [mya]) for the ingroup root node was obtained from paleontological estimates of the timing of the split between the Heteromyidae and the Geomyidae (Fahlbusch 1985; Wahlert 1993). The mean and *SD* for the prior distribution of the rate of evolution at the ingroup node (rtrate and rtratesd) was determined following the procedure of Jansa et al. (2006a). The Markov chain was initialized by randomly selecting the initial parameter value and each chain was sampled every 100 cycles for 10^6 generations with a burn-in of 10^5 cycles. Analyses were performed twice to ensure stationarity.

The earliest fossils of extant heteromyid genera were used as calibration points for the molecular dating analyses (see Wahlert 1993 and included references). Only the fossil records for *Perognathus* and *Dipodomys* were judged to be adequate for use here. The oldest fossils for *Perognathus* and *Dipodomys* date to the Hemingfordian and the Barstovian North American Land Mammal ''Ages,'' respectively. The paleontological literature does not distinguish between *Perognathus* and *Chaetodipus* (see Wahlert 1993), but the oldest *Perognathus* sensu lato is a fossil from the uppermost John Day Formation, Oregon (James 1963), estimated to be 22–20 mya (T. Fremd, pers. comm., 2006). Hence, 22–20 mya is used to calibrate our molecular time analyses for the perognathine node. Fossil dates of 15.9–12.5 mya (the Barstovian—Prothero 1998) are associated with the oldest fossil *Dipodomys* (Reeder 1956) and were used for calibration of the divergence of the dipodomyine node. Our rationale for calibrating at the subfamilial nodes with these fossils is that our method provides more conservative estimates of the oldest ages for the *Dipodomys*, *Perognathus*, and *Chaetodipus* clades than would be achieved if calibrations were set at the generic nodes.

Possible differences in branch lengths (relative rates of nucleotide substitution) across the major heteromyid clades were evaluated using Kruskal–Wallis and Mann–Whitney *U*-tests to examine heterogeneity among means for patristic (i.e., phylogenetic) distance data. Patristic distances (using the 3-gene data set) between all 34 heteromyid taxa and an outgroup (*Zygogeomys*) were calculated 2 ways: distance based on absolute number of differences (produced from a neighbor-joining analysis constrained with the best maximum-likelihood tree topology and using absolute number of differences); and patristic distance data based on our best maximum-likelihood model (GTR+I+Γ model of evolution; genetic data were treated as noncoding). Each of the 3 subfamilies was treated as a group and means for the patristic distances were determined. Tests were conducted to evaluate mean branch-length heterogeneity among the 3 heteromyid subfamilies, between any 2 subfamilies, and between genera within the Dipodomyinae and the Perognathinae.

Results

Sequence characteristics.—None of the heteromyid taxa showed a significant departure from expected base composition for 12S, 16S, or *COI* codon positions ($\chi^2 < 17.18$, $d.f. = 3$). Following the methods of Barker and Lanyon (2000), a plot of uncorrected *p*-distance versus transitions showed no evidence for saturation for 12S, 16S, or the 1st and 2nd codon positions of *COI*. However, saturation was evident for 3rd codon

positions of *COI*. According to the *g*-statistics of Hillis and Huelsenbeck (1992), all 3 genes exhibited high levels of phylogenetic signal (and thereby nonrandom structure; $g_1 = -0.46$, $P < 0.01$). The incongruence length difference test revealed no significant conflict between codon positions of the *COI* gene ($P = 1$), therefore all codon positions were combined in subsequent analyses.

Regardless of the gene examined, sequence divergence values between the subfamilies were roughly the same (Table 1). However, sequence divergence between *Perognathus* and *Chaetodipus* appeared to be somewhat larger than divergences between the major clades of the other 2 subfamilies (Table 1). Sequence divergence within and among genera and subfamilies is highest for the *COI* gene, and noticeably higher for 16S and 12S when ambiguously aligned sites were included (data available upon request).

Mitochondrial DNA analysis.—Of the 2,381 bp examined for the 3-gene data set, 834 bp were potentially parsimony informative as follows: 610 bp (of total 1,542 bp) for *COI*, 120 bp (of 371 bp) for 12S, and 104 (of 468 bp) for 16S. Parsimony analysis of the 3-gene data set yields 2 equally parsimonious trees (not shown; length = 6,715; consistency index = 0.239; retention index = 0.45; rescaled consistency index = 0.107). Phylogenetic analyses using maximum-parsimony, maximum-likelihood, and Bayesian (nonpartitioned and partitioned) methods for the 34 ingroup and 4 outgroup taxa yielded trees that differed only slightly in topology at terminal branches (analyses using single genes did not show significantly different topologies; data available upon request). Monophyly of the Heteromyidae was supported strongly in all analyses (Fig. 2). Support for the 3 currently recognized subfamilies was also very high in all analyses as was support for separate clades representing the genera *Dipodomys*, *Microdipodops*, *Chaetodipus*, and, to a lesser extent, *Perognathus*. None of our analyses supported monophyly of the genus *Liomys* (Fig. 2). Maximum-parsimony and maximum-likelihood bootstrap support values across the heteromyid tree were broadly similar for the analyses. Posterior probabilities were similar to, but generally higher than, maximum-likelihood bootstrap support values (Fig. 2; Cummings et al. 2003; Erixon et al. 2003).

All phylogenetic trees reconstructed in our analyses supported a sister relationship between Perognathinae and Heteromyinae (compare Figs. 1B and 2). However, constraint trees (i.e., Perognathinae and Dipodomyinae, or Dipodomyinae and Heteromyinae as sister taxa) were not significantly different from the best tree based on the Kishino–Hasegawa test ([maximum parsimony] $P > 0.81$), Kishino–Hasegawa test ([maximum likelihood] $P > 0.27$), Shimodaira–Hasegawa test ([maximum likelihood] $P > 0.14$), and likelihood-ratio tests ($P > 0.05$). However, alternative phylogenetic hypotheses based on the Bayesian nonpartitioned and partitioned analyses all were rejected ($P < 0.001$).

Estimates of divergence dates and branch lengths.—Likelihood-ratio tests rejected a molecular clock ($P < 0.01$) in the heteromyid sequence data, reinforcing the use of divergence dating methods that relax the assumption of a molecular clock. Results of the semiparametric (penalized-likelihood) and parametric (parametric Bayesian) time estimates (Fig. 3; Table 2) based on specified calibration points and the inferred topology (Fig. 2) were similar. However, because of the lack of a basal calibration, the penalized-likelihood method tends to slightly overestimate divergence dates at older nodes (Table 2). Our results showed that basal diversification of the extant heteromyid clades occurred about 22.3 mya and all 3 of the heteromyid subfamilial clades were evident shortly thereafter (within 2 million years). Time estimates (Fig. 3; Table 2) also showed that basal divergence within the Perognathinae seemed to occur much earlier (approximately 20.6 mya) than basal cladogenesis in the Dipodomyinae (about 15.4 mya) and in the Heteromyinae (about 15.2 mya). Date estimates based on each gene individually yielded similar results, although trees based on 12S and 16S genes were less precise in their estimates of ages of younger nodes (data available upon request). As other studies have shown (Noonan and Chippindale 2006; Yoder and Yang 2004), more accurate and precise dating estimates were derived from multiple, rather than single, loci.

Means of patristic distances to the outgroup (*Zygogeomys*) for the Dipodomyinae ($n = 14$), the Perognathinae ($n = 12$), and the Heteromyinae ($n = 8$), based on absolute number of substitutions (and based on the best maximum-likelihood model

Table 1.—Mean pairwise uncorrected *P*-values (in percentage) among the major clades in the Heteromyidae for each gene separately and combined (all). In all comparisons, ambiguously aligned sites were excluded.

Taxonomic comparison	12S	16S	*COI*[a]	All
Dipodomys versus *Microdipodops*	12.1	6.5	17.9	14.8
Dipodomys versus *Perognathus*	15.2	9.3	20.3	17.4
Dipodomys versus *Chaetodipus*	15.4	10.6	20.0	17.4
Dipodomys versus *Liomys salvini*	16.0	11.1	18.9	16.9
Dipodomys versus other *Liomys* and *Heteromys*	16.7	10.6	19.4	17.3
Dipodomys versus Perognathinae	16.7	11.0	21.9	19.0
Dipodomys versus Heteromyinae	16.6	10.6	19.3	17.2
Microdipodops versus *Perognathus*	15.2	10.0	20.4	17.6
Microdipodops versus *Chaetodipus*	15.1	10.1	20.2	17.4
Microdipodops versus *Liomys salvini*	16.3	12.4	18.9	17.3
Microdipodops versus other *Liomys* and *Heteromys*	18.1	11.0	20.2	18.1
Microdipodops versus Perognathinae	15.1	10.1	20.3	17.5
Microdipodops versus Heteromyinae	17.9	11.2	20.1	18.0
Perognathus versus *Chaetodipus*	13.4	9.8	20.1	17.1
Perognathus versus *Liomys salvini*	14.2	12.4	20.9	18.2
Perognathus versus other *Liomys* and *Heteromys*	13.3	11.5	20.8	17.8
Perognathus versus Dipodomyinae	15.2	9.4	20.3	17.4
Perognathus versus Heteromyinae	13.4	11.6	20.8	17.8
Chaetodipus versus *Liomys salvini*	16.9	10.9	19.6	17.5
Chaetodipus versus other *Liomys* and *Heteromys*	15.8	10.7	20.2	17.7
Chaetodipus versus Dipodomyinae	15.3	10.5	20.0	17.4
Chaetodipus versus Heteromyinae	15.9	10.7	20.1	17.6
Liomys salvini versus other *Liomys* and *Heteromys*	8.8	5.8	17.4	13.8
Liomys salvini versus Dipodomyinae	16.1	11.3	18.9	17.0
Liomys salvini versus Perognathinae	15.8	11.5	20.1	17.8
Other *Liomys* and *Heteromys* versus Dipodomyinae	16.9	10.6	19.5	17.4
Other *Liomys* and *Heteromys* versus Perognathinae	14.7	11.0	20.4	17.7
Dipodomyinae versus Perognathinae	15.3	10.1	20.1	17.4
Dipodomyinae versus Heteromyinae	16.8	10.7	19.4	17.3
Perognathinae versus Heteromyinae	14.9	11.1	20.4	17.7

[a] *COI* = cytochrome *c* oxidase subunit I.

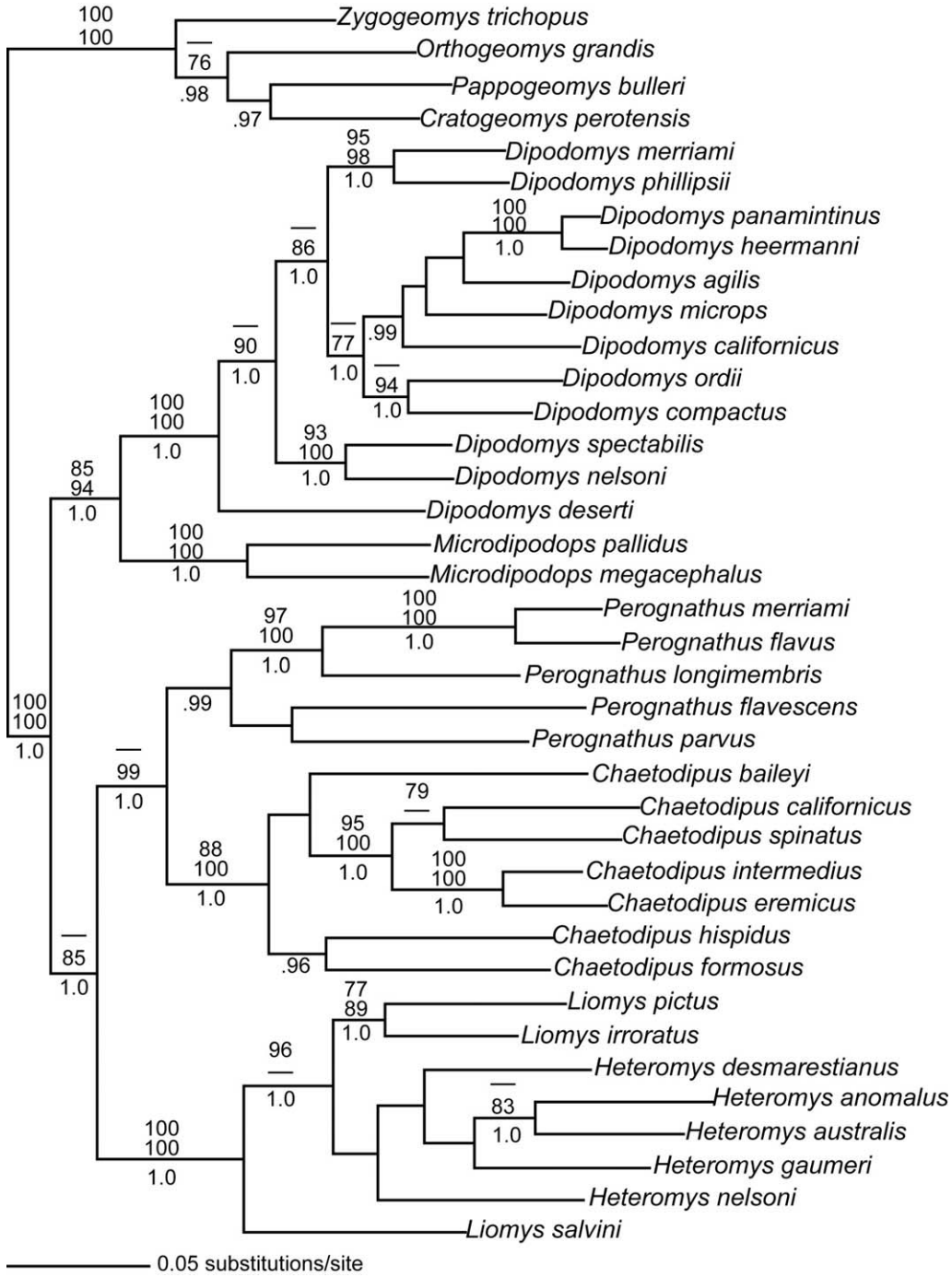

FIG. 2.—Maximum-likelihood phylogram based on 3 genes and unambiguous sites only showing basal clades in the Heteromyidae. Maximum-parsimony and maximum-likelihood bootstrap support values (≥75) are shown above the nodes and Bayesian posterior probabilities (≥0.95) are shown below the nodes. There are subtle differences between the partitioned and the nonpartitioned analyses in tree topology involving some terminal taxa only (data available upon request); see Alexander and Riddle (2005) and Rogers and Vance (2005) for comprehensive resolution of terminal taxa.

of evolution [in parentheses]) were 403.398 (1.037), 430.407 (1.212), and 427.567 (1.202), respectively. There was significant heterogeneity in mean absolute distance (Kruskal–Wallis test statistic = 23.497; $P < 0.001$) and mean maximum-likelihood patristic distance (Kruskal–Wallis test statistic = 24.008, $P < 0.001$) among the 3 subfamilies. There was no significant difference between the mean distance measures of the Perognathinae and the Heteromyinae (Mann–Whitney $U = 38.000$, $P = 0.440$ for absolute distance and $U = 46.000$, $P = 0.877$ for maximum-likelihood distance). However, the means of the distance measures for the dipodomyines were significantly different from the corresponding means for both the perognathines and the heteromyines (all 4 Mann–Whitney U-tests show $P < 0.001$). There were no significant differences

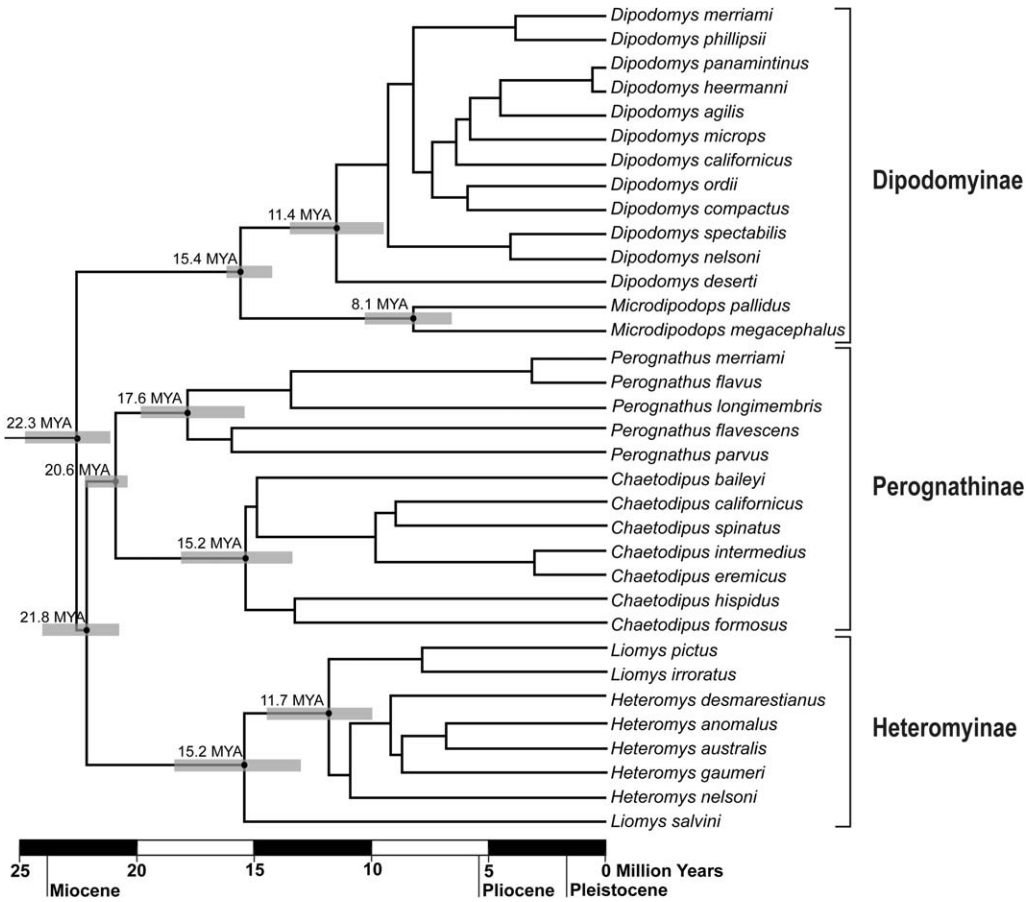

Fig. 3.—Chronogram for the Heteromyidae. This rate-smoothed tree was obtained from the parametric Bayesian divergence dating analysis using *multidivtime* (Kishino et al. 2001; Thorne and Kishino 2002). Divergence dates were estimated on the maximum-likelihood topology resulting from the combined 3-gene analysis and fossil calibrations of 15.9–12.5 mya for Dipodomyinae and 22–20 mya for Perognathinae. Numbers at nodes indicate mean divergence date and gray bars represent the 95% credibility intervals.

in the means of branch lengths between *Dipodomys* and *Microdipodops* nor between *Perognathus* and *Chaetodipus* (results of all Mann–Whitney *U*-tests were nonsignificant, $P > 0.05$).

Discussion

Monophyly of the Heteromyidae.—We found strong support for the recognition of the Heteromyidae as a monophyletic clade relative to the outgroup, the geomyid pocket gophers. Although analyses of individual genes and combined data sets yielded trees that did not differ significantly in tree topology, only when the 3 genes were combined was there strong support at both deep and shallower levels, as seen in other molecular studies (Delpero et al. 2001; Gates et al. 1992; Hafner et al. 2006; Kuznetsova et al. 2002; Ledje and Arnason 1996; Olson et al. 2005; Ruedas and Morales 2005). This finding of monophyly supports early phenetic perspectives on heteromyid evolution (e.g., Wood 1935) and agrees with phylogenetic analyses involving allozymic and immunological data (Hafner 1982), cranial and dental morphological traits (Wahlert 1985, 1991), and myological characters (Ryan 1989). The conflict between this finding of monophyly and the suggestion of Brylski (1990) and DeBry (2003) that the family is paraphyletic may be resolved if Brylski's (1990) single character (stapedial artery absent in adults of *Thomomys* and the spiny pocket mice) was lost independently in these groups and if DeBry's (2003) analyses supporting paraphyly resulted from insufficient taxon sampling.

Recognition of the subfamilies.—Although not as slowly evolving as nuclear genes, simultaneous use of *COI*, 12S, and 16S seems to provide a useful systematic tool for resolving deeper as well as shallower nodes in the family. The 3 traditional subfamilies, Heteromyinae, Perognathinae, and Dipodomyinae, were recognized and supported strongly in all analyses. *Microdipodops* was a sister clade to *Dipodomys* in the Dipodomyinae, a finding that corroborates all other phylogenetic studies based on a wide spectrum of characters (e.g., Alexander and Riddle 2005; Hafner 1982; Hafner and Hafner 1983; Hafner et al. 2006; Rogers 1990; Ryan 1989; Wahlert 1985) and should lay to rest this long-standing controversy.

The Perognathinae, consisting of the sister clades of *Perognathus* and *Chaetodipus*, was supported well in all of our analyses. This arrangement was recognized early on by rodent systematists and has been corroborated by subsequent cladistic analyses (e.g., Hafner 1982; Hafner and Hafner 1983;

Table 2.—Estimates of divergence time for major heteromyid nodes obtained from semiparametric penalized-likelihood (PL) and parametric Bayesian (PB) methods and calibrated with reference fossils (see "Materials and Methods"). The node "*Heteromys* + *Liomys*" does not include *L. salvini*. Values shown are the average and *SD* (in parentheses) in millions of years for the PL analyses, and the average and credibility interval (in parentheses) in millions of years for the PB analyses.

Node	PL	PB
Heteromyidae	24.47 (0.99)	22.27 (20.68, 24.25)
Perognathinae	20.00 (0.06)	20.59 (20.02, 21.76)
Dipodomyinae	15.90 (0.00)	15.35 (14.10, 15.88)
Heteromyinae	11.95 (0.95)	15.19 (12.71, 18.01)
Dipodomys	10.14 (0.80)	11.35 (9.38, 13.30)
Microdipodops	7.09 (0.66)	8.06 (6.34, 10.01)
Perognathus	16.76 (0.92)	17.58 (15.54, 19.71)
Chaetodipus	13.91 (0.87)	15.15 (12.84, 17.58)
Heteromys + *Liomys*	8.66 (0.80)	11.66 (9.66, 13.98)
Heteromyidae + Perognathinae	22.66 (0.75)	21.83 (20.37, 23.66)

Ryan 1989). However, it does contrast with the findings of Alexander and Riddle (2005), who found no support for the traditionally defined Perognathinae. Although Alexander and Riddle (2005) relied on molecular phylogenetic methods, they acknowledged that they used mitochondrial genes that were too rapidly evolving to resolve the deeper clades in the family.

The species of *Heteromys* and *Liomys* used in our analysis formed a monophyletic clade supporting recognition of the traditional subfamily Heteromyinae. The findings that the genus *Liomys* is paraphyletic relative to *Heteromys* and that *L. salvini* forms a well-supported sister clade relative to all other heteromyines corroborate the findings of Rogers and Vance (2005). Distinctiveness of *L. salvini* (along with a closely related species, *L. adspersus*) was documented by Rogers (1990) using allozymic data, but use of those data could not resolve the basal heteromyine lineages. Rogers and Vance (2005), using cytochrome-*b* sequence data, were 1st to recognize that *L. salvini* and *L. adspersus* form a clade that is basal to other heteromyine lineages, and examination of our data supports their finding.

Subfamilial relationships.—A key goal of our study was to resolve evolutionary relationships among the 3 heteromyid subfamilies. Tree topologies from all phylogenetic methods employed in this study demonstrated a sister relationship between the Heteromyinae and the Perognathinae (Fig. 1B). Alternative phylogenetic hypotheses (Figs. 1A and 1C) could not be rejected using Kishino–Hasegawa, Shimodaira–Hasegawa, and likelihood-ratio tests; however, some of these methods may be inappropriate for comparing alternative hypotheses when using combined or partitioned analyses, or both (Weins et al. 2005). In fact, Bayesian analyses, which may be more appropriate for these data, rejected all alternative hypotheses. Taken together, these tests suggest that recognition of the Heteromyinae + Perognathinae clade is superior statistically to any of the alternative phylogenetic hypotheses for our data.

Other workers have stressed the importance of partitioning data appropriately for accurate phylogenetic reconstruction (Brandley et al. 2005; Castoe et al. 2004; Castoe and Parkinson 2006; Lemmon and Moriarty 2004; Mueller et al. 2004). In our study, however, there were no differences between partitioned and nonpartitioned data in the branching patterns reconstructed at the deeper portions of the heteromyid clade. Although our intent here is to focus on resolving basal clades in the family, we did note that there were subtle differences between the partitioned and the nonpartitioned analyses in tree topology involving some terminal taxa (data available upon request).

The finding that the heteromyines and perognathines are sister taxa relative to the dipodomyines was unanticipated. Based on examination of limited fossil and Recent material, Wood (1931) suggested the possible affinity between *Heteromys*, *Liomys*, and *Perognathus* (he did not distinguish between *Perognathus* and *Chaetodipus*), but reversed himself by placing the heteromyines as a sister clade to the other heteromyids in his monographic account of the family (Wood 1935). Rodent systematists throughout the 20th century viewed the heteromyines as an evolutionarily independent lineage quite removed from the pocket mice, kangaroo rats, and kangaroo mice (Genoways 1973; Hafner 1978; Reeder 1956; Wood 1935). Moreover, detailed cladistic analyses of morphological features by Walhert (1985, 1991) and Ryan (1989) supported the phylogeny of Wood (1935) by uniting the Dipodomyinae and the Perognathinae as sister taxa relative to the heteromyines. Our results showing the sister relationship of the heteromyines and the perognathines refute previous cladistic interpretations based on morphology. Actually, the initial suggestion of Wood (1931) regarding subfamilial affinities based on phenotypic similarity appears to be correct in unifying all genera of pocket mice.

Heteromyid fossils for calibration.—The paleontological literature was surveyed for information on the earliest fossils representing extant heteromyid genera for calibrating our molecular time analyses. Incorporating the fossil record presents challenges; in addition to the fragmentary nature of the fossil record for the heteromyids, the paleontological literature does not distinguish between *Perognathus* and *Chaetodipus* (see Wahlert 1993). However, the fossil record for *Perognathus* sensu lato and *Dipodomys* is sufficiently long and plentiful (see Wahlert 1993) that fossil time estimates for these genera are more likely to be reliable for calibration purposes. Wahlert (1993) mentioned that the earliest fossils of *Perognathus* sensu lato are of Hemingfordian age and from the Split Rock Local Fauna of Wyoming and the John Day Formation of Oregon. Inasmuch as the fossil from the John Day (University of California Museum of Paleontology [UCMP] specimen 56279) is older (22–20 mya—T. Fremd, pers. comm., 2006) than the Split Rock fossil (17 mya—Munthe 1979) we use the former fossil for calibration of the perognathine node. Calibration of the dipodomyine node was based on the oldest known fossils of *Dipodomys* (uncatalogued UCMP material) that were reported by Reeder (1956) from the Barstovian of California (15.9–12.5 mya). Fossil material for the other extant genera (*Microdipodops*, *Liomys*, and *Heteromys*) is so uncommon and fragmentary that we did not include those data in our analyses. Specifically, there is no published fossil record for *Heteromys*, *Liomys* is known only from the late Pleistocene

(Rancholabrean to sub-Recent—Wahlert 1993), and *Microdipodops* is known only from the late Blancan (about 2.9–1.9 mya—Jefferson and Lindsay 2006) and the late Pleistocene (Rancholabrean—Wahlert 1993).

Branch lengths and timing of divergence.—Molecular divergence among heteromyid clades did not occur in a clocklike fashion and there was significant heterogeneity among the principal subfamilial clades in terms of mean patristic distances. In particular, the dipodomyine clade had significantly shorter distances to the outgroup taxon and, hence, we infer that the dipodomyines, on average, show retarded rates of molecular evolution relative to the other 2 subfamilies. This general pattern of branch-length difference also was evident in Fig. 2. The argument for slower rates of molecular evolution in the Dipodomyinae receives some support from Hafner's (1982) immunological study of geomyoid rodents; although Hafner (1982) relied on only 2 dipodomyines (*D. heermanni* and *M. megacephalus*), he reported particularly small immunological distances between *M. megacephalus* and the geomyid and carnivore outgroups and only moderate distances for *D. heermanni*. Our study also showed short branches for *Microdipodops* but not for *D. heermanni* (Fig. 2).

The chronogram (Fig. 3) represents a testable proposition for future workers. Not only does the chronogram portray molecular phylogenetic relationships among the major heteromyid clades, but it incorporates the best available fossil evidence in estimating times of divergence. Divergence time estimates of about 22 mya for the initial diversification of the Recent heteromyid lineages (Fig. 3) are reasonably in accord with other molecular and paleontological studies that estimate the timing of the split between the Heteromyidae and the Geomyidae: Montgelard et al. (2002) estimated 30.4 mya; Hafner (1993) recalculated the data in Hafner (1982) and determined 32–30 mya; Wahlert (1993) suggested before the Arikareean (about 30 mya); and Fahlbusch (1985) indicated the late Oligocene (about 26 mya). Our time estimates for the initial divergence of the subfamilies also are in general agreement with Wahlert's (1993) paleontological perspective; although Wahlert (1993:30) believed that the perognathines are more closely related to the dipodomyines (and not the heteromyines, as we show in this study), he suggested that common ancestry of the subfamilies ''must have been in the early Miocene or late Oligocene'' (about 24 mya). Wahlert (1993:26) indicated that the fossil record shows that the ''heteromyid subfamilies were not sharply divided in the Miocene . . .''; however, our chronogram disagreed with the paleontological perspective in showing clear separation of the subfamilies in early Miocene time.

The chronogram (Fig. 3) suggests that the 3 subfamilies diverged almost simultaneously and that the genera *Perognathus* and *Chaetodipus* are relatively ancient lineages. Among the 3 subfamilies, divergence within the heteromyines appears to have occurred most recently (about 15.2 mya); this may seem somewhat surprising because the heteromyines have long been considered to be the most morphologically primitive members of the Recent heteromyids (e.g., Alexander and Riddle 2005; Kelly 1969; Ryan 1989; Williams et al. 1993; Wood 1935). The relatively old (8.1-mya) split of the 2 species of *Microdipodops* also is somewhat surprising, but recent work (J. C. Hafner et al., in litt.) indicates that the 2 currently recognized species actually represent species groups. Contrary to the rather recent (early Pleistocene) evolution of the genus as postulated by Hafner (1978), kangaroo mice appear to be an old lineage, with divergence from the other dipodomyines dating to about 15.4 mya. Alexander and Riddle (2005) also noted the deep divergence between *Microdipodops* and *Dipodomys*.

Historical biogeography.—The longevity of ancient lineages within the Heteromyidae during the dramatic landscape evolution of western North America in the Cenozoic offers an opportunity to examine the impact of specific geological and climatic events on these diversifying lineages. Major events that pertain to the phylogeny of the Heteromyidae have been summarized thoroughly by Alexander and Riddle (2005), but lack of resolution of deeper evolutionary nodes precluded any consideration of causal correlation of lineage diversification with tectonic and climatic changes. Prothero (1998) and Woodburne (2004) reviewed and revised the Cenozoic chronostratigraphy and biostratigraphy, and they detailed paleoclimatic and paleogeographic events that affected mammalian evolution in North America. Rather than attempt a separate overview of these formative events, we emphasize here several salient events that appear to have had major influence on lineage divergence within the Heteromyidae.

It is probable that Mexico and Central America represent the center of geomyoid evolution (Wahlert and Souza 1988). Divergence of the Geomyoidea into the Geomyidae and Heteromyidae (~30 mya) and subsequent subfamilial differentiation of the Heteromyidae (22.3–20.6 mya) follows the early Oligocene cooling shift that Prothero (1998:18) considered ''the most dramatic temperature shift during the entire Cenozoic . . .'' (to that time) resulting in ''the most extreme paleobotanical change in all of the Cenozoic.'' This marked an abrupt shift in vegetation from mesic species to drier, drought-tolerant species, with a concomitant shift in land snails, amphibians, and reptiles (Prothero 1998). Subsequent warming led to increased complexity in vegetative zones (Woodburne 2004). This period witnessed evolutionary ''experimentation'' resulting in morphologically flamboyant forms in both heteromyid and geomyid lineages, as evidenced by extinct fossil forms as well as the origin of extant lineages of heteromyids.

Following the relatively warm and quiescent early Miocene, the middle Miocene experienced a temperature shift even more dramatic than that of the early Oligocene. This rapid cooling shift led to the return of permanent ice sheets to the Antarctic, initiation of modern ocean circulation patterns, and the beginning of a cooling and drying trend that has led to the current glacial episodes (Prothero 1998; Woodburne 2004). Western North America experienced increased aridity, decreased temperatures, spread of grasslands, fragmentation of vegetation previously adapted to Mediterranean climate, and major dispersal pulses of mammals (Woodburne 2004). In addition, the western landscape was altered tremendously: initiation of extensive block-faulting created the Basin and Range province; intensified plate movement shifted the Pacific

coastline; the Gulf of California was opened (with transfer of the Cape Region to the Pacific Plate); and the Baja California Peninsula drifted northward relative to the mainland.

The impact of the major climatic shift and tectonic events of the middle Miocene was most evident in the 2 northern, arid-adapted heteromyid subfamilies, Dipodomyinae and Perognathinae. Desert shrubland was emerging in western North America in response to continued cooling, increased aridity, and continental effects (Woodburne 2004). It is reasonable to assume that the distribution of the subfamily Heteromyinae never extended much further north than the current northernmost distribution along the United States–Mexican border because the fossil record of the family is predominantly from the United States and Canada and no extinct fossil forms are assigned to this subfamily (Wahlert 1993). Instead, this more mesic-adapted, semitropical subfamily experienced little lineage divergence relative to the other subfamilies, and no major differentiation from the basic heteromyine (murinelike) *Bauplan*. In contrast, the 2 northern subfamilies experienced similar (and coincident) adaptive radiations, producing 4 different forms that have invaded colder, continental zones: *Microdipodops*, *Perognathus*, and, within the genus *Chaetodipus*, *C. formosus* and *C. hispidus*. Further lineage divergence in these genera was in response to climatic and geological events of the Pliocene and Pleistocene, including continued opening of the Gulf of California, ongoing uplift of major mountain chains and plateaus, and climatic oscillations of the Pleistocene glaciations (Alexander and Riddle 2005; Hafner and Riddle 2005; Riddle and Hafner 2006; Riddle et al. 2000a, 2000b).

Taxonomic conclusions.—Our study clearly resolves the evolutionary relationship among basal clades within the Heteromyidae. We recognize 3 basal clades that represent 3 subfamilies: the Dipodomyinae (including *Dipodomys* and *Microdipodops*) and the sister clades Perognathinae (including *Perognathus* and *Chaetodipus*) and Heteromyinae (including *Heteromys* and *Liomys*).

In the subfamily Heteromyinae, the paraphyletic nature of the genus *Liomys* necessitates taxonomic change. Merriam (1902) proposed the name *Liomys* and indicated *H. alleni* (= *L. irroratus alleni* [see Genoways 1973]) as its type species. Concern regarding the taxonomic status of the genus *Liomys* 1st surfaced in the studies by Rogers (1986, 1990). Using allozymic data, Rogers (1986, 1990) was unable to demonstrate monophyly of *Liomys* and stated that additional evidence was needed before making taxonomic changes. Rogers and Vance (2005) used mitochondrial DNA data to demonstrate the paraphyletic nature of *Liomys*, and corroborative data from Anderson et al. (2006) and our study affirmed that conclusion. In their study using allozymes and morphology, Anderson et al. (2006:1218) urged future workers to consider the ''likely paraphyletic nature'' of *Liomys* but were unable to demonstrate paraphyly conclusively because of weak branch support. Importantly, there are no known morphological or molecular synapomorphies to distinguish *Liomys* from *Heteromys* (Anderson et al. 2006; Rogers 1986, 1990; Rogers and Vance 2005), and all available evidence from nuclear and mitochondrial markers is consistent with *Liomys* paraphyly; no evidence contradicts this interpretation.

If genus-level groups are to retain approximate equivalence within the family Heteromyidae, then taxonomic decisions at the generic level in any heteromyid subgroup should be made in the broader context of previous taxonomic decisions within the entire family. Previous systematic studies of heteromyid rodents, many of them contributed by the authors of this paper, have maintained a high level of taxonomic equivalence across the family. Heteromyid genera are not merely diagnosable clades (of which there are many in the family; Fig. 3); instead they represent relatively old, monophyletic lineages that show substantial morphological, ecological, physiological, and behavioral differences from other heteromyid genera. Anderson et al. (2006) seemed to favor recognition of 3 heteromyine genera if future studies corroborated the paraphyly of *Liomys*. In our judgment, recognition of more than a single genus in the subfamily Heteromyinae would disrupt taxonomic equivalence in the family by elevating to generic status lineages that are not only geologically younger but, in our experience, also show less morphological and ecological differentiation than do major lineages within the genera *Chaetodipus* and *Perognathus* (Fig. 3). Unless we opt to recognize additional genera within *Chaetodipus* and *Perognathus*, the morphological and ecological differentiation evident in the subfamily Heteromyinae is best reflected at the subgeneric level. Given the evidence accumulated over the past 2 decades, taxonomic consistency within the Heteromyidae demands that *Liomys* be placed in synonymy with *Heteromys*. Below, we provide a formal taxonomic description of the genus *Heteromys*.

Family Heteromyidae Gray, 1868:201

Comments.—Following Wahlert (1991), the family includes 4 subfamilies: Harrymyinae (includes extinct taxa only), Dipodomyinae, Perognathinae, and Heteromyinae. Wahlert (1993) provides a summary of extinct genera of heteromyid rodents.

Subfamily Heteromyinae Gray, 1868:201

Comments.—This subfamily contains 1 Recent genus, *Heteromys*, and no extinct heteromyid genera are included in this subfamily (Wahlert 1993).

Genus *Heteromys* Desmarest, 1817

Heteromys Desmarest, 1817:181. Type species *Mus anomalus* Thompson, 1815. Synonyms include *Xylomys* Merriam, 1902, and *Liomys* Merriam, 1902.

Diagnosis.—With *Liomys* placed in synonymy, *Heteromys* remains as the single, recognized genus in the Heteromyinae. Hence, the diagnosis of the subfamily Heteromyinae provided by Williams et al. (1993:99) is generally applicable to the genus *Heteromys* with its new conformation. Heteromyines exhibit the murine body form and have long been considered as the most structurally primitive members of the family (e.g., Ryan 1989; Wahlert 1993; Williams et al. 1993; Wood 1935).

Although identifying synapomorphies for the heteromyines has proven difficult because of their retention of ancestral characters (Ryan 1989:98), we identify the following morphological features that serve as synapomorphies for the Heteromyinae: male phallus is nonspinous (Genoways 1973; Hafner and Hafner 1983; Kelly 1969); ventral root of anterior-alar fissure rising above M3 (Wahlert 1985); masticatory and buccinator foramina united (Wahlert 1985); stapedial and sphenofrontal foramina absent (Wahlert 1985); stapedial artery absent (Wahlert 1991); troughed overhairs with troughs containing longitudinal lines that are formed by sculptured fusiform cortical cells underlying the cuticular scales (Homan and Genoways 1978).

Included taxa.—Fourteen species are now included in the genus *Heteromys*, including *Heteromys adspersus*, *H. anomalus*, *H. australis*, *H. desmarestianus*, *H. gaumeri*, *H. irroratus*, *H. nelsoni*, *H. nubicolens*, *H. oasicus*, *H. oresterus*, *H. pictus*, *H. salvini*, *H. spectabilis*, and *H. teleus*. This listing includes 8 species listed in Patton (2005), a newly described species, *H. nubicolens* (Anderson and Jansa 2007; Anderson and Timm 2006), and 5 species previously referred to *Liomys* (*adspersus*, *irroratus*, *pictus*, *salvini*, and *spectabilis*).

Comments.—Relative to the dipodomyines and perognathines, the heteromyines have long been recognized to be the most distinct and internally cohesive lineage within the family (Hafner 1993; Wood 1935). In discussing the morphology of the heteromyines, Wood (1935:249) commented that they were more distinct than the other 2 subfamilies; specifically, Wood (1935:230) noted that *Liomys* and *Heteromys* possessed an interesting admixture of more primitive (skeletal) features and more specialized (dental) characters. Homan and Genoways (1978:758) found the hair morphology of *Liomys* and *Heteromys* to be more derived than other members of the family and agreed with Wood's (1935) conclusion of the morphological distinctiveness of the subfamily. Ryan (1989:98) noted that ''*Heteromys* and *Liomys* have many primitive characters relative to other heteromyids and finding synapomorphies for this clade (Heteromyinae) had always been problematic.'' In his myological study, Ryan (1989) was not able to identify a synapomorphy for the Heteromyinae. Hafner and Hafner (1983:7) examined the descriptions of the glans penes of heteromyine rodents from Kelly (1969) and Genoways (1973) and suggested 3 possible synapomorphies for the family: spineless phalli, glans long relative to bacular length, and unique urethral lappet morphology. Our reexamination of the figures and descriptions of the phalli in Kelly (1969) and Genoways (1973) supports the interpretation by Hafner and Hafner (1983) that the nonspinous phallus is a synapomorphy for the Heteromyinae, but the other 2 features appear less reliable; the relative length of the glans is somewhat arbitrary and there is some disagreement between Kelly (1969) and Genoways (1973) regarding the morphology of the urethral lappets.

Rogers and Vance (2005) presented information from the mitochondrial cytochrome-*b* gene that supports the recognition of a basal clade within the heteromyines that includes *H. salvini* and *H. adspersus*. Although the present study does not include *H. adspersus*, our results agree with Rogers and Vance (2005) in showing *H. salvini* as a sister clade to all other heteromyines. Molecular phylogenetic studies currently are underway to evaluate evolutionary relationships among the species of *Heteromys* (R. P. Anderson, pers. comm.). Although it is premature to suggest taxonomic changes regarding species groupings within *Heteromys*, it would not be surprising if future workers recognized the *salvini* + *adspersus* clade at the subgeneric level.

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APPENDIX I

Specimens examined in the genetic analyses (molecular data were obtained from 1 specimen per species). Specimens are grouped by heteromyid subfamily. Collection abbreviations are as follows: Angelo State Natural History Collection (ASNHC); M. L. Bean Life Science Museum, Brigham Young University (BYU); Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA); Louisiana State University Museum of Natural Science (LSUMZ); University of California Museum of Vertebrate Zoology (MVZ); Moore Laboratory of Zoology, Occidental College (MLZ); New Mexico Museum of Natural History (NMMNH); Texas Cooperative Wildlife Collection (TCWC).

Dipodomys agilis.—MEXICO: Baja California: 6 km S, 17 km E Valle de la Trinidad, 905 m (MVZ 153957).

Dipodomys californicus.—UNITED STATES: California: Tehama County; 2.5 miles S, 0.2 miles E Paynes Creek, 1,960 feet (MLZ 2061).

Dipodomys compactus.—UNITED STATES: Texas: Cameron County; 4.5 miles N, 3.6 miles E Port Isabel (ASNHC 4327).

Dipodomys deserti.—UNITED STATES: Nevada: Clark County; Corn Creek Desert Wildlife Refuge (NMMNH 5374).

Dipodomys heermanni.—UNITED STATES: California: San Luis Obispo County; 15.0 miles S, 8.2 miles E Simmler, 2,300 feet (MLZ 1852).

Dipodomys merriami.—MEXICO: Chihuahua; 6 miles NW Ricardo Flores Magón, 1,500 m (NMMNH 4548).

Dipodomys microps.—UNITED STATES: California: Inyo County; 6.0 miles N, 0.5 mile W Bishop, 4,200 feet (MLZ 1765).

Dipodomys nelsoni.—MEXICO: Coahuila; 5 km S, 16 km W General Cepeda (NMMNH 4703).

Dipodomys ordii.—UNITED STATES: New Mexico: Grant County; 2.6 miles N, 1.8 miles E Redrock, 1,241 m (NMMNH 4377).

Dipodomys panamintinus.—UNITED STATES: California: San Bernadino County; 8.9 miles N, 1.1 miles E Red Mountain, 3,150 feet (MLZ 1879).

Dipodomys phillipsii.—MEXICO: Zacatecas; 2 miles E San Jerónimo, 2,350 m (CNMA 42050).

Dipodomys spectabilis.—UNITED STATES: New Mexico: Hidalgo County; 6 miles SE Portal (Cochise County, Arizona) (NMMNH 4399).

Microdipodops megacephalus.—UNITED STATES: California: Mono County; 5 miles N Benton, 5,600 feet (MLZ 1741).

Microdipodops pallidus.—UNITED STATES: Nevada: Nye County; 3 miles S, 4.3 miles E Gold Reed, 5,330 feet (MLZ 1959).

Heteromys anomalus.—VENEZUELA: Miranda; 40 km N Altagracia (TCWC 39720).

Heteromys australis.—PANAMA: Province Darién; 6 km NW Cana, N Slope Cerro Pirré, approximately 1,200 m (LSUMZ 25452).

Heteromys desmarestianus.—MEXICO: Veracruz; 8 km ENE Catemaco, 480 m (LSUMZ 36300).

Heteromys gaumeri.—MEXICO: Campeche; 7 km W Escarcega (ASNHC 7118).

Heteormys nelsoni.—MEXICO: Chiapas; Cerro Mozotal, 2,930 m (BYU 20645).

Liomys irroratus.—MEXICO: Puebla; 6 km N Tilapa, 1,300 m (LSUMZ 36295).

Liomys pictus.—MEXICO: Veracruz; Biological Station La Mancha, 0 m (CNMA 41912).

Liomys salvini.—COSTA RICA: Puntarenas Province; 5 km S, 6 km W Esparza (LSUMZ 28358).

Chaetodipus baileyi.—UNITED STATES: New Mexico: Hidalgo County; Doubtful Canyon, 8 miles N, 1 mile W Steins, 1,380 m (NMMNH 4421).

Chaetodipus californicus.—UNITED STATES: California: San Luis Obispo County; 15.9 miles S, 7.2 miles E Simmler, 3,200 feet (MLZ 1843).

Chaetodipus eremicus.—UNITED STATES: New Mexico: Doña Ana County; 1 mile S junction I-10 and Picacho Avenue, 1,314 m (NMMNH 4433).

Chaetodipus formosus.—UNITED STATES: California: San Bernardino County; 8.9 miles N, 1.1 miles E Red Mountain, 3,150 feet (MLZ 1863).

Chaetodipus hispidus.—UNITED STATES: Texas: Hidalgo County; Mission, 2519 Inspiration Road (LSUMZ 36375).

Chaetodipus intermedius.—UNITED STATES: New Mexico: Grant County; 2.6 miles N, 1.8 miles E Redrock, 1,241 m (NMMNH 4371).

Chaetodipus spinatus.—MEXICO: Baja California Sur; 4 miles SE (by road) Migriño, 90 m (MVZ 153946).

Perognathus flavescens.—UNITED STATES: New Mexico: Bernalillo County; Albuquerque (LSUMZ M4670).

Perognathus flavus.—MEXICO: Puebla; 3.1 km SW El Veladero (LSUMZ 36254).

Perognathus longimembris.—UNITED STATES: Nevada: Lyon County; 10.3 miles S, 2.2 miles E Yerington, 4,600 feet (MLZ 2046).

Perognathus merriami.—MEXICO: Coahuila; Plan de Guadalupe, 1,040 m (NMMNH 4728).

Perognathus parvus.—UNITED STATES: Nevada; Lincoln County; 6 miles N, 31 miles W Hiko, 4,800 feet (MVZ 159055).

Zygogeomys trichopus.—MEXICO: Michoacán; 6 km N, 2 km W Tancítaro, 2,000 m (LSUMZ 34340).

Orthogeomys grandis.—MEXICO: Puebla; 6 km N Tilapa, 1,300 m (CNMA 41821).

Pappogeomys bulleri.—MEXICO: Jalisco; 8.6 km (by road) SW La Huerta, 374 m (CNMA 41923).

Cratogeomys perotensis.—MEXICO: Veracruz; 9 km NE Perote, 2,440 m (CNMA 41911).