MOLECULAR PHYLOGENETICS OF THE CHIROPTERAN FAMILY VESPERTILIONIDAE

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INTRODUCTION

Vesper bats constitute the largest chiropteran family (Vespertilionidae) with about 44 genera and 350 species of small, primarily insectivorous mammals (Corbet and Hill 1991; Nowak 1999). Only murid rodents display greater mammalian diversity. Vespertilionids are most diverse in warmer parts of the world, but their unique versatility in metabolism and behavior (and ability to fly) has set few limits on geographic distribution; worldwide essentially wherever there is ample vegetation to sustain sufficient insect life, including subalpine and subpolar locations and all but the most remote islands (Koopman 1970; Rosevear 1965). Phenotypes are simple and non-descript compared to members of other chiropteran families, which in practice makes distinguishing Vespertilionidae relatively easy. Formal description of the family is more difficult, requiring combinations of several external and internal characters (i.e., each of which is shared with 1 or more other families): muzzle and lips simple and unadorned; ears widely separate with conspicuous, pointed, or slightly curved tragi; tail long and essentially included to tip within wide interfemoral membrane; wings generally not broad; finger joints numerous; secondary or "double" articulation between scapula and humerus well-developed; ulna extremely

rudimentary; teeth essentially normal (Koopman 1994; Miller 1907). A derived morphologic feature defining the family has yet to be discovered (Koopman 1994; Simmons 1998).

Present systematics of the family is based almost entirely on criteria derived from taxonomic interpretations of traditional anatomical characters (Miller 1907; Tate 1941a, 1942). Five groups are recognized and typically regarded as subfamilies (Kerivoulinae, Miniopterinae, Murininae, Nyctophylinae, Vespertilioninae). Another subfamily (Tomopeatinae), containing a single species known only from Peru (*Tomopeas ravus*), also has been recognized traditionally; however, morphologic and molecular evidence clearly document its affinity with Molossidae (Barkley 1984; Pierson 1986; Simmons 1998; Simmons and Geisler 1998; Sudman et al. 1994). Each subfamily except Vespertilioninae is well-defined morphologically, includes few genera and species, and is confined to the Old World. The majority of vesper bats (>82% of genera and species) are placed in Vespertilioninae, but assuming ill-defined criteria: non-descript and without the special modifications distinguishing the other subfamilies. Vespertilioninae is the only subfamily with members in all zoogeographic regions and most islands occupied by the family. It is typically divided by dental characteristics into 6 tribes (Antrozoini, Lasiurini, Myotini, Nycticeiini, Plecotini, Vespertilionini) with half of these, about 140 species of *Pipistrellus*-like bats, placed in Vespertilionini. Four of these tribes are

widely distributed with members in both New and Old Worlds, whereas Antrozoini and Lasiurini are exclusively New World.

Various 20th century authors generally have agreed with this view of higher-level relationships, with few or no principal discrepancies regarding monophyletic assemblages even among individual classifications (Corbet and Hill 1991; Hill and Smith 1984; Koopman 1984, 1985, 1993, 1994; Koopman and Cockrum 1967; Kuzjakin 1950; McKenna and Bell 1997; Nowak 1999; Simpson 1945). With minor alterations, arrangements of Miller (1907) and Tate (1941a, 1942) still remain widely accepted (excepting Tomopeatinae). However, morphologic criteria supporting the traditional classification offer limited resolution for relationships among genera or among tribes and subfamilies.

Furthermore, apparent stability of higher-level taxa in 20th century classifications of vesper bats is misleading considering the contradictory evidence that has accumulated in the past 30 years. Specifically, data show that many morphologic characters traditionally used in vespertilionid systematics have little phyletic information (e.g., Hill and Topál 1973; Topal 1970; Zima and Horácek 1985), and study of several new types of data (e.g., embryology, DNA, immunology, karyology, non-classical morphology) have questioned monophyly of the family, of several subfamilies and tribes, and of numerous genera. However, there is a general lack of consensus among recent studies, and no synthesis of the new information into a well-supported contemporary

classification. An important argument both for a lack of consensus among recent studies and against classificatory synthesis is that monophyly of nearly all higher-level vespertilionid taxa remains to be tested by rigorous taxonomic sampling and explicit phylogenetic analysis.

The most comprehensive phylogenetic analysis of vespertilionid relationships is that of Volleth and Heller (1994a; stemming from Volleth's 1989 dissertation). They examined banded karyotypes from 50 species representing 23 genera and all subfamilies of Vespertilionidae, but sampled only 1 New World species [*Rhogeessa* (*Baeodon*) *alleni*]. Cladistic analysis afforded little resolution to deep branching patterns except for a basal position for Miniopterinae and for monophyly of Vespertilioninae excluding *Myotis* (Fig. 1). Other noteworthy findings included support for classifying Vespertilionini into 3 tribes (Eptesicini, Pipistrellini, Vespertilionini) and *Pipistrellus* into 4 genera (*Falsistrellus*, *Hypsugo*, *Pipistrellus*, *Vespadelus*); *Pipistrellus* within Pipistrellini, the others within Vespertilionini (Fig. 1). Additional study of karyotypes supports generic distinction for *Neoromicia* (Volleth et al. 2001), a former subgenus of both *Eptesicus* or *Pipistrellus* (Hill and Harrison 1987; Koopman 1993). Despite providing much needed resolution to relationships among closely related, *Pipistrellus*-like species, chromosomal data leave virtually all deep-branching patterns unresolved and, perhaps

more importantly, monophyly of all cosmopolitan taxa untested.

Mitochondrial DNA (mtDNA) analysis is widely recognized as a robust method for phylogenetic studies of animals (Avise 1986; Moritz et al. 1987; Simon et al. 1994; Wilson et al. 1985), but until recently it has been impractical to collect, align, and analyze large samples (e.g., >100) of orthologous sequences. Collecting sequences is reasonably straightforward now, and expedited by automated techniques using polymerase chain reaction (PCR) products. More efficient algorithms also are available now for personal computers, making alignment and analysis of large data sets workable (e.g., Leaché and Reeder 2002; Orti and Meyer 1997; Whiting et al. 1997). The purpose of this study was to employ mtDNA analysis and extensive taxonomic sampling to test long-standing genealogic hypotheses for vesper bats and to help resolve deep branching patterns within the family. I inferred relationships among 171 taxa by phylogenetic analysis of mtDNA characters (about 2.6 kilobases) encompassing 3 adjacent genes (12S rRNA, $tRNA^{Val}$, 16S rRNA).

MATERIALS AND METHODS

Taxon Sampling

I set out to sample about 1/3 of all vespertilionid species to represent taxonomic, morphologic, ecologic, behavioral, and geographic diversity equally within each subfamily, tribe, and (when appropriate) genus. Four years of acquiring samples by field collections or institutional

loans or from GenBank (http://ncbi.nlm.nih.gov/) resulted in a sample of 120 vespertilionids representing 110 species, 37 of 44 genera, and all subfamilies: Kerivoulinae, 3 of 22 species, 1 of 2 genera; Miniopterinae, 6 of 11 species, 1 of 1 genus; Murininae, 2 of 16 species, 2 of 2 genera; Nyctophylinae, 2 of 9 species, 1 of 2 genera; Vespertilioninae, 97 of 293 species, 32 of 38 genera (Appendix 1). I also sampled 51 bats representing all other families (except Craseonycteridae; Appendix 1). I sampled Molossidae relatively well (11 of 16 genera) as previous hypotheses have implied a close relationship between molossids and some vespertilionids (e.g., *Antrozous*; Simmons 1998; Simmons and Geisler 1998).

I relied on species identifications made by institutional collections. A voucher specimen for nearly all samples (Ruedas et al. 2000) is deposited in 1 of the following mammal collections: American Museum of Natural History, Carnegie Museum of Natural History, Field Museum of Natural History, Indiana State University Vertebrate Collection, Museum d'Histoire Naturelle de Geneve, Museum of Southwestern Biology at the University of New Mexico, Museum of Texas Tech University, National Museum of Natural History, Natural History Museum of Bern, Oklahoma State University Collection of Vertebrates, Royal Ontario Museum, Senckenberg Natural History Museum, Texas Cooperative Wildlife Collection at Texas A&M University, Transvaal Museum, Universidad Autónoma Metropolitana-Iztapalapa, Universidad Nacional

Autónoma de Mexico City, University of Memphis, Mammal Collection, University of Wisconsin Zoological Museum (Appendix 1). I was unable to locate voucher information for 14 samples, 7 of which were vespertilionids. There also was limited voucher information (e.g., sampling locality) for all 6 sequences obtained from GenBank, 2 of which were vespertilionids (Appendix 1).

Molecular Methods

I extracted genomic DNA from skeletal muscle or organ tissue samples with standard phenol methods (Longmire et al. 1997). I followed Van Den Bussche and Hoofer's (2000) methods to amplify and sequence a 2.6 kilobase-fragment of mtDNA encompassing 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes. Thus, I sequenced all 3 genes entirely in both directions with an assortment of external and internal primers (Van Den Bussche and Hoofer 2000).

Multiple Sequence Alignment

I aligned sequences in CLUSTAL X software (Thompson et al. 1997) following methods of Hoofer et al. (in press), who used 15.00:6.66 (default) and 5:4 values for gap cost ratio (Hickson et al. 2000). I refined both alignments by eye according to secondary structural models (Anderson et al. 1982; De Rijk et al. 1994; Springer and Douzery 1996). I also identified regions of alignment where positional homology was uncertain by using the "gap-sliding" method (Lutzoni et al. 2000, criteria 1–3, and 7, pp. 634–635). I was concerned primarily with large regions (e.g., up to 200

sites long) with multiple insertion/deletion events. I excluded all identified regions containing multiple gaps, but not every character (site) containing a gap. Some gapped-regions, typically small regions spanning only a few characters (sites), can be aligned unambiguously. A clear example is when 1 sequence contains 1 inserted nucleotide (or vice versa) within a highly conserved or constant region of nucleotides. In such cases, placement of 1 gap in all but 1 taxon (or 1 gap in 1 taxon) allowed assignment of positional homology among neighboring nucleotides. Alignment and phylogenetic analysis of 2 cost ratios nonetheless provides objectivity for gap placement in the relatively few, unambiguous, and small gapped-regions (Hickson et al. 2000).

Taxon Sets

I analyzed 4 separate sets of taxa to assess relationships at different taxonomic levels (Table 1). I first analyzed all taxa, including all sampled vespertilionids and representatives of all other bat families (except Craseonycteridae), using representatives of Hipposideridae, Pteropodidae, Rhinolophidae, and Rhinopomatidae as outgroups. These overall analyses were designed primarily to allow testing of vespertilionid monophyly without assuming any relationships within Chiroptera. I subsequently analyzed 3 truncated sets of taxa chosen to allow more appropriate analysis of relationships at different taxonomic levels: 1) within Vespertilionidae (128 taxa); 2) among all *Pipistrellus*-like bats (62 taxa); and 3)

within *Myotis* (39 taxa). I selected each taxon set, especially the outgroups, based on results from overall analyses and other studies (Hoofer et al. in press; Simmons and Geisler 1998; Teeling et al. 2000, 2002; Van Den Bussche and Hoofer 2001; Volleth and Heller 1994a; Volleth et al. 2001). For each taxon set, I performed new sequence alignments (with 2 gap-cost ratios) and assessed positional homology as described above, and assessed possible effects associated with choice of outgroup by including, and analyzing separately (for both alignments), multiple putative outgroups (Table 1). Thus, I analyzed 6 different alignments (2 per taxon set), and 8 total, including the overall taxon set.

Phylogenetic Inference

I coded nucleotides as unordered, discrete characters (G, A, T, C), multiple states as polymorphisms, and gaps as missing. I analyzed complete sequences for all 3 genes together, rather than by each gene separately, because all mitochondrial genes are linked and should have identical phylogenetic histories (Brown 1985; Wiens 1998), and it was impractical to perform separate and combined analyses as described for each alignment, outgroup choice, and taxon set.

I inferred phylogenetic relationships by using 2 optimality criteria: Bayesian Likelihood (Li 1996; Mau 1996; Rannala and Yang 1996) and Parsimony. I ran Bayesian analyses in MrBayes 2.01 (Huelsenbeck and Ronquist 2001) at least 1 million generations with 1 cold and 3 incrementally

heated Markov chains, random starting trees for each chain, and trees sampled (saved) every 10 generations. For both alignments within each taxon set, I ran a minimum of 9 independent analyses (sets of 3 analyses for 3 different taxa designated as the outgroup) to assess whether chains converged on the same posterior probability distribution, likelihoods reached stable values (Huelsenbeck et al. 2002), and outgroup choice affected topology. I also ran several other analyses using other outgroup species (but not sets of 3 analyses) to further assess affects of outgroup choice on topology and posterior probability distribution. I estimated burn-in values (initial set of unstable generations to be ignored) by empirical evaluation of likelihoods. The general time reversible (GTR) model with allowance for gamma distribution of rate variation (Γ) and for proportion of invariant sites (I) best fit the data regardless of taxon set (Modeltest; Posada and Crandall 1998). I did not define values for model parameters (from Modeltest) *a priori*, but instead treated them as unknown variables (with uniform priors) in each Bayesian analysis (Leaché and Reeder 2002).

I ran Parsimony analyses in PAUP* (test version 4.0b10; Swofford 2002), treated all characters and substitution types with equal probability, conducted heuristic searches with 10 random additions of input taxa and tree-bisection-reconnection (TBR) branch swapping (Swofford and Olsen 1990), and assessed reliability of clades via bootstrapping with 200 iterations (Felsenstein 1985). I

chose not to employ differential weighting schemes under Parsimony because they are poor attempts to correct for the same biological phenomena addressed by Bayesian analysis with the GTR + Γ + I model.

RESULTS

Alignments

Complete sequence for 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes averaged about 2,600 base pairs, ranging from 2,571 (*Otonycteris hemprichii*, Vespertilionidae) to 2,626 (*Diphylla ecaudata*, Phyllostomidae). Alignment of all sequences (default settings) resulted in 2,851 characters (12S, 37%; tRNA, 2.5%; 16S, 60.5%). I excluded 888 characters because of ambiguity in assessment of positional homology. This left 1,963 characters for analysis, 985 (50%) were constant, and 187 (10%) were parsimony-uninformative. The 3 truncated sets of taxa with progressively fewer taxa showed less divergence among sequences, fewer inserted gaps, fewer ambiguous characters, more characters available for analysis, and more characters constant among taxa (Table 2). The number of parsimony-uninformative characters also generally increased in smaller taxon sets (except in Vespertilionidae taxon set). Within taxon sets, alignments with the smaller gap-cost ratio (5:4) always resulted in more characters (i.e., more inserted gaps) and more ambiguous characters, but slightly fewer characters available for analysis ("Analyzed"; Table 2). The number of constant and parsimony-uninformative characters was

nearly identical between default and 5:4 alignments (within taxon sets).

Bayesian Analyses

Bayesian analysis of mtDNA provided considerable resolution to relationships across taxonomic levels. Approximately 70% of nodes for each taxon set were supported by posterior probabilities ≥0.95 (Figs. 2–5). Within taxon sets, Bayesian topologies and posterior probabilities essentially were identical regardless of alignment or choice of outgroup. There were only a few instances where support for a node ($P \geq 0.95$) was produced by analysis of 1 alignment but not the other. I treated these nodes as unresolved (denoted "?" in Figs. 2–5).

Among taxon sets, topologies and support values also were essentially identical, with regard to taxa shared between them. There were no supported conflicts ($P \geq 0.95$) between any analysis, and clades with significant posterior probabilities ($P \geq 0.95$) from analyses of more inclusive taxon sets also were significant in analyses of truncated taxon sets (Figs. 2–5). There were very few cases of greater resolution for truncated taxon sets, which included slightly more characters (Table 2). All differences essentially were limited to the specific value at which likelihoods stabilized (Table 3), specific estimates of model parameters (Table 3), and nodes with non-significant posterior probabilities ($P < 0.95$; Figs. 2–5).

Parsimony Analyses

Parsimony analysis provided about the same supported resolution (i.e., bootstrap values ≥ 50%) as Bayesian analysis, although not in analyses of overall taxon set and not with regard to some deep branching patterns within Vespertilionidae (Figs. 2–5). About 20% fewer nodes were supported by analyses with all sampled taxa (Fig. 2), and several critical nodes defining relationships among tribes and subfamilies of Vespertilionidae received weak support (i.e., bootstrap values < 50%; Figs. 3–5). Bootstrap topologies and support were essentially identical between analyses of alternative alignments within taxon sets, with only slight variation in specific lengths of bootstrap trees, exact bootstrap proportions, and consistency and retention indices (Table 4). They also were essentially identical between analyses based on different taxon sets (Figs. 2–5). There were no supported conflicts between analyses based on Parsimony and Bayesian methods, and nearly all nodes receiving support from 1 phylogenetic method also were supported by the other.

DISCUSSION

Phylogenetic Utility and Alignment of Ribosomal Gene Sequences

Bayesian and Parsimony analyses of mtDNA sequences from 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes provide a novel assessment of vespertilionid systematics. Resolution with concomitant support was afforded to the majority of relationships and at various taxonomic levels, among closely

related species and genera (Figs. 4 and 5), and among more distantly related subfamilies and families (Figs. 2 and 3). Ribosomal gene sequences are known for their versatile applicability in systematics, having been used successfully to resolve a wide range of relationships, from subspecific affinities (e.g., Leaché and Reeder 2002) to deepest branches in tree of life (e.g., Gouy and Li 1989; Perasso et al. 1989). They also have been used extensively in chiropteran systematics to resolve more intermediate-level relationships within and among families other than Vespertilionidae (Hoofer et al. in press; Hoofer and Van Den Bussche 2001; Lee et al. 2002; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2002). Such versatile applicability is facilitated not only by the volume of characters available for analysis, but also by secondary and tertiary structural elements and concomitant variation in rate of evolution along the length of RNA molecules (reviewed by Simon et al. 1994). These characteristics were present in all alignments regardless of taxon set, a fact exemplified by the number of sites along lengths of alignments that were ambiguous with regard to positional homology (Lutzoni et al. 2000) and excluded from phylogenetic analysis (Table 2).

Truncating taxa and performing new alignments for each set had several theoretical and realized advantages. Analysis of 4 sets of taxa and use of 2 phylogenetic methods, 2 independent alignments, multiple independent runs, and >30 designated outgroups allowed assessment of repeatability

(Figs. 2–5). It also addressed potential concerns with the Bayesian approach, namely subjectivity of prior distributions (e.g., initial tree topology) and mixing behavior and convergence of Markov chains (Huelsenbeck et al. 2002). Other advantages of truncating taxa were related to decreased divergences among ingroup and outgroup sequences. There was a corresponding decrease in homoplasy, ambiguity in gapped regions, and computer time. Sequence alignment always becomes increasingly problematic as more taxa are included, especially more divergent taxa, and this was my motivation for analyzing smaller sets of taxa.

Accordingly, the greatest difference between taxon sets involved the 2 sets with the largest and smallest number of taxa. For example, there were about 500 more characters available for analysis in the *Myotis* taxon set as compared to the overall taxon set. Although bootstrap support increased slightly for some nodes in the *Myotis* taxon set versus the overall set, resolution and branch support from all analyses essentially were the same for shared taxa. The simple explanation is that, although some informative characters were "salvaged" by truncating taxa and re-assessing positional homology, most were parsimony-uninformative.

Whereas ribosomal gene sequences have characteristics that contribute to their overall utility in studies of systematics, such characteristics also have important implications concerning provisional statements of homology (i.e., sequence alignment; Giribet and Wheeler 1999).

Alignment of orthologous sequences always is an important early step in evolutionary studies, but it is a critical early step for ribosomal gene sequences (mitochondrial and nuclear; Wheeler 1995; Wheeler et al. 1995). It can be problematic and (by implication) can affect phylogenetic reconstruction.

The crux of the difficulty is 2-fold: how to insert gaps (and maintain positional homology) in areas along the molecule that apparently have been riddled with several insertion/deletion events; and whether or not to exclude data that appears ambiguously-aligned. A corollary of the latter is how to delimit ambiguous data objectively. Both have been the source of debate recently (Hickson et al. 2000; Lutzoni et al. 2000; and citations therein). Sequence alignment typically is accomplished by 1 of several computer programs, yet different optimal alignments may be favored by different programs and by different parameter values (De Salle et al. 1994; Fitch and Smith 1983; Gatesy et al. 1993; Hickson et al. 2000; Lake 1991; Lutzoni et al. 2000; Mindell 1991; Morrison and Ellis 1997; Wheeler 1995; Wheeler and Gladstein 1991). The key parameter that can be modified for all programs is the cost ratio for opening and extending a gap. Hickson et al. (2000) demonstrated that alignments from the programs CLUSTAL, Divide and Conquer, and TreeAlign are robust over a range of cost ratios (i.e., insensitive to small changes), and that small opening gap costs (smaller than default values in a number of popular programs)

generally give more accurate results relative to a "known" phylogeny.

Previous study of mitochondrial ribosomal genes in bats has explored this possibility. Van Den Bussche and Hoofer (2001) found essentially no effect of gap-cost ratios (5:4, 10:5, 20:8, 30:5) on tree topology, bootstrap support, or consistency indices. The present study and Hoofer et al. (in press) found no supported differences in results with widely divergent ratios (15.00:6.66 and 5:4). Differences in alignments almost exclusively were in regions of ambiguous alignment regardless of choice of program or parameter values (see also Lutzoni et al. 2000). In this study, after excluding ambiguous blocks of data, choice of specific cost ratio had no effect on phylogeny reconstruction.

It is common practice in molecular systematics to exclude ambiguous blocks of data, with the correct intention of examining only homologous characters (e.g., Berbee 1996; Bruns et al. 1992; Hoofer and Van Den Bussche 2001; Lutzoni 1995; Springer 1997; Turbeville et al. 1992; Van Den Bussche and Hoofer 2001). This conservative approach clearly is preferred over the opposite extreme of including all sites with gaps coded as a 5^{th} character state, but the question remains of how to delimit potential ambiguous characters objectively. Subjectivity in defining ambiguous data can lead to different phylogenetic results depending on which mixture of characters is excluded (e.g., Mysticeti/Physeteroidea debate; Cerchio and Tucker 1998).

More objective criteria have been introduced recently to help define ambiguous data: alignment-ambiguous sites (Gatesy et al. 1993; Lake 1991; Waterman et al. 1992); elision (Wheeler 1995); "gap-sliding" (Lutzoni et al. 2000).

Alignment-ambiguous and elision criteria both employ information obtained from different alignments based on a wide range of gap-cost ratios (e.g., from 2:3 to 300:1). Characters that are not constant among all alignments are deemed ambiguous, and either are deleted (alignment-ambiguous) or downweighted (elision). Although this method is objective, it still requires arbitrary choice of the number and range of cost ratios. Furthermore, with extreme cost ratios otherwise unambiguous regions may be unstable among alignments, such that sites not violating positional homology are deleted (Lutzoni et al. 2000).

In this study, I used a slightly modified version of the "gap-sliding" approach of Lutzoni et al. (2000, pp. 634–635):

1. Inspect each region with at least one gap.
2. Slide the gap(s) laterally, in an outward direction from where they are located, to determine whether the nucleotide compositions at adjacent sites, and the secondary structure, can provide any justification for alternative position(s) for the gap(s).
3. Continue this outward sliding of gaps, in both directions, until the sliding of gaps, by one more

position cannot be justified, thus marking the boundaries for that region.

7. A first approximation of the limits of these regions can be made by using invariant flanking regions as a guide.

These criteria are easily employed when examining relatively few sequences, but more difficult with relatively large data sets (e.g., 171 taxa). With 9-point font on a 15-inch monitor, only about 40 taxa at a time can be visualized, requiring about 5 complete page scrolls between the 1st and 171st taxon, not to mention the approximately 100 page scrolls separating the beginning and end of a 2.6 kilobase alignment. I therefore relied on criterion #7 almost exclusively, defining boundaries of ambiguous regions by conserved, invariant flanking regions, such that the 1st and last sites of nearly every ambiguous region were invariant. This resulted in conservative assessments of positional homology, with about 500 to 1,000 sites excluded depending on taxon set. Probably some sites were excluded that did not violate positional homology, and perhaps even were parsimony-informative. However, a conservative approach seems more appropriate even if some informative characters (and resolution) are lost when aligning >100 ribosomal DNA sequences, rather than risking the inclusion of many non-homologous characters by attempting to salvage as many sites as possible region by region. Resolution afforded in the present study, based on this conservative approach, is

not heavily burdened by or highly sensitive to alignment of ambiguous regions.

Methods of Inference

I employed 2 phylogenetic methods that have different logical frameworks: Maximum Parsimony and the Bayesian approach to Maximum Likelihood. The approach under Parsimony searches for the tree with the fewest character conflicts (i.e., homoplasies; Swofford et al. 1996). Bayesian analysis is a relatively new approach to phylogeny reconstruction that operates under the same logical framework as Maximum Likelihood analysis (reviewed by Hall 2001; Huelsenbeck et al. 2002; Larget and Simon 1999; Lewis 2001). Both are optimality criteria that elicit information from the data through the likelihood function and employ character-based data and complex models of sequence evolution to search for trees and branch lengths most consistent with the data and specified model. These characteristics offer several advantages over Parsimony analysis (and other methods): 1) an objective system with which to estimate and choose character weights (Felsenstein 1981); 2) a more efficient system with which to reconcile important biologic phenomena for molecular data (e.g., among-site rate variation, unequal base frequencies, non-independence of substitutions); 3) access to the maximum amount of information in a set of DNA sequences (Whelan et al. 2001); and 4) more reliable estimates of phylogeny reconstruction under a variety of conditions (Huelsenbeck 1995; Yang 1996).

Bayesian and Maximum Likelihood analyses differ, however, because Bayesian analysis connects the likelihood function with prior and posterior distributions, and thereby provides posterior probabilities for hypotheses (i.e., trees and branch lengths) given the data and specified model of evolution. Maximum Likelihood analysis provides likelihood probabilities of data, given a hypothesis (i.e., tree and branch lengths) and specified model of evolution. This principal difference is what makes Bayesian analysis of large data sets feasible with current computer technology, and why Bayesian analysis is fast-becoming a preferred alternative when Maximum Likelihood analysis (especially with subsequent bootstrapping) requires an inordinate amount of computing time (e.g., Buckley et al. 2002; Hoofer et al. in press; Leaché and Reeder 2002; Murphy et al. 2001b). Furthermore, Bayesian analysis might eventually replace Maximum Likelihood analysis because reliability for inferred relationships (i.e., branch support) not only accompanies the tree estimation process, but also is a straightforward, parametric estimate. Reliability estimates for Maximum Likelihood trees (i.e., non-parametric bootstrapping) are de-coupled from the tree estimation process, computationally expensive or prohibitive, and controversial with regard to statistical probability (Efron et al. 1996; Hillis and Bull 1993).

Despite computational efficiency, Bayesian analysis is not without pitfalls. Two important concerns include sensitivity to chosen prior distributions and convergence and

mixing behavior of Markov chains (Huelsenbeck et al. 2002). Methods employed in the present study address both concerns. There were virtually no differences between analyses of multiple taxon sets, each with 2 independent alignments (=8 different sets of data) and multiple independent runs of at least 1 million generations with 1 cold and 3 incrementally heated Markov chains, random starting trees for each chain, and >30 designated outgroups (Figs. 2–5).

Furthermore, the Bayesian and Parsimony analyses showed marked agreement in topologies and levels of support. All relationships receiving strong support under Parsimony (≥ 75% bootstrap proportions) were supported by the Bayesian method ($P \geq 0.95$). A few relationships received weak Parsimony support but were supported strongly by Bayesian methods, and none that showed the reverse.

Despite subtle differences in levels of support from the 2 methods, none affected inferences of relationship. All of the following taxonomic recommendations are supported by ≥ 0.95 Bayesian probabilities and in ≥ 50% of the bootstrap proportions under Parsimony.

Superfamily Vespertilionoidea

With 1 exception, all traditional families (other than Vespertilionidae) for which I examined ≥2 representatives were supported as monophyletic assemblages. The exception was Hipposideridae relative to *Triaenops* (Fig. 2). The position of *Triaenops* may have been spurious, however, resulting from inadequate sampling of taxa within

Hipposideridae and closely related families; 3 hipposiderids (including *Triaenops*), 1 megadermatid, 1 rhinolophid, and 1 rhinopomatid. The small number of sampled taxa produced long branch lengths, a situation that can lead to decreased efficiency of phylogeny estimation (especially Parsimony). Furthermore, the terminal branch for *Triaenops* also was long. Whereas likelihood-based methods (e.g., GTR + Γ + I) typically help to overcome problems associated with long branches, it is better to break up potentially long branches by adding closely related taxa (Graybeal 1998; Hillis 1998; Poe 1998; Swofford et al. 1996). The purpose of this study was not to sample all bat families with equal density, but only to provide some representation of nearly all non-vespertilionid families. Further study with better focus on and sampling of hipposiderids and related families is necessary before making conclusions about this group.

This study affirms the long-held view that Vespertilionidae is closely associated with Molossidae and Natalidae (= superfamily Vespertilionoidea; Koopman 1984; Koopman and Jones 1970; Miller 1907; Smith 1976). Traditional classification of Vespertilionoidea, which is heavily weighted by characters of the wing and shoulder joint, includes several other families (Furipteridae, Mystacinidae, Myzopodidae, Thyropteridae), but there is no consensus for affinities of these 5 families (Koopman 1984, 1993; Miller 1907; Smith 1907, 1980). Recent studies of morphologic and molecular data contradict this traditional

classification, and suggest that all 5 families share greater affinities with noctilionoid families, or at least that they did not share a recent common ancestry with Molossidae, Natalidae, and Vespertilionidae (Hoofer et al. in press; Kennedy et al. 1999; Kirsch et al. 1998; Pierson 1986; Simmons and Conway 2001; Teeling et al. 2002; Van Den Bussche and Hoofer 2000, 2001).

The present study supports the revision by Hoofer et al. (in press) for superfamily Vespertilionoidea to include Molossidae, Natalidae, and Vespertilionidae, with Natalidae representing the basal lineage (Fig. 2). Although their study of mitochondrial and nuclear DNA sequences (about 4 kilobases) supported monophyly of Vespertilionidae, it included relatively few taxa, and in paticular did not include *Miniopterus*. Thus, the present study supports Hoofer et al. (in press) but, as discussed at length below, also recognizes a 4th family, Miniopteridae, within Vespertilionoidea.

Family Vespertilionidae

This study supports monophyly of traditional Vespertilionidae, with the notable exclusion of *Miniopterus* (Figs. 2 and 3). Thus, this study contradicts previous suggestions for removing Kerivoulinae (Sige 1974; Van Valen 1979) or Antrozoini (Simmons 1998; Simmons and Geisler 1998) from Vespertilionidae. Bayesian analyses gave no supported resolution among clades representing *Miniopterus*, Molossidae, and Vespertilionidae. All possible branching orders within

this trichotomy were depicted in various Bayesian analyses, but nodes received essentially no support (2 of 3 possibilities are shown; Figs. 2 and 3). Parsimony analyses gave moderate bootstrap support (66%) for *Miniopterus* and Molossidae as sister-taxa (Fig. 3), a relationship supported by immunologic distance data (Pierson 1986). Bayesian analyses also depicted *Miniopterus* sister to Molossidae but without statistical support.

Miniopterus also was as divergent or more divergent from Vespertilionidae than any recognized family (Figs. 2 and 3). I explored the possibility that my biased sampling of vespertilionids relative to other families somehow affected divergence estimates for *Miniopterus* or its phylogenetic placement and level of support. I performed several analyses that included only about 20 representatives of Vespertilionidae and all 6 *Miniopterus* (trees not shown), none of which affected phylogenetic inference for *Miniopterus* (Figs. 2 and 3). Furthermore, it is unlikely that inadequate sampling of *Miniopterus* or Vespertilionidae explains the extreme divergence and phylogenetic position of *Miniopterus*. I sampled multiple representatives of all putative subfamilies and tribes and most genera within the family, both New and Old World members, and sampled both taxonomic and geographic variation within *Miniopterus* reasonably well; 6 of 11 recognized species and 4 of 5 subgenera (*sensu* Koopman 1994) representing Australian, Ethiopian, Indomalayan, and Palearctic regions.

Miniopterus simply stands apart from Vespertilionidae based on explicit phylogenetic analysis of mtDNA sequences (Figs. 2 and 3), a fact not surprising considering they also appear markedly divergent in a number of other morphologic and biochemical aspects (Table 5). Some authors even have suggested removing *Miniopterus* from Vespertilionidae to its own family, Miniopteridae: Mein and Tupinier (1977) based on the observation that *Miniopterus*, but not Vespertilionidae, possesses a supplementary vestigial tooth between upper canine and 1st premolar; Gopalakrishna and Karim (1980) and Gopalakrishna and Chari (1983) based on a number of important embryologic features – *Miniopterus* apparently differs from vespertilionids in development of blastocyst, amniotic cavity, and yolk sac, and from all other mammals (let alone bats) in pattern of placental development; Tiunov (1989) based on uncharacteristic differences in morphology of tongue and male accessory glands; Pierson (1986) based on explicit analysis of immunologic distance data supporting reciprocal monophyly of Vespertilionidae and *Miniopterus*, Tomopeatinae, and Molossidae (Table 5).

Few have followed in recognizing Miniopteridae. To my knowledge, all syntheses of chiropteran systematics have favored Miller's (1907) arrangement (excepting Tomopeatinae), relegating Miniopteridae subfamily rank within Vespertilionidae (e.g., Corbet and Hill 1991, 1992; Koopman 1984, 1993, 1994; McKenna and Bell 1997; Yoshiyuki 1989). Explicit arguments against recognizing Miniopteridae

apparently are rare, but phyletic utility of mentioned characters most certainly has been an important concern. Dental characteristics have long-been perceived as adaptive and unreliable phyletic criteria, especially when characterizing families (e.g., Hill and Topál 1973; Topál 1970; Van Valen 1979). In this regard, all other mentioned characters are thought to be more reliable. How much more reliable (and at what taxonomic level) is a matter of debate, but the relative importance of some mentioned characters, namely developmental characters, and their role in systematics for classifying higher categories of mammals and other vertebrates is without doubt (Mossman 1987). In mammals, developmental characters are relatively conservative, possibly a result of their progression inside the maternal uterus (except monotremes) relatively free from direct environmental influences (Mossman 1953, 1987; Torpin 1976). For recognition of Miniopteridae, a greater concern more likely has been that none of the mentioned studies employed rigorous taxonomic sampling and/or explicit methods of phylogenetic analysis.

Volleth and Heller's (1994a) analysis of banded karyotypes ostensibly supported monophyly of Vespertilionidae including *Miniopterus* (Fig. 1). On 1 hand, their study is very important to vespertilionid systematics because it overcomes most criticisms leveled against previous studies. They studied a rather thorough taxonomic sample (primarily Old World members), including all putative subfamilies, and

employed explicit methods for phylogenetic analysis. Furthermore, chiropteran karyotypes are conservative at the genus level and seem especially useful for inferring inter-generic relationships of bats (Baker 1970; Bickham 1979; Volleth and Heller 1994a; Zima and Horácek 1985). Accordingly, others also have pointed to the study as positive evidence for including *Miniopterus* within Vespertilionidae (e.g., Simmons 2000, pp. 33–34). On the other hand, however, Volleth and Heller's (1994a) explicit methods provided no test of ingroup monophyly (i.e., monophyly of Vespertilionidae including *Miniopterus*) and do not validate their conclusion that "the subfamily Miniopterinae belongs to the Vespertilionidae and does not represent a separate family" (p. 31). The outcome of Volleth and Heller's (1994a) analysis was predetermined: a monophyletic clade, the ingroup, containing Vespertilionidae and *Miniopterus* (Fig. 1).

Their methods for dealing with outgroup taxa are typical of karyotypic studies, which usually follow (and cite) the outgroup comparison method of Maddison et al. (1984); Volleth and Heller (1994a) did not cite the outgroup comparison method explicitly, but described the same procedure nonetheless [see Hoofer and Van Den Bussche (2001, p. 132) for similar criticisms of another karyotypic study]. They inferred an hypothetical ancestor (or hypothetical ancestral states for each character) from multiple outgroups (1 molossid, *Molossus ater;* 2 natalids, *Natalus stramineus* and

N. tumidirostris) to polarize each character and maximize global parsimony relative to the ingroup. The inferred ancestor represented 1 taxon, and subsequently represented the designated outgroup in parsimony analysis of relationships among *Miniopterus* and vespertilionids. Because this method assumes ingroup monophyly, monophyly of Vespertilionidae inclusive of *Miniopterus* was untested. Ingroup monophyly is tested, at least minimally, only by concurrent phylogenetic analysis of ingroup and multiple successive outgroups (Baverstock and Moritz 1996). Considering these facts, it is noteworthy that karyotypic synapomorphies support monophyly of a clade containing *Myotis*, Kerivoulinae, Murininae, and Vespertilioninae (but no resolution among them) to exclusion of *Miniopterus*, and that the *Miniopterus* karyotype appears relatively distinct from Vespertilionidae, being unique by 6 autosomes and the X-chromosome (Volleth and Heller 1994a).

Simmons and Geisler's (1998) parsimony analysis of "total evidence" (superceding that of Simmons 1998) is another study suggesting monophyly of Vespertilionidae including *Miniopterus* (but excluding *Antrozous*). As discussed by the authors, however, relationships involving *Miniopterus* appeared in most-parsimonious reconstructions but received essentially no support from bootstrap or decay analyses. It is not surprising either, considering the study employed an abbreviated sampling scheme for "vespertilionids" emphasizing relationships among all chiropteran families, and

was based on extremely divergent and perhaps inappropriate outgroup taxa (i.e., Scandentia and Dermoptera; see Murphy et al. 2001; Teeling et al. 2000, 2002).

From the foregoing accounts, it is apparent that *Miniopterus* is markedly divergent in a number of characteristics from Vespertilionidae, with which it has been grouped almost universally in the past. Furthermore, whereas evidence supporting monophyly of Vespertilionidae inclusive of *Miniopterus* is limited, primarily to classical inferences based on certain morphologic features (e.g., Miller 1907; see Simmons 1998), several lines of evidence support monophyly of Vespertilionidae excluding *Miniopterus* (e.g., morphology, immunology, karyology, embryology, mtDNA). Evidence available for phylogenetic affinities of *Miniopterus* is in fact without consensus, pointing toward 2 alternative relationships: sister to Vespertilionidae; or sister to Molossidae. This study cannot exclude either hypothesis, but certainly adds to the list of evidence distinguishing *Miniopterus* from Vespertilionidae (Table 5), and from other recognized families as well.

It also seems appropriate to consider criteria previously used to assign family rank within Chiroptera. Miller's (1907) family-level assignments, based on comparative anatomy of wing, shoulder girdle, sternum and associated ribs, and dental formulae, provide the basis of current classification (e.g., Corbet and Hill 1991; Koopman 1993; McKenna and Bell 1997). Given the arbitrary nature of

assigning family rank, it is noteworthy that only 1 of Miller's (1907) 17 families is no longer recognized (Desmodontidae), and only 2 families have been added (Craseonycteridae and Mormoopidae). Craseonycteridae represents an addendum to Miller's arrangement, as it contains only 1 species (*Craseonycteris thonglongyai*) unknown to science until the 1970s (Hill 1974); it differs markedly from all other morphologic families (Hill and Smith 1981).

Justification for reclassifying the other 2 taxa was more circumstantial, and based on explicit presentations of several types of corroborating evidence: Forman et al. (1968) presented evidence from immunology, karyology, and sperm morphology to justify relegating Desmodontidae (*Desmodus*, *Diaemus*, *Diphylla*) subfamily rank within Phyllostomidae (Desmodontinae); and Smith (1972) presented new morphologic evidence combined with considerable correlative evidence from echolocation, hair structure, karyology, ectoparasites, brain morphology, and immunology to justify recognition of Mormoopidae (formerly a subfamily within Phyllostomidae containing *Mormoops* and *Pteronotus*). Furthermore, recognition of Mormoopidae has been almost universal since Smith's (1972) thesis, despite ample morphologic and molecular evidence for a sister-taxon relationship between Mormoopidae and Phyllostomidae (e.g., Baker et al. 2000; Hoofer et al. in press, Simmons and Conway 2001; Van Den Bussche et al. 2002; Van Den Bussche and Hoofer 2000, 2001). Thus, many of the same types of evidence used

previously to justify family-level assignments, also distinguish *Miniopterus* from Vespertilionidae.

There seems good justification for separating *Miniopterus* (subfamily Miniopterinae) from Vespertilionidae, based on results of this study alone or in combination with correlative information from several other data sources, and for recognizing *Miniopterus* in its own family, Miniopteridae. Pending further study I suggest Miniopteridae be placed *incertae sedis* within Vespertilionoidea (*sensu* Hoofer et al. in press), specifically within the clade containing Molossidae and Vespertilionidae. This nomenclatural arrangement facilitates recognition of both similarities and differences among vespertilionoid groups (Natalidae, Molossidae, Miniopteridae, Vespertilionidae).

Subfamilies of Vespertilionidae

This study supports monophyly of only 2 of the traditional subfamilies within Vespertilionidae (*sensu stricto*), Murininae and Kerivoulinae (*sensu* Miller 1907). Nyctophilinae (*sensu* Corbet and Hill 1991; Hill and Harrison 1987; Miller 1907) clearly "has no real validity" (Koopman 1985, p. 27). For mtDNA, *Nyctophilus* nestled deeply within a clade of *Pipistrellus*-like bats. Furthermore, Vespertilioninae (*sensu* Koopman 1994; McKenna and Bell 1997; Miller 1907) is paraphyletic relative to Murininae and Kerivoulinae. *Myotis* is markedly divergent from Vespertilioninae, and is sister to a clade containing Kerivoulinae + Murininae.

Whereas Bayesian and Parsimony analyses both supported monophyly of Kerivoulinae, Murininae, and *Myotis* (and close-association among them) regardless of outgroup or taxon set, support for Vespertilioninae (excluding *Myotis*) varied somewhat, and deserves comment. Bayesian analyses supported the Vespertilioninae clade ($P > 0.95$) regardless of taxon set or outgroup, but support from Parsimony analyses differed depending on choice of outgroup (compare Figs. 3 and 4). Bootstrap support was <5% (Fig. 3) from analyses with distantly related taxa designated as outgroups (i.e., pteropodids, rhinolophids, natalids, miniopterids), but ≥94% (Fig. 4) when less divergent taxa were the outgroups (i.e., kerivoulines, murinines).

Weak support for Vespertilioninae (excluding *Myotis*) with distantly related outgroups apparently was caused by instability in placement of *Corynorhinus*, *Lasiurus*, and *Scotophilus*; their positions always received weak support from bootstrap analyses with distantly related outgroups. Each of these clades has undergone long periods without cladogenesis or, equivalently, high rates of evolution (i.e., long branch lengths). Thus, using highly divergent taxa as outgroups may have caused misleading tree-estimation because of sequence divergence and resultant losses of genealogic information at the ends of those long branches (Felsenstein 1978). Parsimony analysis employing equal weight to all types of nucleotide changes provides no correction for substitution rate variation or among-site rate variation. On

the other hand, Bayesian analysis, which employs complex models of sequence evolution (Huelsenbeck et al. 2002; Whelan et al. 2001), supported the Vespertilioninae clade with both distantly and closely related outgroups ($P \geq 0.95$).

The present study, therefore, agrees with karyotypic data for Vespertilioninae exclusive of *Myotis* (Volleth and Heller 1994a). Based on the mtDNA tree (Figs. 2 and 3), there is more than 1 option available for subfamily assignment. For example, the clade comprising *Myotis*, Kerivoulinae, and Murininae could be placed into a single subfamily with each respective lineage given tribal status. However, I follow Volleth and Heller's (1994a) suggestion for recognizing *Myotis* in its own subfamily, Myotinae. This retains traditional subfamily names (i.e., Kerivoulinae and Murininae) and recognizes the distinctiveness and remarkable radiation of the myotine lineage. Unranked names can be employed in lieu of formal ranked names, facilitating phylogenetic classification (de Quieroz and Gauthier 1990, 1992, 1994). Simmons (1998) also recognized Myotinae, and actually was the 1st to use the subfamily name formally; however, mtDNA analysis does not support her reclassification of Vespertilioninae, which excludes both Antrozoini (*Antrozous* + *Bauerus*) and *Myotis*.

It is difficult to compare the mtDNA results with previous studies because there has been little previous resolution of deep branching patterns within the family. However, the mtDNA phylogeny is compatible with general

notions about vespertilionid evolution based on morphology and palaeomorphology. Despite Miller's (1907) placement of *Myotis* within Vespertilioninae, he specifically pointed out several features shared between *Myotis*, Kerivoulinae, and Murininae. For example, his remarks for *Murina* (p. 230) included, "External form peculiar in the projecting tubular nostrils only, the animals otherwise resembling the species of *Myotis* or *Kerivoula*..."

Additionally, the prevailing view of vespertilionid evolution holds that primitive forms had complete dentition (38 teeth), identical to presumed ancestral condition for all bats (i.e., as found in *Icaronycteris index*, the oldest fossil known for bats; Horácek 2001; Tate 1942). All vespertilionids apparently exhibit a generalized cranial and dental constitution that, unlike other family groups, essentially was unaffected by any specific rearrangements (Horácek 2001). Within the family, this general dental design has been modified somewhat, primarily by "clade-specific" reductions in incisive or premolar teeth, and presumably in connection with feeding adaptations (Tate 1942). Only 3 vespertilionid genera, *Myotis* (Myotinae) and *Kerivoula* and *Phoniscus* (Kerivoulinae), retain the primitive condition of 38 teeth. Although shared primitive characters give no indication of genealogy, *Myotis* and Kerivoulinae nonetheless have long-been regarded as the most primitive members of the family (Tate 1942). The fact that *Myotis*-like and kerivouline-like bats predominate the early fossil record

of Vespertilionidae certainly strengthens this argument (Czaplewski et al. in press; Horácek 2001). They have been placed in separate subfamilies, however, because *Myotis* lacks the skeletal peculiarities of Kerivoulinae (Miller 1907; Tate 1942). The mtDNA phylogeny is compatible with these views, and suggests that tooth reduction occurred independently in 2 lineages: early on in the evolution of Vespertilioninae; and subsequently during the evolution of Murininae.

Volleth and Heller's (1994a) karyotypic analysis provides additional support for a close relationship among Myotinae, Kerivoulinae, and Murininae (with no further resolution), but their results differed depending on which character-states were assumed ancestral for Vespertilionidae. Two sets of assumptions supported a clade containing Myotinae, Murininae, and Kerivoulinae, whereas a 3rd set left relationships of all subfamilies unresolved (their Fig. 6, p. 23). Volleth and Heller (1994a) chose to use the 3rd set of assumptions when constructing an overall tree for the family (which is shown in my Fig. 1) evidently because it "enables the first branch to be that of *Miniopterus* and avoids a closer relationship between *Myotis*, *Murina* [Murininae] and *Phoniscus* [Kerivoulinae], representatives of three subfamilies" (p. 24). Their actions may or may not be justified, but do seem conservative when making taxonomic conclusions. All relationships within Vespertilioninae were identical regardless of karyotypic assumptions.

The mtDNA phylogeny also is congruent with recent studies of the nuclear genome. For example, the same relationships among subfamilies were supported by analyses of DNA sequences from the Dentin Matrix Protein 1 gene (*DMP1*; Van Den Bussche et al. 2003) and Recombination Activating gene 2 (*RAG2*; Hoofer et al. in press). Analyses of DNA sequences from the von Willebrand Factor (vWF) gene and of short interspersed elements (SINEs) furthermore support a close-association between *Myotis* and Murininae (Kawai et al. 2002; kerivoulines were not sampled). Results from all 3 studies probably should be interpreted as tenative, however, until more vespertilionids can be examined. These studies focused on interfamilial relationships of bats and/or sampled relatively few species.

Subfamily Myotinae

Support for classifying *Myotis* in its own subfamily, Myotinae, contradicts its long-standing, morphologic association with the monotypic genus *Lasionycteris* (i.e., Myotini *sensu* Koopman 1970; McKenna and Bell 1997; Tate 1942). These results are not surprising because, other than cranial and dental similarity, there is little evidence supporting "Myotini;" *Lasionycteris* and *Myotis* differ in various morphologic characters (Miller 1907), including the baculum (Hamilton 1949; Hill and Harrison 1987), and have markedly different karyotypes (*Lasionycteris*, 2N = 20, FN = 48; *Myotis*, 2N = 44, FN = 50–53; Baker and Patton 1967; Zima and Horácek 1985). There has not been, until now, an

explicit test of "Myotini" monophyly. Neither Simmons (1998) nor Volleth and Heller (1994a) sampled *Lasionycteris*. Thus, their recommendation elevating "Myotini" to subfamily rank should be interpreted only with regard to *Myotis*, not for supporting monophyly of "Myotini." Myotinae as understood here includes only *Myotis*.

Myotis represents a remarkable radiation, with some 90 species in a distribution "equalled among mammals only by man and some of his commensals" (Findley 1972, p. 31). Despite diversification, species of *Myotis* have a rather undifferentiated phenotype, usually exhibiting subtle differences corresponding to feeding adaptations (piscivory, aerial planktonic feeding, terrestrial gleaning). As a result, classical inferences of species relationships have been difficult. Karyotypic studies have been of little help as well because *Myotis* is 1 of the most karyotypically conservative genera within Vespertilionidae (2N = 44, FN = 50–52; Bickham 1979a, 1979b; Bickham et al. 1986; McBee et al. 1986).

Current systematics of *Myotis*, chartered by Miller and Allen (1928) and Tate (1941b), essentially follows Findley (1972), who undertook a numerical taxonomic analysis of nearly all species known at that time. The analysis distinguished 3 phenetic groups, corresponding more or less to 3 major modes of flight and food procurement (=ecomorphs). Findley (1972) recognized each as subgenera: *Leuconoe*, typical foragers over water surfaces; *Selysius*, typical

aerial planktivores; *Myotis*, typical terrestrial gleaners. Each subgenus is about equally diverse (20–30 species each) and distributed widely throughout both the New and Old worlds. Koopman (1994) followed Findley's (1972) classification, but also recognized 2 rare South African species in a 4th subgenus, *Cistugo*; karyotypically, *Cistugo* probably warrants full generic rank (Rautenbach et al. 1993).

mtDNA analysis of nearly 1/3 of all recognized extant species of *Myotis*, including representatives from all zoogeographic regions and all subgenera except *Cistugo* (Appendix 1), provides well-supported resolution for many relationships within the genus. Mapping the subgeneric classification (= ecomorphs) onto the mtDNA tree suggests polyphyletic origins for each subgenus examined (Fig. 5). Thus, based on mtDNA data, morphologic and ecologic similarity as a rule do not reflect close relationship. For example, *M. lucifugus* is morphologically and ecologically the Nearctic equivalent of the Palearctic *M. daubentoni*, the type species of *Leuconoe* (relatively small bats with short ears that typically forage over water surfaces; Fenton and Barclay 1908; Jones and Rayner 1988). However, mtDNA analysis supports placement of these species into separate clades. Additionally, several mtDNA clades contain members of 2 or all 3 of the examined subgenera. Thus, morphologic and ecologic similarities defining each of the 3 subgenera represent convergent evolution.

In contrast, mtDNA analysis groups species according to geography, supporting a primary divergence between New and Old World *Myotis* (Fig. 5). Within the New World clade, mtDNA analysis supports 3 groups: 1 containing only Nearctic species (*ciliolabrum, septentrionalis, thysanodes, volans*); another containing only Neotropical species (*elegans, keaysi, riparius, ruber*); and a 3rd containing both Nearctic (*austroriparius, lucifugus, yumanensis*) and Neotropical (*albescens, dominicensis, fortidens, levis, nigricans*) species. The examined Old World species fall into either an Ethiopian clade (*bocagei, welwitschii*) or Indomalayan clade (*adversus, capaccinii, muricola, ridleyi*). Positions of the 2 Palearctic species sampled (*daubentoni, myotis*) essentially were unresolved within the Old World clade.

These results for *Myotis* agree markedly with a recent study by Ruedi and Mayer (2001), who reconstructed phylogenetic history of 13 American, 11 Palaearctic, and 6 other Old World species of *Myotis* based on DNA sequence data from 2 other mitochondrial genes (cytochrome *b* and *nd1*). Their separate and combined analyses of mitochondrial protein-coding genes provided no support for monophyly of any of the 3 subgenera (*Leuconoe, Myotis, Selysius*). The results supported 2 clades, 1 comprising all New World *Myotis* plus the Old World species *blythii*, and 1 comprising the rest of the sampled Old World species. Ruedi and Mayer (2001) sampled several species not sampled here (and vice versa), including various sibling species. For example, in addition

to *M. thysanodes*, they also sampled *M. natteri*, which together represent the Nearctic and Palearctic members of "fringed bats," respectively, and sometimes are recognized in a distinct subgenus, *Isotus* (Corbet and Hill 1991; Tate 1941b). All of their analyses contradicted monophyly of *Isotus*, placing the 2 species in widely divergent clades, suggesting that remarkable similarities in morphology and ecology are the result of convergent evolution.

Overall, relationships supported in this study and that of Ruedi and Mayer (2001) require reassessment of the evolutionary history of *Myotis*. Current classification suggests that 3 major ecomorphs within *Myotis* each evolved once during the early radiation of the genus, and the present worldwide distributions reflect secondary dispersal events across continents. In contrast, the mtDNA results suggest a less complex zoogeographic history for *Myotis*, and that much of the morphologic and ecologic similarity (i.e., ecomorphs) reflects repeated episodes of convergent evolution in different parts of the world. "This kind of deterministic evolution [(Losos et al. 1998)] has led to the situation in which a species [of *Myotis*] found today in America appears morphologically almost identical to its European counterparts, yet both are completely unrelated on the phylogenetic tree" (Ruedi and Mayer 2001, p. 447). Other lines of evidence either contradict the current classification or give credence to the mtDNA hypothesis, or both.

First, based on dental characteristics of mainly Old World species of *Myotis*, both Menu (1987) and Godawa Stormark (1998) concluded that the current classification (based on external morphology) does not reflect phylogeny. Second, independent evolution of *Myotis* species in different parts of the world with subsequent convergent adaptive radiations certainly is not an isolated case among bats or other vertebrate groups. The Old World fruit bats or flying foxes (Alvarez et al. 1999; Hollar and Springer 1997), along with cichlid fishes (Verheyen et al. 1996), ranid frogs (Bossuyt and Milinkovitch 2000), Caribbean anoles (Beuttell and Losos 1999), and river dolphins (Cassens et al. 2000) all represent well-documented examples. Third, the fossil record for *Myotis* does not contradict an early separation of New and Old World species. Whereas the earliest fossil bat assignable to *Myotis* is from early Oligocene of Europe (*Myotis misonnei*; Quinet 1965), similar, *Myotis*-like fossil bats (e.g., *Oligomyotis*) also were present in North America in the Oligocene, with the main radiation of *Myotis* in both Worlds occurring in the Miocene (Czaplewski et al. in press; Horácek 2001).

More species of *Myotis* need to be examined before making firm conclusions about the largest adaptive radiation of bats. The relationships supported in this study and the apparent polyphyly of currently recognized subgenera indicates that a full review of *Myotis* is needed. Full taxonomic revision of *Myotis* is beyond the scope of the

current study. However, mtDNA analysis suggests a classification reflecting geography, principally New and Old World clades. Provisionally, therefore, I suggest broadening the subgenus *Myotis* (type species *M. myotis*) to include the sampled Old World species, and allocating the sampled New World species to another subgenus. *Aeorestes* Fitzinger, 1870, which was applied to 4 New World species (*M. albescens*, *M. levis*, *M. nigricans*, and *M. villosissimus*; i.e., no type species was designated by Fitzinger), would be the oldest available name for this subgenus. Such classification may or may not prove universal for all New and Old World species (e.g., *M. blythii*; Ruedi and Mayer 2001), but it does provide a working hypothesis for future tests. mtDNA analysis also suggests further geographic structuring of monophyletic species assemblages within the New and Old World clades. Future studies with dense sampling of species should provide insight into the tempo and mode of the *Myotis* radiation.

Subfamily Vespertilioninae

mtDNA analysis provides little resolution to deep branching patterns within Vespertilioninae, which are characterized by short, internodal distances (Figs. 3 and 4). Such patterns often yield topologic instabilities and, therefore, weak statistical support, because cladogenesis apparently was rapid relative to the rate of molecular divergence (Avise et al. 1994; Pitra and Veits 2000). It is important to note that the primary vespertilionine lineages in which resolution is problematic for the mtDNA data is that

where traditional classifications also have failed. A reasonable interpretation of the inability of molecular and morphologic characters to resolve these basal relationships is to favor a contemporaneous diversification for many (if not all) primary vespertilionine lineages within a short period of time. However, mtDNA analysis does resolve several generic and suprageneric relationships that generally agree with previous hypotheses of relationship, especially with those based on the baculum and karyotype. At the same time, several of these relationships are inconsistent with existing classifications (e.g., Corbet and Hill 1991; Koopman 1984, 1985, 1993, 1994; McKenna and Bell 1997), and deserve some preface.

Vespertilioninae (*sensu stricto*) is an enormous complex of "closely interrelated genera separated in some instances by comparatively slender or even rather arbitrary distinctions, the patterns of relationship often obscured by parallelism or convergence" (Hill and Harrison 1987, p. 229). As such, classical studies of morphology (primarily of tooth reduction) have yielded unsatisfactory and incongruent results (reviewed by Hill and Harrison 1987). Numerous studies employing less-adaptive characters, most notably the baculum and karyotype, confirm this contention. They also have helped to define problematic genera (e.g., *Pipistrellus*, *Eptesicus*) and, to a much lesser extent, to discover relationships among them. However, there has been no comprehensive phylogenetic study of vespertilionine bats —

Hill and Harrison's (1987) bacular study was comprehensive, but their classification was based on general trends in bacular similarity and has been criticized for its subjectivity (e.g., see Frost and Timm 1992). Thus, the state of vespertilionine systematics is such that formal classifications reflect mostly traditional arrangements of genera and tribes, presumably for purposes of convenience, despite obvious indications of paraphyly or polyphyly. Two of the best known examples include *Pipistrellus* and Nycticeiini (e.g., *sensu* McKenna and Bell 1997), both of which clearly represent unnatural assemblages based on inferences from this and several other "non-classical" studies (Bickham 1979; Heller and Volleth 1984; Hill and Harrison 1987; Horácek 1991; McBee et al. 1986, 1987; Menu 1984, 1985, 1987; Morales et al. 1991; Ruedi and Arllettaz 1991; Volleth and Heller 1994a, 1994b; Volleth et al. 2001; Volleth and Tidemann 1991).

The following subdivisions of this section discuss tribal relationships as depicted in Figures 3 and 4, but also refer to a somewhat abbreviated phylogeny for Vespertilioninae that more clearly depicts resolution supported by mtDNA analysis (Fig. 6). A separate section is devoted to generic and tribal relationships of *Pipistrellus*-like bats.

Lasiurini.—This study supports monophyly of the tree bats in the New World genus *Lasiurus* (Fig. 3), which, owing to its extreme dental and cranial constitution, almost always

has been given special status within Vespertilioninae (i.e., Lasiurini *sensu* Tate 1942). Tate (1942, p. 229) wrote, "The Lasiurini may be regarded as having diverged farthest of all from the early vespertilionine bats." Karyology (Bickham 1979, 1987) and biochemical data (Baker et al. 1988) support this view. mtDNA analysis likewise distinguishes Lasiurini, but provides no supported resolution of its relationship among vespertilionines (Fig. 5).

Within *Lasiurus*, mtDNA analysis gives further support for monophyly of 2 recognized species groups (red bats, represented by *attratus*, *borealis*, *blossevillii*, *seminolus*; and yellow bats, represented by *ega* and *xanthinus*) and for distinction of a 3rd recognized group (hoary bats, represented by *cinereus*). Recognition of yellow bats as a distinct genus (*Dasypterus*) has been debated. Based on morphology, Tate (1942) and Hill and Harrison (1987) recognized *Dasypterus*, whereas Handley (1960) and Hall and Jones (1961) regarded all tree bats as congeneric (*Lasiurus*). Recent studies of karyotypes (Bickham 1979, 1987), allozymes (Baker et al. 1988), and restriction sites (Morales and Bickham 1995) favor recognition of only 1 genus. In contrast, mtDNA analysis demonstrates marked separation between yellow and red bats (and hoary bats), but this may not warrant generic revision because the position of hoary bats is unresolved. Previous recognition of *Dasypterus* was based primarily on support for sister relationship between red and hoary bats, a relationship clearly unresolved in this study (Fig. 3).

Antrozoini.—This study supports monophyly of Antrozoini (*Antrozous pallidus* + *Bauerus dubiaquercus* — *sensu* McKenna and Bell 1997). Based primarily on peculiarities of the muzzle, these 2 New World bats have always been considered a distinct vespertilionid lineage, but with uncertain affinities. Antrozoini traditionally was allied with the Australian *Nyctophilus* and *Pharotis* (subfamily Nyctophylinae *sensu* Miller 1907; Koopman and Jones 1970), a relationship later considered superficial (Koopman 1970; Pine et al. 1971). More recently, Antrozoini was given family rank (Antrozoidae) and allied with Molossidae (within "Molossoidea") based on "total evidence" analyses (Simmons 1998; Simmons and Geisler 1998). However, there was essentially no statistical support for this placement of Antrozoini. Also, all analyses of Simmons (1998) and Simmons and Geisler (1998) were based on the assumption that Vespertilioninae (including Nyctophylinae) excludes Antrozoini (and Myotini), presumably because Antrozoini possesses unique muzzle morphology. Thus, character states of each character for the single taxon "Vespertilioninae" apparently were formulated through combined observations of several vespertilionines ("Pipistrellini" + "Eptesicini" + "Nycticeiini" + "Plecotini" + "Lasiurini" + "Vespertilionini") without regard to Antrozoini, an unwarranted assumption based on mtDNA analysis and several studies of morphology, karyology, and ecology (e.g., Bickham

1979; Breed and Inns 1985; Freeman 1998; Hill and Harrison 1987; Pine et al. 1971).

The present study supports *Antrozous* + *Bauerus* within Vespertilioninae as part of an unresolved trichotomy with *Baeodon* and *Rhogeessa*, and with conspicuously little divergence relative to other relationships within the subfamily (Fig. 3). Both *Baeodon* and Rhogeessa contain few species, all endemic to the New World, that are extremely similar morphologically (Miller 1907; Tate 1942). Some authors have relegated *Baeodon* subgeneric rank within *Rhogeessa* (Jones et al. 1988; Koopman 1993; McKenna and Bell 1997). Results from mtDNA analysis provisionally support generic recognition for *Baeodon* (Corbet and Hill 1991; Miller 1906, 1907; Hill and Harrison 1987; Tate 1942); divergence between *Baeodon* and *Rhogeessa* is about twice that within *Rhogeessa* (Fig. 3).

A close relationship between *Baeodon*, *Rhogeessa*, and Antrozoini might be considered surprising because of their dissimilarity in external morphology. However, *Baeodon* and *Rhogeessa* essentially are no more different from Antrozoini than from *Otonycteris*, with which they have been allied traditionally (Nycticeiini; *sensu* Koopman and Jones (1970). A close relationship among these taxa is plausible zoogeographically and is suggested by karyotypes (Baker et al. 1985; Bickham 1979; see also Volleth and Heller 1994a). I suggest recognizing this close relationship by placing

Baeodon and *Rhogeessa* in the tribe Antrozoini, along with *Antrozous* and *Bauerus*.

Scotophilini.—This study supports monophyly of *Scotophilus*, including several Ethiopian and 2 Indomalayan species (Koopman 1994; Nowak 1999), and adds further evidence for its distinction, perhaps early separation from other vespertilionines. *Scotophilus* traditionally has been grouped within "Nycticeiini," but Hill and Harrison (1987) concluded that the baculum of *Scotophilus* was sufficiently distinct among vespertilionines to warrant tribal status. They noted that *Scotomanes* possesses several bacular similarities with *Scotophilus*, and recognized both genera within the tribe Scotophilini. mtDNA analysis contradicts any close association between *Scotomanes* and *Scotophilus* (and traditional "Nycticeiini"), but agrees with bacular data in distinguishing *Scotophilus*. In its mtDNA, *Scotophilus* is the most divergent genus (or tribe) examined within Vespertilioninae (Fig. 3).

This study offers no resolution to the relationship of *Scotophilus* among other vespertilionines. Other data also offer little resolution, although some morphologic and karyotypic evidence favors an association between *Scotophilus* and *Antrozous*, *Rhogeessa*, or *Otonycteris* (Baker et al. 1985; Bickham 1979; Hill and Harrison 1987; Volleth and Heller 1994a). Without consensus of relationship, and in light of results of this and Hill and Harrison's (1987) study, it

seems reasonable to assign *Scotophilus* to its own tribe (Scotophilini) pending further study.

mtDNA analysis provides resolution of relationships among species of *Scotophilus*, suggesting a distant relationship between the 2 Indomalayan forms (*heathi* and *kuhlii*) and close relationship among 4 Ethiopian forms (*borbonicus*, *dinganii*, *leucogaster*, *nux*; Fig. 3). However, taxonomy of *Scotophilus*, especially Ethiopian forms, has been controversial with little consensus for definition of species (e.g., see Koopman 1994; Robbins et al. 1985). Application of some species names within *Scotophilus* (e.g., *borbonicus*, *nux*, *viridus*) is so unreliable and confused that I reserve making certain conclusions until I examine the voucher specimens and verify their identifications. Based on mtDNA analysis, Ethiopian and Indomalayan forms of *Scotophilus* represent a monophyletic assemblage, and sequence divergence among all forms examined are typical of at least species-level comparisons (Fig. 3).

Plecotini.—The plecotine bats, or large-eared bats, comprise 11 species of the genera *Barbastella*, *Corynorhinus*, *Euderma*, *Idionycteris*, and *Plecotus* (Nowak 1999), and represent the only suprageneric group within Chiroptera that is Holarctic in distribution (Koopman 1970). Although rarely tested with explicit methods, there is considerable morphologic and karyotypic evidence supporting monophyly of Plecotini (Frost and Timm 1992; Handley 1959; Leniec et al. 1987; Tate 1942), as demonstrated in a recent consensus

analysis of published trees (i.e., "super"-tree analysis; Jones et al. 2002). The present study neither supports nor refutes monophyly of Plecotini; each genus was supported as monophyletic (for which I sampled ≥ 2 members), but there was no supported relationship among them (Fig. 6). One exception was Bayesian support for a sister relationship between *Euderma* and *Idionycteris*, a relationship previously inferred from morphologic and karyotypic data (e.g., Bogdanowicz et al. 1998; Tumlison and Douglas 1992).

There also has been some debate over rank status of some plecotine genera (e.g., *Corynorhinus*, *Idionycteris*). This study favors Tate's (1942) opinion for distinction of 5 plecotine genera, as each is as divergent or more divergent from each other than are other recognized genera (e.g., *Antrozous* versus *Rhogeessa*; Fig. 3). If monophyly of Plecotini is assumed, this study suggests an early separation of the group, as well as each respective genus, from the common ancestor of Vespertilioninae, an observation that may explain why there is little consensus for relationships and rank status among plecotine genera (e.g., Bogdanowicz et al. 1998; Frost and Timm 1992; Handley 1959; Hill and Harrison 1987; Tumlison and Douglas 1992).

Otonycteris.—Affinities of *Otonycteris hemprichii*, the sole species of the genus endemic to semi-arid parts of the Palearctic, have long been a source of debate. Although traditionally allied with *Nycticeius*, *Rhogeessa*, and *Scotophilus* (Nycticeiini *sensu* Koopman and Jones 1970),

recent studies of phallus morphology (Pine et al. 1971), other morphologic data (Horácek 1991), and karyotypic data (Baker et al. 1985; Bickham 1979; see Volleth and Heller 1994a) indicate a possible close association between *Otonycteris* and *Antrozous* + *Bauerus*, as well as some traditional "nycticeiines." Other studies of karyotypes (Qumsiyeh and Bickham 1993; Zima et al. 1992), morphology and karyotypes (Bogdanowicz et al. 1998), and to some extent bacular morphology (Hill and Harrison 1987) have allied *Otonycteris* with Plecotini. The present study contradicts any close association between *Otonycteris* and *Nycticeius*, but it cannot exclude either hypothesis of relationship with Antrozoini (including *Baeodon* and *Rhogeessa*) or plecotine genera (Fig. 6). Considering these results, and without consensus of relationship from other sources, I suggest *incertae sedis* placement *Otonycteris* within Vespertilioninae.

These results differ somewhat from Hoofer and Van Den Bussche (2001), who published a subset of the present study (same mtDNA sequences, smaller taxonomic sample) with specific focus on taxonomic position of *Otonycteris*. Unlike the present study, their parsimony analyses supported *Otonycteris* as sister to Antrozoini (including *Baeodon* and *Rhogeessa*; bootstrap value = 94%). There are several likely explanations for differences in supported resolution between this study and that of Hoofer and Van Den Bussche (2001). First, they examined a much smaller taxonomic sample, which undoubtedly reduced overall homoplasy. Second, they employed

differential weighting schemes under parsimony analysis. Without such weighting schemes, particularly successive weighting (Farris 1969), the majority of relationships in their tree including position of *Otonycteris* was unresolved. Third, Hoofer and Van Den Bussche (2001) did not exclude ambiguous characters from sequence alignment, resulting in nearly 1,000 characters more than in the present study. These additional characters, some of which would have exhibited ambiguous positional homology, and the various weighting schemes, probably account for incongruence with the present results. Thus, Hoofer and Van Den Bussche's (2001) results should be interpreted with caution as they are not affirmed in the present study and perhaps were influenced by "ambiguous" data.

Pipistrellus-like Bats

There is considerable uncertainty regarding relationships within and among the relatively large, cosmopolitan complex of bats that, for purposes of convenience, typically is referred to as *Pipistrellus*-like bats (or "pipistrelloid" bats). The group was originally recognized by Tate (1942), who described cranial and dental characteristics within Vespertilioninae and placed all "genera coderived with *Pipistrellus*," characterized by a shortened rostrum and reduction of tooth number, into a single tribe that he called "Pipistrellini." Subsequent classifications have recognized the group but by the name of Vespertilionini, presumably because *Vespertilio* Linnaeus,

1758 has priority over *Pipistrellus* Kaup, 1829 (Koopman 1984; McKenna and Bell 1997). The group also has been redefined several times since Tate (1942), but essentially the only consensus has been for the removal of *Barbastella* (*barbastellus* and *leucomelas*) and its placement within Plecotini (Bogdanowicz et al. 1998; Handley 1959; Hill and Harrison 1987; Koopman 1984, 1985; McKenna and Bell 1997).

Hill and Harrison's (1987) bacular study redefined the group by including *Scoteanax*, *Scotorepens*, and *Scotozous* (formerly regarded as "nycticeiines"), and by dividing Vespertilionini into 2 tribes, formally recognizing a distinction between *Pipistrellus*-types (Pipistrellini) and *Eptesicus*-types (Vespertilionini; Table 6). Their classification also recognized 7 subgenera within *Pipistrellus* (*Pipistrellus*, *Hypsugo*, *Falsistrellus*, *Perimyotis*, *Arielulus*, *Vespadelus*, *Neoromicia* — the latter 2 formerly classified within *Eptesicus*); some of which were given full generic rank after detailed morphologic or biochemical analyses (*Hypsugo*, Horácek and Hanak 1985–1986; Ruedi and Arlettaz 1991; *Falsistrellus*, Adams et al. 1987a,b; Kitchener et al. 1986; *Perimyotis*, Menu 1984, 1987; *Arielulus*, Csorba and Lee 1999).

Karyotypic studies also have helped elucidate relationships among *Pipistrellus*-like bats (Volleth 1987, 1989; Volleth et al. 2001; Volleth and Heller 1994a; Volleth and Tidemann 1989, 1991). They redefined the group as a whole by including *Nyctophilus*, whose specialized morphology

has always been translated into at least tribal status within Vespertilioninae if not subfamilial status within the family. They further confirmed the polyphyletic origin of *Pipistrellus* (*sensu* Hill and Harrison 1987), recognizing 2 closely related tribes and elevating several subgenera to generic rank: Pipistrellini, including true *Pipistrellus* (i.e., subgenus *Pipistrellus*) along with *Glischropus*, *Nyctalus*, and *Scotozous*; and Vespertilionini, including members of 4 former subgenera (*Falsistrellus*, *Hypsugo*, *Neoromicia*, *Vespadelus*) and *Chalinolobus*, *Nyctophilus*, *Philetor*, *Scotorepens*, *Tylonycteris*, and *Vespertilio*. *Eptesicus*, together with *Hesperoptenus*, formed a 3rd, more distantly related tribe (Eptesicini; Fig. 1).

The present study is congruent with bacular and, especially, karyotypic revisions of *Pipistrellus*-like genera and tribes. For example, mtDNA analysis supports the inclusion of *Nyctophilus* within the *Pipistrellus*-like bats, and provides no validation for Nyctophilini (*sensu* McKenna and Bell 1997) or Nyctophilinae (*sensu* Miller 1907; Hill and Harrison 1987). The mtDNA results differ somewhat in supporting inclusion of the New World genera *Lasionycteris* and *Nycticeius*, and exclusion of the 2 New World "*Pipistrellus*" (*hesperus* and *subflavus*); however, none of these New World taxa were studied by Volleth and Heller (1994a), or by any other comprehensive phylogenetic analysis. The present study also supports classification of *Pipistrellus*-like bats into 3 tribes (Nycticeiini,

Pipistrellini, Vespertilionini), corresponding closely with Volleth and Heller's (1994a) arrangement and further documenting a sister relationship between Pipistrellini and Vespertilionini.

There are only 2 principle differences between mtDNA and karyotypic results (Volleth and Heller 1994a). First, the position of *Vespertilio* was unresolved within the clade containing Pipistrellini and Vespertilionini rather than supported within Vespertilionini (*sensu* Volleth and Heller 1994a). This unresolved placement, although not contradictory to monophyly of Vespertilionini (*sensu* Volleth and Heller 1994a), suggests further study is needed to assess certain affinities of *Vespertilio*. The 2nd difference deals with nomenclature, resulting from differences in taxonomic sampling. mtDNA analysis agrees with karyotypic data for distinction of *Eptesicus* (tribe Eptesicini) from other *Pipistrellus*-like bats (i.e., tribes Pipistrellini and Vespertilionini), but also documents a similar distinction for other genera that were not studied karyologically (i.e., *Glauconycteris*, *Histiotus*, *Lasionycteris*, *Nycticeius*, *Scotomanes*). Volleth and Heller's (1994a) Eptesicini included only *Eptesicus* and *Hesperoptenus*. If only 3 tribes of *Pipistrellus*-like bats are to be recognized, as supported by this study, then Nycticeiini (rather than Eptesicini) is the valid name for the tribe that includes *Nycticeius* (Fig. 6); *Nycticeius* Rafinesque 1819 and Nycticeini Gervais, 1855

have priority over *Eptesicus* Rafinesque 1820 and Eptesicini Volleth and Heller 1994a, respectively.

Thus, mtDNA analysis agrees markedly with karyotypic data in supporting 3 major groups of *Pipistrellus*-like bats, tribes Nycticeiini, Pipistrellini, and Vespertilionini (Fig. 6). Support for such classification also has several implications at the genus level, nearly all of which are congruent with either karyotypic or bacular data, or both.

Polyphyly of "Pipistrellus".—mtDNA analysis affirms the often-discussed polyphyletic origin of *Pipistrellus* (*sensu* Hill and Harrison 1987), agreeing with karyotypic data in confining true *Pipistrellus* (i.e., subgenus *Pipistrellus*; Hill and Harrison 1987) to tribe Pipistrellini. Within Pipistrellini, mtDNA analysis also suggests that *Pipistrellus* (*sensu stricto*) may be paraphyletic with regard to *Nyctalus*; *Nyctalus* is related to *Pipistrellus* subgroup (*pipistrellus* and *nathusii*) more closely than either the *coromandra* (*coromandra* and *tenuis*) or *javanicus* (*abramus* and *javanicus*) subgroups (Hill and Harrison 1987).

Thus, the true definition of *Pipistrellus* remains uncertain, and according to mtDNA analysis *Nyctalus* may be treated as a member of *Pipistrellus*, or as a separate genus. The latter case would, to avoid paraphyletic taxa, require introduction of a new genus to include both *coromandra* and *javanicus* subgroups of Hill and Harrison (1987) due to position of *Pipistrellus pipistrellus* (i.e., types species of *Pipistrellus*). This in fact may be preferred eventually, as

karyotypic analysis suggests a similar paraphyletic situation for *Pipistrellus* (within Pipistrellini), with *Scotozous* being related to the *coromandra* and *javanicus* subgroups more closely than *pipistrellus* (*pipistrellus* and *nathusii*) or *kuhlii* (*kuhlii*) subgroups (Volleth and Heller 1994a; Fig. 1). Such revision is beyond the scope of this study and more thorough examinations will be necessary to resolve the situation. I suggest provisionally treating *Nyctalus* as a member of *Pipistrellus* (as proposed by Simpson 1945).

mtDNA analysis affirms previous contentions for distinction of *Hypsugo*, *Neoromicia*, and *Vespadelus* from *Pipistrellus* (*sensu stricto*), as sampled members of each taxon are supported in the tribe Vespertilionini (not Pipistrellini). Thus, these results also strongly corroborate previous reclassifications of the genus *Eptesicus* that excluded *Neoromicia* and *Vespadelus* (Heller and Volleth 1984; Hill and Harrison 1987; Volleth 1987, 1989; Volleth et al. 2001). Although not well-supported, mtDNA analysis does not refute monophyly of *Vespadelus* (Fig. 3), and supports karyotypic data for close affinities between *Vespadelus* and other Australian genera (*Chalinolobus*, *Nyctophilus*; Volleth et al. 2001; Volleth and Heller 1994a; Volleth and Tidemann 1991).

Within Vespertilionini, however, mtDNA analysis contradicts monophyly of both *Hypsugo* and *Neoromicia* (*sensu* Hill and Harrison 1987): *N. brunneus* and *N. rendalli* are supported as monophyletic, but *N. somalicus* is supported

sister to *Laephotis;* all 3 sampled species of *Hypsugo* are distantly related, with the position of *H. savii* essentially unresolved within Vespertilionini, position of *H. nanus* unresolved within a clade of *Neoromicia* and *Laephotis,* and position of *H. eisentrautii* supported sister to *Nycticeinops.*

Thus, as with *Pipistrellus* (*sensu stricto*) the definitions of *Hypsugo* and *Neoromicia* are questionable. Volleth and Heller (1994a) also documented polyphyly of *Hypsugo* (*sensu* Hill and Harrison 1987), resulting in them transferring the species *stenopterus* from *Hypsugo* (back) to *Pipistrellus.* Also, mtDNA analysis clearly refutes an association of species *hesperus* with *Hypsugo* or *Pipistrellus* (discussed below). Pending further study, this study supports restricting the genus *Hypsugo* to the type species *H. savii* (Kolenati) 1856 and transferring the species *eisentrautii* from *Hypsugo* to *Nycticeinops.*

The situation with (*Hypsugo) nanus* is confounded somewhat by poylyphyly of *Neoromicia.* Whereas *N. brunneus* and *rendalli* clearly represent a monophyletic group, the type species of *Neoromicia, N. somalicus* (= *Eptesicus zuluensis;* Roberts 1926), clearly is sister to *Laephotis.* In avoiding polyphletic taxa, the name *Neoromicia* would be unavailable for *brunneus* and *rendalli.* Provisionally, therefore, I recommend retaining the genus *Neoromicia* (i.e., not lumping it within *Laephotis*), but restricting it to the type species *N. somalicus.* I further suggest provisional allocation of (*Hypsugo*) *nanus* and (*Neoromicia*) *brunneus* and *rendalli* to a

separate, as yet unnamed genus. This seems the best alternative pending further study of additional putative members of *Hypsugo* (*sensu lato*), *Laephotis*, and *Neoromicia* (*sensu lato*).

mtDNA analysis revealed no support for including the 2 New World "*Pipistrellus*" (*hesperus* and *subflavus*) within any of the 3 tribes of *Pipistrellus*-like bats, further documenting polyphyly of *Pipistrellus* (and *Hypsugo; sensu* Hill and Harrison 1987). mtDNA analysis also documents marked divergence between *hesperus* and *subflavus*, affirming what has been suspected for nearly a half-century. For example, Hamilton (1949) discovered "very great dissimilarity" between bacula of *hesperus* and *subflavus* (and *Pipistrellus pipistrellus;* Leydig 1857), leading him to suggest "generic, or at least subgeneric differences" for the 2 American species. Baker and Patton (1967) likewise documented "extremely significant" differences between *hesperus* and *subflavus* karyotypes, leading them to posit, "It would seem doubtful that these two species are very closely related, for such would necessitate the complete loss of a major chromosome in the evolution of *P. hesperus* from *P. subflavus* or a common ancestor. Possibly, the 2 species are distantly related, acquiring their distinctive karyotypes through a series of changes from the karyotype of some remote ancestor" (p. 281).

Subsequent studies of both *hesperus* and *subflavus* confirm these early assertions, and further distinguish each

from *Pipistrellus* (*sensu lato*). Menu (1984) placed *subflavus* in a new genus that he called *Perimyotis*, based on a comparative study of dental, skeletal, and bacular characters among vespertilionine bats. Horácek and Hanak (1985, 1985–1986) likewise distinguished *subflavus* (=genus *Perimyotis*), and furthermore placed *hesperus* in a new genus that they called "*Parastrellus*," based on fundamental differences in several anatomical characters (dentition, cranium, baculum, skeleton) [However, the name "*Parastrellus*" is not properly available under the rules of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999) and as such was a *nomen nudem* in these publications. I use "*Parastrellus*" in this paper to facilitate discussion of this taxon. I intend, in an appropriate publication, to make the existing *nomen nudem*, "*Parastrellus*," available as the valid name for this genus.]

Despite these recommendations, Hill and Harrison (1987) opted to retain both *subflavus* and *hesperus* within *Pipistrellus*, although they placed the former in its own subgenus (*Perimyotis*), and separated the latter from true *Pipistrellus* (i.e., subgenus *Pipistrellus*) in the subgenus *Hypsugo*. Most recent authors have followed Hill and Harrison's (1987) recommendations (e.g., Koopman 1985, 1993; McKenna and Bell 1997).

The present study represents the 1st study of *hesperus*, *subflavus*, and several other representatives of *Pipistrellus*

(*sensu lato*) since Hill and Harrison (1987), and provides further justification for recognizing "*Parastrellus*" and *Perimyotis*. Considering the breadth of morphologic evidence associating both taxa with other *Pipistrellus*-like bats (e.g., Hill and Harrison 1987; Tate 1942), a reasonable interpretation of the mtDNA results is to essentially restate Baker and Patton's (1967) opinion: "*Parastrellus*" *hesperus* and *Perimyotis subflavus* each represent distantly related lineages that perhaps separated very early from other *Pipistrellus*-like bats. However, whether these taxa shared a common ancestry with *Pipistrellus*-like bats or have closer affinities with other vespertilionine tribes is clearly unresolved in this study. I recommend *incertae sedis* placement for "*Parastrellus*" and *Perimyotis* within Vespertilioninae.

Chalinolobus and Glauconycteris.—Australian *Chalinolobus* and Ethiopian *Glauconycteris* almost always have been allied together, with *Glauconycteris* frequently regarded as a subgenus of *Chalinolobus*, principally due to external similarity; although members of both taxa share several cranial and dental characteristics, they are united at once by the conspicuous, rather unusual characteristic of fleshy, outwardly projecting lobes at corners of mouth (Corbet and Hill 1991; Dobson 1875, 1878; Hayman and Hill 1971; Koopman 1971, 1993; McKenna and Bell 1997; Miller 1907; Peterson 1982; Peterson and Smith 1973; Ryan 1966; Skinner and Smithers 1990). The present study provides further

justification for generic distinction between *Chalinolobus* and *Glauconycteris*. Also, like bacular data (Hill and Harrison 1987), mtDNA data refute a recent shared ancestry between them, associating *Glauconycteris* with *Eptesicus* and its allies (tribe Nycticeiini), and *Chalinolobus* with other, primarily Australian *Pipistrellus*-like bats (tribe Vespertilionini; Fig. 6). *Glauconycteris* has yet to be included in a comprehensive study of karyotypes, but mtDNA results are congruent with Volleth and Heller's (1994a) placement of *Chalinolobus* within Vespertilionini (Fig. 1).

Nycticeius.—Definition of *Nycticeius* has been modified continually in the past century, but by the mid-1980s finally was restricted to include only 2 species, the Nearctic *humeralis* and Ethiopian *schlieffeni* (Corbet and Hill 1986; Kitchener and Caputi 1985; reviewed by Hill and Harrison 1987). Hill and Harrison (1987) subsequently placed *schlieffeni* in a new genus, *Nycticeinops*, a placement affirmed by karyology (Bickham 1979; Ruedas et al. 1990); although, karyotypes of *humeralis* and *schlieffeni* have yet to be analyzed concurrently.

The present study, therefore, is further justification for generic distinction between *Nycticeius humeralis* (tribe Nycticeiini) and *Nycticeinops schlieffeni* (tribe Vespertilionini; Fig. 6). As defined here and by bacular data, the genus *Nycticeius* is monotypic including only *humeralis*. Unlike bacular data, which defined *Nycticeinops* as monotypic (including only *schlieffeni*), the present study

supports provisional allocation of the species *eisentrautii* from *Hypsugo* to *Nycticeinops* (along with *schlieffeni*).

Histiotus and Laephotis.—The genera *Histiotus* and *Laephotis* are 2 more groups of long-eared bats whose affinities always have been speculative. Classical studies of morphology, primarily specializations of the ear and bullae (i.e., large ears), indicate a close association between the 2 groups, suggesting that together they represent a specialized offshoot from "the *Eptesicus* stem" (*sensu lato;* Miller 1907; Tate 1942). Even early on the association seemed doubtful. For example, in his remarks for *Laephotis* Miller (1907, p. 215) wrote, "The very striking similarity of this African genus to the South American *Histiotus* may be the result of parallel development from some *Eptesicus*-like ancestry."

The present study confirms Miller's suspicion. mtDNA analysis agrees with bacular data (Hill and Harrison 1987) in supporting a close relationship between *Histiotus* and *Eptesicus* (*sensu stricto;* tribe Nycticeiini), and between *Laephotis* and *Neoromicia* (*sensu stricto;* tribe Vespertilionini; Fig. 6). *Neoromicia* (and *Vespadelus*) has been removed from *Eptesicus* only recently, 1st placed in *Pipistrellus* and subsequently elevated to full generic rank. Thus, Miller (1907) and Tate (1942) were correct when referring to an *Eptesicus*-like ancestry for both *Histiotus* and *Laephotis*.

Additionally, mtDNA analysis suggests paraphyly of the genus *Eptesicus* (*sensu* Hill and Harrison 1987) relative to the position of *Histiotus*. Specifically, *Histiotus* is related to New World species of *Eptesicus* (*brasiliensis*, *diminutus*, *furinalis*, *fuscus*) more closely than Old World species (*hottentotus* and *serotinus*). Thus, the true definition of *Eptesicus* once again is called into question, and according to mtDNA data *Histiotus* may be treated as a separate genus, or as a member of *Eptesicus*. The former case would give continued recognition to the auditory specializations of *Histiotus*, but avoidance of polyphyletic taxa would require the introduction of a new genus to include Old World members of *Eptesicus* (i.e., due to position of *E. fuscus*, type species of *Eptesicus*). On the other hand, including *Histiotus* as a member of *Eptesicus* would underscore cranial and dental similarities between *Histiotus* and *Eptesicus* (*sensu stricto*), and it de-emphasizes the fact that large ears were gained secondarily in *Histiotus* after divergence between New and Old World *Eptesicus*. Very large ears and their attendant auditory specializations in the skull have been gained or lost independently numerous times within Vespertilioninae (e.g., see Tate 1942). Including *Histiotus* within *Eptesicus* also may be preferred based on chromosomal evidence, as it would emphasize the unique karyotype uniting the 2 groups; *Histiotus* possesses the "true *Eptesicus* karyotype" (2N = 50, FN = 48), with acrocentric autosomes only, that differs from all other vespertilionid

genera, including *Laephotis* (2N = 34, FN = 50; Heller and Volleth 1984; McBee et al. 1987; Rautenbach et al. 1993; Volleth 1987; Volleth et al. 2001; Volleth and Heller 1994a; Volleth and Tidemann 1989; Williams and Mares 1978).

Ultimately the decision of whether to include *Histiotus* within *Eptesicus* or, conversely, to retain the genus *Histiotus* and elevate the Old World species to generic status is arbitrary. Obviously more thorough examinations of *Histiotus* and New and Old World *Eptesicus* will be necessary to resolve the situation and to test relationships suggested here. However, the relationship of *Histiotus* to New World species of *Eptesicus* supported by mtDNA analysis is not arbitrary, and leaves *Eptesicus*, as currently understood, paraphyletic. Provisionally, therefore, I suggest honoring the "true *Eptesicus* karyotype" by relegating *Histiotus* subgeneric status within *Eptesicus*. Regarding paraphyly of subgenus *Eptesicus* (and *serotinus* subgroup; *sensu* Hill and Harrison 1987), mtDNA analysis provisionally suggests a classification that reflects geography, restricting subgenus *Eptesicus* (type species *fuscus* Rafinesque, 1820) to include the sampled New World members (*brasiliensis*, *diminutus*, *furinalis*, *fuscus*), and allocating the remaining Old World species (*hottentotus*, *serotinus*) to another subgenus. *Cnephaeus* Kaup, 1829 with type species *Vespertilio serotinus* Schreber (= *E. serotinus*) would be the oldest available name for this subgenus.

SUMMARY

Present systematics of Vespertilionidae is based almost entirely on criteria derived from taxonomic interpretations of traditional anatomical characters, which offer limited resolution of relationships among genera and essentially none of relationships among tribes and subfamilies. Furthermore, data accumulated in the past 30 years contradict many traditional groupings, and many traditional characters used in vespertilionid systematics have little phyletic utility. Bayesian and Parsimony analyses of mtDNA sequences from 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes provide well-supported resolution for vespertilionid relationships, at various taxonomic levels.

Ribosomal gene sequences are known for their applicability in studies of systematics at various taxonomic levels, facilitated primarily by secondary and tertiary structural elements and concomitant variation in rate of evolution along the length of RNA molecules. At the same time, such characteristics complicate multiple sequence alignment. I implemented a 2-tier approach to help avoid complications: independent analysis of 3 sets of taxa truncated from the overall taxon set; and a rather conservative estimate of positional homology, delimiting and excluding about 500 to 1,000 ambiguously aligned characters (sites) depending on taxon set. Resolution afforded in the present study, based on these conservative methods, is not heavily burdened by alignment of ambiguous regions of

mitochondrial ribosomal sequences. Truncating taxa and performing new alignments for each set provided an existential test of results and a measure of robustness; analysis of 4 sets of taxa that employed 2 independent alignments, multiple independent runs, and >30 designated outgroups provided essentially the same resolution and branch support regarding shared taxa. Topologies and levels of support produced by 2 methods of phylogenetic inference (Bayesian and Parsimony) also agreed markedly. Despite some subtle differences between levels of support from individual methods, none affected inferences of relationship.

mtDNA analysis suggests relationships that in many respects support traditional classification but which also support several changes, at various taxonomic levels. The majority of "contradictory" relationships also receives support from other data sources, particularly bacular and karyotypic data. The present study also provides supported resolution to several relationships, some of which contradict traditional classification, that have long been recognized but rarely tested, if ever, by phylogenetic methods. Following is a numbered summary of the taxonomic conclusions and recommendations supported by both Bayesian and Parsimony analyses of ribosomal gene sequences (discussions for each are referenced by page numbers in parentheses):

1) Traditional Vespertilionidae is monophyletic, but notably to the exclusion of *Miniopterus*.

Miniopterus (subfamily Miniopterinae) is recognzied in its own family, Miniopteridae (pp 28–36).

2) Only 2 of the traditional subfamilies within Vespertilionidae (*sensu stricto*) are monophyletic, Murininae and Kerivoulinae. Nyctophilinae has no validity and Vespertilioninae is paraphyletic relative to the position of *Myotis* (pp 36–42).

3) *Myotis* is sister to a clade containing Kerivoulinae and Murininae and is recognized in its own subfamily, Myotinae (pp. 36–42).

4) Myotini (*Myotis* + *Lasionycteris*) does not represent a natural assemblage (pp. 42–49).

5) *Myotis* subgenera *Leuconoe*, *Selysius*, and *Myotis* are polyphyletic. A subgeneric classification reflecting geography is suggested, broadening subgenus *Myotis* to include the sampled Old World species, and allocating the sampled New World species to another subgenus. The name *Aeorestes* Fitzinger, 1870 is available (pp. 42–49).

6) Vespertilioninae (excluding *Myotis*) is monophyletic. Deep branching patterns within Vespertilioninae are characterized by short, internodal distances, suggesting contemporaneous diversification for many (if not all) primary lineages within the subfamily. Several generic and suprageneric relationships are supported (pp. 49–74).

7) Lasiurini, including only *Lasiurus*, is monophyletic. Within *Lasiurus*, 3 traditional species groups (red bats, yellow bats, hoary bats) are each monophyletic (pp. 51–52).

8) Antrozoini, including *Antrozous* and *Bauerus*, is monophyletic, and closely allied with *Baeodon* and *Rhogeessa*. The latter 2 genera are allocated to tribe Antrozoini (pp. 52–55).

9) Scotophilini, including *Scotophilus*, is monophyletic and distinguished as the most divergent tribe (genus) within Vespertilioninae (pp. 55–56).

10) Monophyly of traditional Plecotini (i.e., excluding *Otonycteris*) is neither supported nor refuted. Recognition of 5 plecotine genera (*Barbastella*, *Corynorhinus*, *Euderma*, *Idionycteris*, *Plecotus*) is supported (pp. 57–58).

11) Position of *Otonycteris* is unresolved, and the genus is placed within Vespertilioninae *incertae sedis* (pp. 58–60).

12) Nycticeiini as traditionally recognized (*Otonycteris*, *Nycticeius*, *Rhogeessa*, *Scotophilus*) does not represent a natural assemblage.

13) *Pipistrellus*-like bats (i.e., traditional Vespertilionini) are divided into 3 tribes: Nycticeiini; Pipistrellini; and Vespertilionini (pp. 60–64).

14) *Pipistrellus* as traditionally recognized is polyphyletic. True *Pipistrellus* are confined to the tribe Pipistrellini. *Nyctalus* is treated as a member of *Pipistrellus* pending further study (pp. 64–67).

15) *Hypsugo*, *Neoromicia*, and *Vespadelus* are valid genera distinct from *Pipistrellus*, as each belongs to the tribe Vespertilionini (not Pipistrellini) (pp. 65–67).

16) True definitions of *Hypsugo* and *Neoromicea* remain questionable. Pending further study, *Hypsugo* is restricted to the type species, *H. savii*, and *Neoromicia* is restricted to the type species, *N. somalicus*; (*H.*) *eisentrautii* is transferred to *Nycticeinops*, and (*H.*) *nanus* and (*N.*) *brunneus* and *rendalli* are allocated to a separate, as yet unnamed genus (pp. 65–67).

17) "*Parastrellus*" *hesperus* and *Perimyotis subflavus* are generically distinct from true *Pipistrellus* and from each other. Affinities of both genera among other groups is uncertain, and each is placed *incertae sedis* within Vespertilioninae. "*Parastrellus*" currently is a *nomen nudem*, but will be made available as the valid name for this genus in an appropriate publication (pp. 67–69).

18) *Chalinolobus* (tribe Vespertilionini) and *Glauconycteris* (tribe Nycticeiini) are distinct

genera and do not form a monophyletic group (pp. 69–70).

19) *Nycticeius* (tribe Nycticeiini) and *Nycticeinops* (tribe Vespertilionini) are distinct genera and do not form a monophyletic group. *Nycticeius* is monotypic including only *humeralis*. *Nycticeinops* includes *schlieffeni*, but also *eisentrautii* (transferred from *Hypsugo*) (pp. 70–71).

20) The genus *Eptesicus*, subgenus *Eptesicus*, and *serotinus* subgroup within *Eptesicus* are paraphyletic relative to position of *Histiotus*. *Histiotus* is relegated subgeneric rank within *Eptesicus*. The subgenus *Eptesicus* is restricted to include the sampled New World species. The sampled Old World species are allocated to a separate genus, for which the name *Cnephaeus* Kaup, 1829 is available (pp. 71–74).

Overall, the present study offers a robust working hypothesis for vespertilionid systematics. Whereas mtDNA analysis provides a solid beginning to the goal of well-resolved, well-supported genealogic hypotheses for vespertilionid bats, there are numerous hypotheses that remain essentially untested due to insufficient taxonomic or data sampling, or both. Nearly 2/3 of the family waits to be analyzed.

At the onset of this study, I had hoped to employ objective cladistic methods, ancestral-area analysis (Bremer 1992), to assess zoogeographic patterns and history of various lineages within Vespertilionidae. Lack of supported resolution within and among several widely distributed taxa, not to mention that 2/3 of the family was not represented, severely limited the effectiveness of such analyses. However, a pattern apparent in the mtDNA tree is that geographic origin of these bats appears to predict their phylogenetic position better than ecology or morphology, upon which the current classification is based. For example, the current classification suggests that 3 phenetic groups (=ecomorphs) within *Myotis* each evolved once during the early radiation of the genus, and the present worldwide distributions reflect secondary dispersal events across continents. mtDNA analysis, however, suggests that much of the ecologic and morphologic similarity within *Myotis* reflects repeated episodes of convergent evolution.

mtDNA analysis also corroborates karyotypic data (Volleth and Tidemann 1991) for a shared common ancestry of the majority of Australian vespertilionids, which radiated into a wide range of niches, ultmately producing a diversity of phenotypes, most of which resemble those of vespertilionids from other continents. Vesper bats traditionally regarded as Australian *Pipistrellus* and *Eptesicus* are not related closely to members of either genus. mtDNA analysis suggests similar trends for other traditional

morphologic groups, such as the New World "*Pipistrellus*," traditional *Nycticeius*, traditional *Eptesicus*, *Chalinolobus* and *Glauconycteris*, and *Histiotus* and *Laephotis*.

These results are intriguing, but it remains to be seen whether or not such trends are affirmed by future study or are found for other vespertilionid groups. As shown for other vertebrate groups, the zoogeographic history of vesper bats, especially regarding New World/Old World disperal events, may have been far less complex than traditionally thought, and imply that much of the morphologic and ecologic similarity has resulted from repeated episodes of convergent evolution. Moreover, perhaps entire (identical) sets of adaptive radiations "replicated" in several parts of the world. Future study of vespertilionids not sampled in this study will be critical before meaningful assessments of evolutionary and zoogeographic hypotheses can be made.

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Appendix 1.—List of specimens examined. Families are arranged phylogenetically, whereas species within genera and genera within families are listed alphabetically. A voucher specimen for most samples is housed in a mammal collection at the American Museum of Natural History (AMNH), Carnegie Museum of Natural History (CM), Field Museum of Natural History (FMNH), Indiana State University Vertebrate Collection (ISUV), Museum d'Histoire Naturelle de Geneve (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB), Museum of Texas Tech University (TTU), National Museum of Natural History (NMNH), Natural History Museum of Bern (NHMB), Oklahoma State University Collection of Vertebrates (OSU), Royal Ontario Museum (ROM), Senckenberg Natural History Museum (SMF), Texas Cooperative Wildlife Collection at Texas A&M University (TCWC), Transvaal Museum (TM), Universidad Autónoma Metropolitana-Iztapalapa (UAM-I), Universidad Nacional Autónoma de Mexico City (UNAM), University of Memphis, Mammal Collection (UM), or University of Wisconsin Zoological Museum (UWZM). Museum catalog numbers are missing for vouchers that are housed but not yet cataloged. Location of voucher specimen was undetermined (***) for 14 specimens examined, 7 of which vespertilionids. Additionally, voucher information was undertermined for all 6 sequences obtained from GenBank (accession numbers given). * indicates type specimen.

Taxon	Tissue Collection No.	Museum Catalog No.	Locality
PTEROPODIDAE			
Nyctimene robinsoni	GENBANK—U93061, AF069536		
Pteropus hypomelanus	GENBANK—U93073, AF069537		
RHINOPOMATIDAE			
Rhinopoma hardwickei	TK 25643	TTU 40639	PALESTINE: WEST BANK
MEGADERMATIDAE			
Macroderma gigas	ECT	***	
HIPPOSIDERIDAE			
Hipposideros abae	AMNH 268375	AMNH 268375	CENTRAL AFRICAN REPUBLIC
Hipposideros cyclops	AMNH 268380	AMNH 268380	CENTRAL AFRICAN REPUBLIC
Triaenops furculus	ECT	***	
RHINOLOPHIDAE			
Rhinolophus alcyone	AMNH 268373	AMNH 268373	CENTRAL AFRICAN REPUBLIC

NYCTERIDAE			
Nycteris argae	AMNH 268371	AMNH 268371	FRENCH GUIANA: PARACOU
Nycteris sp.	TK 21558	CM 90794	GABON: ESTUAIRE PROV.
EMBALLONURIDAE			
Balantiopteryx plicata	ECT	***	
Cormura brevirostris	AMNH 267822	AMNH 267822	FRENCH GUIANA: PARACOU
Diclidurus scutatus	AMNH 267832	AMNH 267832	FRENCH GUIANA: PARACOU
Emballonura atrata	GENBANK—AF203773		
Peropteryx macrotis	TK 70465	***	PERU -- probably
Rhynchonycteris naso	AMNH 267373	AMNH 267373	FRENCH GUIANA: PARACOU
Saccopteryx bilineata	AMNH 267842	AMNH 267842	FRENCH GUIANA: PARACOU
Saccopteryx leptura	TK 70480	***	PERU -- probably
Taphozous nudiventris	TK 16602	CM 62342	EGYPT: GIZA
MYZOPODIDAE			
Myzopoda aurita	OK 4246	USNM 448885	MADAGASCAR: FIANARANTSOA
MYSTACINIDAE			
Mystacina tuberculata	UWZM M27027	UWZM M27027	NEW ZEALAND: NORTH ISLAND

FURIPTERIDAE			
Furipterus horrens	AMNH 272837	AMNH 272837	FRENCH GUIANA: PARACOU
Furipterus horrens	F 34443	ROM 100202	GUYANA: EAST BERBICE-CORENTYNE PROV.
NOCTILIONIDAE			
Noctilio albiventris	TK 86633	***	GUYANA: BERBICE DIST.
Noctilio leporinus	TK 10224	CM 63552	SURINAME: SARAMACCA
MORMOOPIDAE			
Mormoops megalophyla	TK 19311	CM 78267	VENEZUELA: BARINAS
Pteronotus parnellii	TK 17953	CM 77083	SURINAME: MAROWIJNE
PHYLLOSTOMIDAE			
Centurio senex	TK 13110	CM 55731	MEXICO: VERACRUZ
Diphylla ecaudata	TK 13514	***	MEXICO: YUCATÁN
Tonatia brasiliensis	TK 18834	AMNH 267103	FRENCH GUYANA: PARACOU
Trachops cirrhosus	TK 18829	AMNH 267129	FRENCH GUYANA: PARACOU
Vampyrum spectrum	TK 40370	TTU 61071	HONDURUS: ATLANTIDA
THYROPTERIDAE			
Thyroptera discifera	TK 17210	CM 68440	SURINAME: SARAMACCA

Thyroptera tricolor	AMNH 268577	AMNH 268577	FRENCH GUIANA: PARACOU
NATALIDAE			
Natalus stramineus	TK 15660	TTU 31457	DOMINICA: ST JOHN
Natalus micropus	TK 9454	CM 44578	JAMAICA
MOLOSSIDAE			
Eumops auripendula	AMNH 268594	AMNH 268594	FRENCH GUIANA: PARACOU
Chaerephan pumila	FMNH 137634	FMNH 137634	UGANDA: SOUTH BUGANDA
Molossops abrasus	AMNH 267534	AMNH 267534	FRENCH GUIANA: PARACOU
Molossus molossus	AMNH 269102	AMNH 269102	FRENCH GUIANA: PARACOU
Molossus molossus	AMNH 269105	AMNH 269105	FRENCH GUIANA: PARACOU
Molossus sinaloe	GENBANK—U93053, AF203739		
Molossus rufus	AMNH 268595	AMNH 268595	FRENCH GUIANA: PARACOU
Mops condylurus	FMNH 151943	FMNH 151943	MADAGASCAR: TOLIARA PROV.
Mormopterus planiceps	RLH 63	TCWC	AUSTRALIA
Nyctinomops femorosaccus	TK 19552	TTU 37731	MEXICO: JALISCO
Nyctinomops macrotis	TK 78908	TTU 79570	USA: TEXAS
Otomops martiensseni	FMNH 137633	FMNH 137633	BURUNDI: MURAMUYA
Promops centralis	AMNH 269114	AMNH 269114	FRENCH GUIANA: PARACOU

Sauromys petrophilus	SP 7791	CM 105758	SOUTH AFRICA: TRANSVAAL PROV.
Tadarida brasiliensis	OK 430	OSU 12794	USA: NEW MEXICO
MINIOPTERIDAE			
Miniopterus australis	TK 20330	***	PAPUA NEW GUINEA: CENTRAL PROV.
Miniopterus fraterculus	TK 33132	CM 98058	KENYA: RIFT VALLEY PROV.
Miniopterus inflatus	TK 33539	CM 98079	KENYA: WESTERN PROV.
Miniopterus pusillus	F44196	ROM 110871	VIETNAM: LAM DONG
Miniopterus schreibersi	TK 40910	TTU 70985	TUNISIA: BEJA GOVERNMENT
Miniopterus tristis	TK 20337	TTU 36281	PAPUA NEW GUINEA: CENTRAL PROV.
VESPERTILIONIDAE			
Antrozous pallidus	TK 49646	TTU 71101	USA: TEXAS
Baeodon alleni	TK 45023	UNAM	MEXICO: MICHOACAN
Barbastella barbastellus	IZEA 3590	MHNG 1804.094	SWITZERLAND: VALAIS PROV.
Bauerus dubiaquercus	FN 33200	ROM 97719	MEXICO: CAMPECHE
Chalinolobus gouldi	RLH 27	TCWC	AUSTRALIA
Chalinolobus morio	05M3	TCWC	AUSTRALIA
Chalinolbus morio	05M4	TCWC	AUSTRALIA
Chalinolobus tuberculatus	GENBANK—AF321051		NEW ZEALAND

Corynorhinus mexicanus	TK 45849	UAM-I	MEXICO: MICHOACAN
Corynorhinus rafinesquii	TK 5959	TTU 45380	USA: ARKANSAS
Corynorhinus townsendii	TK 83182	TTU 78531	USA: TEXAS
Eptesicus brasiliensis	TK 17809	CM 76812	SURINAME: NICKERIE
Eptesicus diminutus	TK 15033	TTU 48154	VENEZUELA: GUARICO
Eptesicus furinalis	AMNH 268583	AMNH 268583	FRENCH GUIANA: PARACOU
Eptesicus fuscus	SP 844	CM 102826	USA: WEST VIRGINIA
Eptesicus hottentotus	TK 33013	CM 89000*	KENYA: RIFT VALLEY PROV.
Eptesicus serotinus	TK 40897	TTU 70947	TUNISIA: SIDI BOU ZID GOVERNMENT
Euderma maculatum	NK 36260	MSB 121373	USA: UTAH
Glauconycteris argentatus	FMNH 15119	FMNH 15119	TANZANIA: KILIMANJARO REGION
Glauconycteris beatrix	FMNH 149417	FMNH 149417	ZAIRE: HAUTE ZAIRE
Glauconycters poensis	AMNH 268381	AMNH 268381	CENTRAL AFRICAN REPUBLIC
Glauconycteris variegatus	TK 33545	CM 97983	KENYA: WESTERN PROV.
Harpiocephalus harpia	TK 21258	CM 88159	THAILAND: UTHAI THANI PROV.
Histiotus macrotus	FMNH 129207	FMNH 129207	PERU: ANCASH
"Hypsugo" eisentrautii	F 34348	ROM 100532	IVORY COAST

"Hypsugo" nanus	TK 33378	CM 98003	KENYA: EASTERN PROV.
Hypsugo savii	IZEA 3586	MHNG 1804.100	SWITZERLAND: VALAIS PROV.
Idionycteris phyllotis	NK 36122	MSB 120921	USA: UTAH
Kerivoula hardwickei	F 44154	ROM 110829	VIETNAM: DONG NAI
Kerivoula papillosa	F 44175	ROM 110850	VIETNAM: DONG NAI
Kerivoula pellucida	F 35987	ROM 102177	INDONESIA: EAST KALIMANTAN
Laephotis namibiensis	SP 4097	TM 37547	NAMIBIA: LUDERITZ DIST.
Lasionycteris noctivagans	TK 24216	TTU 56255	USA: TEXAS
Lasiurus attratus	F 39221	ROM 107228	GUYANA: POTARO-SIPARUNI
Lasiurus blossevillii	F 38133	ROM 104285	PANAMA: CHIRIQUI
Lasiurus borealis	TK 49732	TTU 71170	USA: TEXAS
Lasiurus borealis	TK 84510	TTU 80739	USA: TEXAS
Lasiurus cinereus	TK 78926	TTU	USA: TEXAS
Lasiurus ega	TK 43132	UNAM	MEXICO: MICHOACAN
Lasiurus seminolus	TK 90686	***	USA
Lasiurus xanthinus	TK 78704	TTU 78296	USA: TEXAS
Murina huttoni	F 42722	ROM 107739	VIETNAM: DAK LAK
Myotis adversus	RLH 62	TCWC	AUSTRALIA

Myotis albescens	TK 17932	CM 77691	SURINAME: MAROWIJNE
Myotis austroriparius	MLK 4079	UM 16629	USA: TENNESSEE
Myotis bocagei	FMNH 150075	FMNH 150075	TANZANIA: TANGA REGION
Myotis capaccinii	TK 25610	TTU 40554	JORDAN: NORTHERN PROV.
Myotis ciliolabrum A	TK 78797	TTU 79325	USA: TEXAS
Myotis ciliolabrum B	TK 24872	TTU 40680	USA: OKLAHOMA
Myotis ciliolabrum C	TK 83155	TTU 78520	USA: TEXAS
Myotis daubentoni	IZEA 2692	MHNG 1805.054	SWITZERLAND: VAUD PROV.
Myotis dominincensis	TK 15613	***	DOMINICA: ST. JOSEPH PARISH
Myotis elegans	F35471	ROM 35471	EL SALVADOR: AHUACHAPAN
Myotis fortidens	TK 43186	***	MEXICO: MICHOACAN
Myotis keaysi	TK 13532	***	MEXICO: YUCATAN
Myotis levi	FMNH 141600	FMNH 141600	BRAZIL: SAO PAULO
Myotis lucifugus A	TK 11929	TTU 46405	USA: TEXAS
Myotis lucifugus B	TK 79170	TTU 78599	USA: TEXAS
Myotis muricola	FMNH 147067	FMNH 147067	PHILIPPINE ISLANDS: MINDANAO ISLAND
Myotis myotis	IZEA 3790	MHNG 1805.062	SWITZERLAND: BERN PROV.

Myotis nigricans	FMNH 129210	FMNH 129210	PERU: AMAZONAS
Myotis ridleyi	F 44086	ROM 110767	VIETNAM: DONG NAI
Myotis riparius	AMNH 268591	AMNH 268591	FRENCH GUIANA: PARACOU
Myotis ruber	F 44409	ROM 111110	BRAZIL: SAO PAULO
Myotis septentrionalis	DWS 608	ISUV 6454	USA: INDIANA
Myotis siligorensis	F 42629	ROM 107649	VIETNAM: TUYEN QUANG
Myotis thysanodes	TK 78800	TTU 79328	USA: TEXAS
Myotis volans	TK 78980	TTU 79545	USA: TEXAS
Myotis welwitschii	FMNH 144313	FMNH 144313	UGANDA: KASESE DIST.
Myotis yumanensis	TK 28753	TTU 43200	USA: OKLAHOMA
Myotis sp.	TK 48587	***	NORTH AMERICA
"Neoromicia" brunneus	TK 21501	CM 90802	GABON: ESTUAIRE PROV.
"Neoromicia" rendalli	TK 33238	CM 97977	KENYA: COASTAL PROV.
Neoromicia somalicus	TK 33214	CM 97978	KENYA: COASTAL PROV.
"Nyctalus" leisleri	FMNH 140374	FMNH 140374	PAKISTAN: MALAKAND DIV.
"Nyctalus" noctula	NHMB 209/87	NHMB 209/87	SWITZERLAND: BERN PROV.
Nycticeius humeralis	TK 26380	TTU 49536	USA: TEXAS
Nycticeinops schlieffeni	TK 33373	CM 97998	KENYA: EASTERN PROV.

Nyctophilus geofroyii	RLH 23	TCWC	AUSTRALIA
Nyctophilus gouldi	09M1	TCWC	AUSTRALIA
Nyctophilus gouldi	1804	SMF 64967	AUSTRALIA: AUSTRALIAN CAPITAL TERR.
Nyctophilus gouldi	RLH 29	TCWC	AUSTRALIA
Otonycteris hemprichii	SP 7882	CM	JORDAN: MAAN GOVERNMENT
"*Parastrellus*" *hesperus*	TK 78703	TTU 79269	USA: TEXAS
Perimyotis subflavus	TK 90671	TTU 80684	USA: TEXAS
Pipistrellus abramus	GENBANK—AB061528		
Pipistrellus coromandra	FMNH 140377	FMNH 140377	PAKISTAN: MALAKAND DIV.
Pipistrellus javanicus	FMNH 147069	FMNH 147069	PHILIPPINE ISLANDS: MINDANAO ISL.
Pipistrellus nathusii	IZEA 2830	MHNG 1806.003	SWITZERLAND: VAUD
Pipistrellus nathusii	IZEA 3406	MHNG 1806.001	SWITZERLAND: VAUD
Pipistrellus nathusii	TK 81167	TTU	UKRAINE: CHORNOBYL DIST.
Pipistrellus nathusii	TK 81169	TTU	UKRAINE: CHORNOBYL DIST.
Pipistrellus pipistrellus	IZEA 3403	MHNG 1806.032	SPAIN: BARCELONE PROV.

Pipistrellus tenuis	FMNH 137021	FMNH 137021	PHILIPPINE ISLANDS: SIBUYAN ISL.
Plecotus auritus	IZEA 2694	MHNG 1806.047	SWITZERLAND: VALAIS PROV.
Plecotus austriacus	IZEA 3722	MHNG 1806.042	SWITZERLAND: VAUD PROV.
Rhogeessa aeneus	TK 20712	TTU 40012	BELIZE: BELIZE DIST.
Rhogeessa mira	TK 45014	UNAM	MEXICO: MICHOACAN
Rhogeessa parvula	TK 20653	TTU 36633	MEXICO: SONORA
Rhogeessa tumida	TK 40186	TTU 61231	HONDURAS: VALLE
Scotophilus borbonicus	TK 33267	CM 98041	KENYA: COASTAL PROV.
Scotophilus dinganii	FMNH 147235	FMNH 147235	TANZANIA: TANGA REGION
Scotophilus heathi	F 42769	ROM 107786	VIETNAM: DAK LAK
Scotophilus kuhlii	FMNH 145684	FMNH 145684	PHILIPPINE ISLANDS: SIBUYAN ISL.
Scotophilus leucogaster	TK 33359	CM 98054	KENYA: EASTERN PROV.
Scotophilus nux	TK 33484	***	KENYA: WESTERN PROV.
Scotophilus viridis	FMNH 150084	FMNH 150084	TANZANIA: TANGA REGION
Scotoecus hirundo	FMNH 151204	FMNH 151204	TANZANIA: KILIMANJARO REGION
Scotomanes ornatus	F 42568	ROM 107594	VIETNAM: TUYEN QUANG

Tylonycteris pachypus	F 38442	ROM 106164	VIETNAM: TUYEN QUANG
Vespadelus regulus	RLH 30	TCWC	AUSTRALIA
Vespadelus sagittula	RLH 20	TCWC	AUSTRALIA
Vespadelus vulturnus	RLH 16	TCWC	AUSTRALIA
Vespertilio murinus	IZEA 3599	MHNG 1808.017	SWITZERLAND: VALAIS PROV.

Table 1.—Three truncated sets of taxa used in phylogenetic analysis. Number of sequences per genus (if ≥ 2) is indicated parenthetically. Most sequences correspond to different species within genera as only 5 species are represented by sequences from multiple individuals. Asterisks (*) denote outgroup taxa designated in phylogenetic analyses of each taxon set .

Taxon sets		
Vespertilionidae (128 taxa)	*Pipistrellus*-like (62 taxa)	*Myotis* (39 taxa)
Natalidae*	Kerivoulinae*	Kerivoulinae*
Natalus (2)	*Kerivoula* (2)	*Kerivoula* (3)
Molossidae*	Murininae*	Murininae*
Eumops	*Harpiocephalus*	*Harpiocephalus*
Molossops	*Murina*	*Murina*
Molossus	Myotinae*	Myotinae
Mops	*Myotis* (2)	*Myotis* (29)
Nyctinomops	Vespertilioninae	Vespertilioninae*

Tadarida

Miniopteridae

Miniopterus (6)

Vespertilionidae

Kerivoulinae

Kerivoula (3)

Murininae

Harpiocephalus

Murina

Myotinae

Myotis (29)

Vespertilioninae

Antrozous

Bauerus

Baeodon

Barbastella

Corynorhinus (3)

Antrozous

Corynorhinus

Chalinolobus (4)

Eptesicus (6)

Glauconycteris (4)

Histiotus

Hypsugo (3)

Laephotis

Lasionycteris

Lasiurus (2)

Neoromicia (3)

Nyctalus (2)

Nycticeinops

Nycticeius

Nyctophilus (3)

"*Parastrellus*"

Perimyotis

Lasionycteris

Lasiurus

Rhogeessa

Scotophilus (2)

Chalinolobus (4)

Eptesicus (6)

Euderma

Glauconycteris (4)

Histiotus

Hypsugo (3)

Idionycteris

Laephotis

Lasionycteris

Lasiurus (8)

Neoromicia (3)

Nyctalus (2)

Nycticeinops

Nycticeius

Nyctophilus (4)

Otonycteris

"*Parastrellus*"

Pipistrellus (7)

Plecotus (2)

Rhogeessa (2)

Scotoecus

Scotomanes

Scotophilus (2)

Tylonycteris

Vespadelus (3)

Vespertilio

Perimyotis

Pipistrellus (9)

Plecotus (2)

Rhogeessa (5)

Scotoecus

Scotomanes

Scotophilus (7)

Tylonycteris

Vespadelus (3)

Vespertilio

Table 2.—Number of characters (=sites) for each taxon set based on 2 separate alignments; 1 with default values for gap cost ratio (15:00:6.66), the other with a smaller ratio (5:4). Value for 5:4 alignment is shown parenthetically. Constant and parsimony-uninformative characters were counted after excluding ambiguous characters.

Characters	Taxon sets			
	All taxa *n* = 171	Vespertilionidae *n* = 128	Pipistrellus-like *n* = 62	*Myotis* *n* = 39
Aligned	2,851 (2,966)	2,799 (2,883)	2,748 (2,816)	2,733 (2,766)
Excluded	888 (1,011)	728 (864)	661 (753)	519 (618)
Analyzed	1,963 (1,955)	2,071 (2,019)	2,087 (2,063)	2,214 (2,148)
Constant	985 (986)	1,104 (1,103)	1,205 (1,200)	1,459 (1,457)
Parsimony-uninformative	187 (185)	165 (159)	220 (216)	204 (195)

Table 3.—Burn-in values and mean estimates for Bayesian analyses (GTR + Γ + I) of 4 sets of taxa. Estimated parameters are -Ln likelihoods (-Lnl), rates (R) of 6 substitution types, base frequencies (π), proportion of invariant sites (p_{inv}), and shape of gamma distribution (α). All values are based on alignments with default settings for gap cost ratio.

	All taxa	Vespertilionidae	*Pipistrellus*-like	*Myotis*
Burn-in	2,000	2,000	2,000	1,500
-Lnl	42608.14	34710.98	22072.97	14052.10
R_{AC}	3.71	3.57	4.74	4.21
R_{AG}	19.00	24.18	30.48	24.84
R_{AT}	3.12	4.06	4.69	5.93
R_{CG}	0.47	0.48	0.49	0.35
R_{CT}	48.69	61.66	68.41	70.46
R_{GT}	1.00	1.00	1.00	1.00
π_A	0.40	0.39	0.38	0.37
π_C	0.19	0.19	0.18	0.20
π_G	0.18	0.18	0.19	0.18

π_T	0.23	0.24	0.25	0.25
p_{inv}	0.41	0.45	0.43	0.50
α	0.62	0.66	0.54	0.60

Table 4.—Lengths and consistency (CI) and retention (RI) indexes for Parsimony bootstrap analyses of 4 sets of taxa. All values are based on alignments with default settings for gap cost ratio.

	All taxa	Vespertilionidae	*Pipistrellus*-like	*Myotis*
Length	9,597	7,528	4,408	2,405
CI	0.13	0.19	0.22	0.42
RI	0.57	0.59	0.45	0.53

Table 5.—Apomorphies distinguishing *Miniopterus* from all other vespertilionids.

	Miniopterus	Vespertilionidae
Anatomy		
Hair structure (Benedict 1957)	Long, entire coronal scales alternating between extremely short hastate scales	Generally hastate scales
Dental formula (Mein and Tupinier 1977; van der Merwe 1985)	Supplementary vestigial tooth present between upper canine and 1st premolar	No tooth between upper canine and 1st premolar
Tongue (papillae) (Tiunov 1989)	Distributed transversely on *torus linguae* like continuous ridges	Distributed unevenly, but with tops pointed to tip of tongue and back of tongue in anterior and posterior regions of *torus linguae*, respectively

2nd phalanx of 3rd finger (Miller 1907)	About 3 times as long as 1st	Usually about as long as 1st (always << 3 times as long)
Tendon locking mechanism (Simmons 1998)	Absent	Present
Rostral and sylvian sulci (Reep and Bhatnager 2000)	Prominent	Slight
Baculum (Mathews 1942)	Absent	Present
Sperm head (Breed and Inns 1985; Mori and Uchida 1982)	Long (9 μm), filled with nucleus and massive acrosome	Short (4–5.5 μm), filled with nucleus and capped with small acrosome
Urethral glands (Tiunov 1989)	Present	Absent
Cowper's glands (Tiunov 1989)	At root of penis with long ducts connected anteriorly just after urethral glands	At root of penis with short ducts connected posteriorly (at root of penis)

Embryology

(Gopalakrishna and Karim 1980; Gopalakrishna and Chari 1983; Karim and Bhatnager 2000; Richardson 1977)

Delayed development	Blastocyst remains free	Blastocyst implants, but development is retarded
Blastocyst attachment	On uterine wall entirely and circumferentially so that lumen is obliterated at nidation level	On antimesometrial side of uterus by embryonic hemisphere so that abembryonic part of blastocyst lies freely in persistent uterine lumen
Roof of amniotic cavity	Developed by uterine endometrial layer (no cavitation)	Developed by cavitation (trophoblastic layer)
Abembryonic yolk	Remains in contact with uterine wall	Remains hanging in persistent uterine lumen
Chorioallantoic placenta	3 types (primary, secondary, tertiary)	1 or 2 types
Sperm storage	Absent	Present

Immunology		
MC'F transferrin distances (Pierson 1986)	Closest to anti-*Tadarida*	Closest to anti-*Antrozous*

Figure 1.—Volleth and Heller's (1994a) cladogram of Vespertilionidae based on parsimony analysis of karyologic features. Topology shown is based on 1 of 3 sets of assumptions for ancestral character states. Under this set of assumptions, dotted line indicates another possibility for relationship between *Eptesicus* and *Scotophilus*. *H.* = *Hesperoptenus*, *Hyps.* = *Hypsugo*, *N.* = *Nyctalus*, *P.* = *Pipistrellus*, *R.* = *Rhogeessa* (=*Baeodon*), *T.* = *Tylonycteris*.

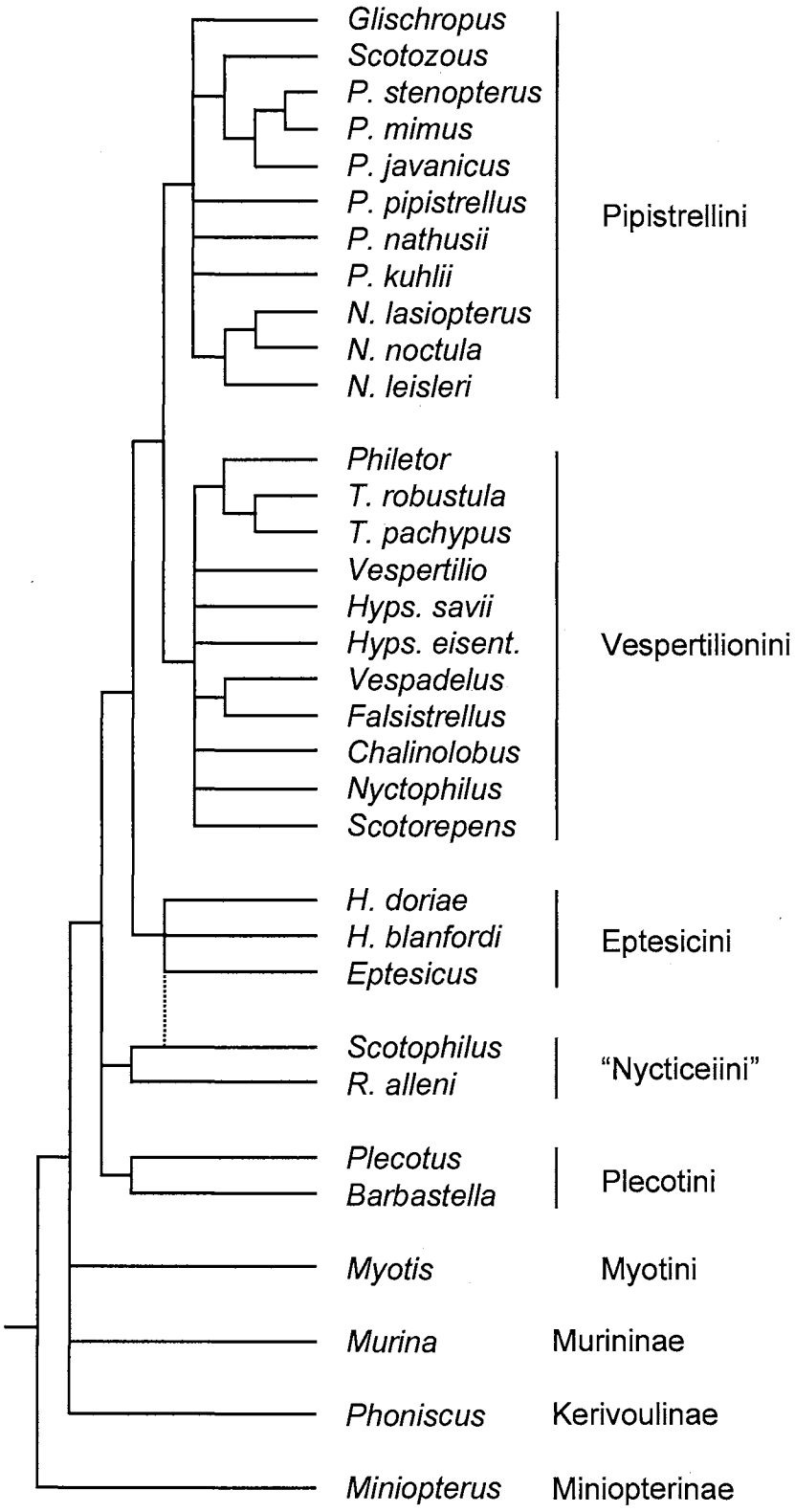

Glischropus
Scotozous
P. stenopterus
P. mimus
P. javanicus
P. pipistrellus
P. nathusii
P. kuhlii
N. lasiopterus
N. noctula
N. leisleri
Pipistrellini
Philetor
T. robustula
T. pachypus
Vespertilio
Hyps. savii
Hyps. eisent.
Vespadelus
Falsistrellus
Chalinolobus
Nyctophilus
Scotorepens
Vespertilionini
H. doriae
H. blanfordi
Eptesicus
Eptesicini
Scotophilus
R. alleni
"Nycticeiini"
Plecotus
Barbastella
Plecotini
Myotis
Myotini
Murina
Murininae
Phoniscus
Kerivoulinae
Miniopterus
Miniopterinae

Figure 2.—Best tree (mean Lnl = -42608.14) from Bayesian analysis (GTR + Γ + I) of ribosomal gene sequences from 171 taxa including all chiropteran families (except monotypic Craseonycteridae). Designated outgroups included representatives of Hipposideridae, Pteropodidae, Rhinolophidae, and Rhinopomatidae. Parameter estimates from Bayesian and Parsimony analyses given in Table 3 and Table 4, respectively. Topology and support values [Bayesian posterior probabilities (*P*) and Parsimony bootstrap percentages (BS)] are abbreviated to family-level relationships and averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and 2 different sequence alignments. "**," $P = 1.0$ and BS ≥ 98% in all analyses regardless of alignment; "*+," $P = 1.0$, 70% < BS < 90% in all analyses regardless of alignment; "*," $P = 1.0$ in all analyses regardless of alignment, but BS < 70%; "▌," $0.95 \leq P < 1.0$ in all analyses regardless of alignment, but BS < 70%. Intermittent shading is only for help visually distinguishing family-level clades.

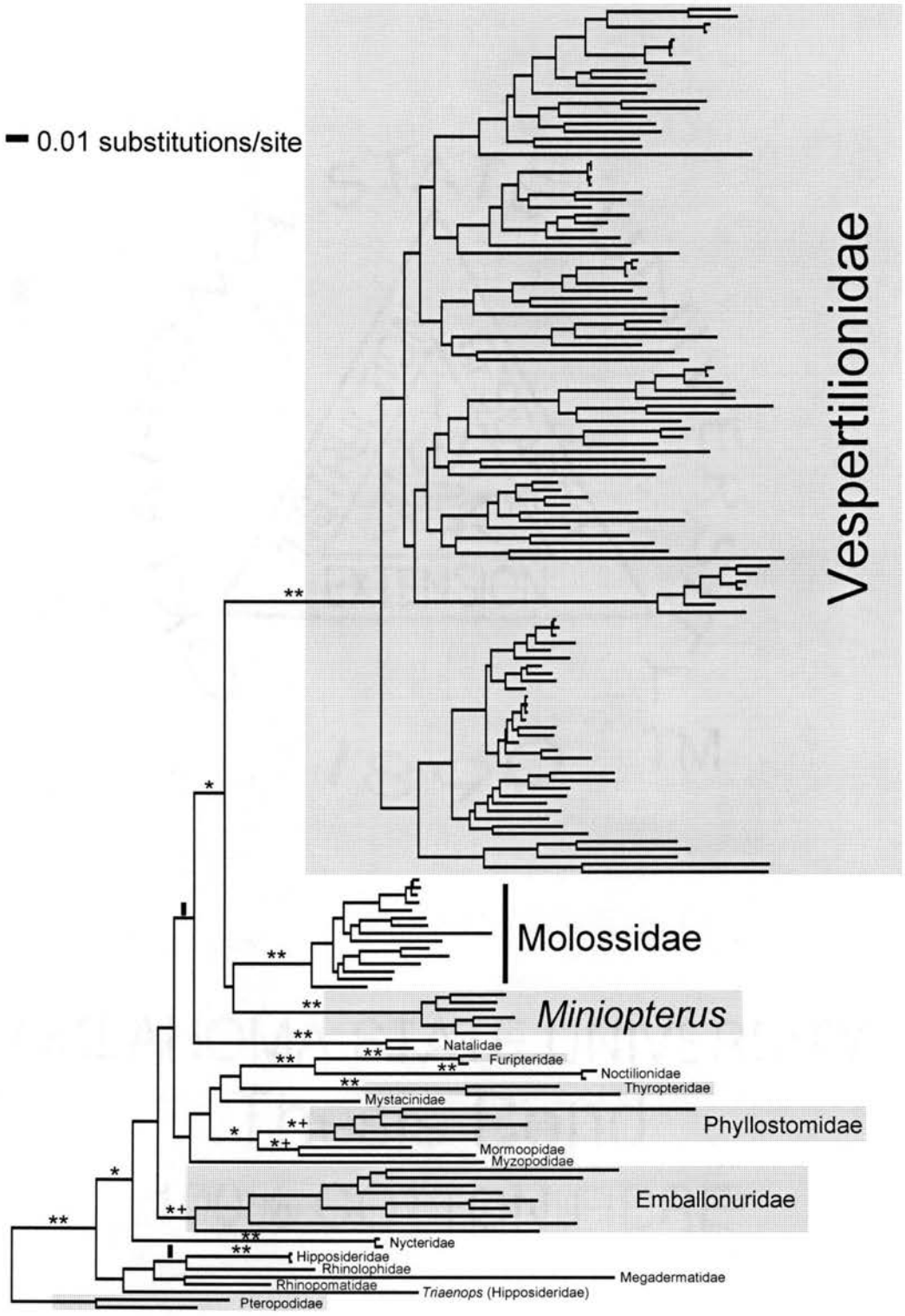
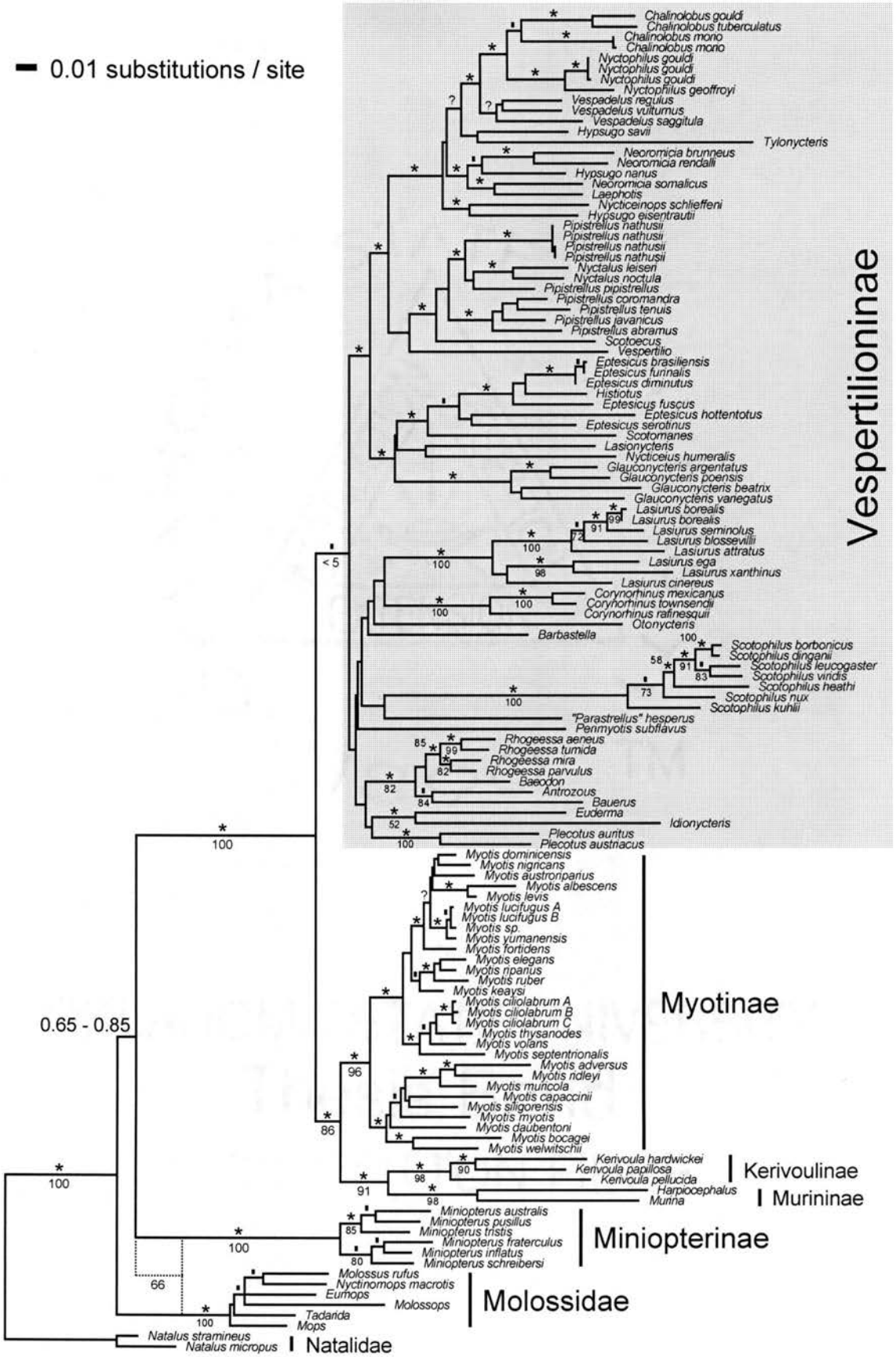

0.01 substitutions/site
Vespertilionidae
Molossidae
Miniopterus
Natalidae
Furipteridae
Noctilionidae
Thyropteridae
Mystacinidae
Phyllostomidae
Mormoopidae
Myzopodidae
Emballonuridae
Nycteridae
Hipposideridae
Rhinolophidae
Megadermatidae
Rhinopomatidae
Triaenops (Hipposideridae)
Pteropodidae

Figure 3.—Best tree (mean Lnl = -34710.98) from Bayesian analysis (GTR + Γ + I) of ribosomal gene sequences from 128 taxa (Vespertilionidae taxon set). Designated outgroups included representatives of Natalidae and Molossidae. Parameter estimates from Bayesian and Parsimony analyses given in Table 3 and Table 4, respectively. Bayesian posterior probabilities (*P*) if ≥ 0.95 are shown above branches (as symbols) throughout the tree and are averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and 2 different sequence alignments. "*," $P = 1.0$ in all analyses regardless of alignment; "▌," $0.95 \leq P < 1.0$ in all analyses regardless of alignment; "?," $P \geq 0.95$ in all analyses based on 1 alignment, but < 0.95 in all analyses based on other alignment. Bootstrap support from Parsimony analysis if > 50% is shown adjacent to or below branches (as percentage of 200 iterations) and also are averaged conservatively over all analyses. Bootstrap support for relationships within Myotinae and among *Pipistrellus*-like bats within Vespertilioninae are not shown here; rather, they are shown in subsequent figures. Dotted line indicates sister relationship between *Miniopterus* and Molossidae supported by Parsimony analysis (66%).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Vespertilioninae
Chalinolobus gouldi
Chalinolobus tuberculatus
Chalinolobus morio
Chalinolobus morio
Nyctophilus gouldi
Nyctophilus gouldi
Nyctophilus gouldi
Nyctophilus geoffroyi
Vespadelus regulus
Vespadelus vulturnus
Vespadelus saggitula
Hypsugo savii
Tylonycteris
Neoromicia brunneus
Neoromicia rendalli
Hypsugo nanus
Neoromicia somalicus
Laephotis
Nycticeinops schlieffeni
Hypsugo eisentrautii
Pipistrellus nathusii
Pipistrellus nathusii
Pipistrellus nathusii
Pipistrellus nathusii
Nyctalus leisleri
Nyctalus noctula
Pipistrellus pipistrellus
Pipistrellus coromandra
Pipistrellus tenuis
Pipistrellus javanicus
Pipistrellus abramus
Scotoecus
Vespertilio
Eptesicus brasiliensis
Eptesicus furinalis
Eptesicus diminutus
Histiotus
Eptesicus fuscus
Eptesicus hottentotus
Eptesicus serotinus
Scotomanes
Lasionycteris
Nycticeius humeralis
Glauconycteris argentatus
Glauconycteris poensis
Glauconycteris beatrix
Glauconycteris variegatus
Lasiurus borealis
Lasiurus borealis
Lasiurus seminolus
Lasiurus blossevillii
Lasiurus ega
Lasiurus xanthinus
Lasiurus cinereus
Corynorhinus mexicanus
Corynorhinus townsendii
Corynorhinus rafinesquii
Otonycteris
Barbastella
Scotophilus borbonicus
Scotophilus dinganii
Scotophilus leucogaster
Scotophilus viridis
Scotophilus heathi
Scotophilus nux
Scotophilus kuhlii
"Parastrellus" hesperus
Penmyotis subflavus
Rhogeessa aeneus
Rhogeessa tumida
Rhogeessa mira
Rhogeessa parvulus
Baeodon
Antrozous
Bauerus
Euderma
Idionycteris
Plecotus auritus
Plecotus austriacus
< 5
100
0.65 - 0.85
Myotis dominicensis
Myotis nigricans
Myotis austroriparius
Myotis albescens
Myotis levis
Myotis lucifugus A
Myotis lucifugus B
Myotis sp.
Myotis yumanensis
Myotis fortidens
Myotis elegans
Myotis nyanus
Myotis ruber
Myotis keaysi
Myotis ciliolabrum A
Myotis ciliolabrum B
Myotis ciliolabrum C
Myotis thysanodes
Myotis volans
Myotis septentrionalis
Myotis adversus
Myotis ndleyi
Myotis muricola
Myotis capaccinii
Myotis siligorensis
Myotis myotis
Myotis daubentoni
Myotis bocagei
Myotis welwitschii
Myotinae
Kerivoula hardwickei
Kerivoula papillosa
Kerivoula pellucida
Kerivoulinae
Harpiocephalus
Murina
Murininae
Miniopterus australis
Miniopterus pusillus
Miniopterus tristis
Miniopterus fraterculus
Miniopterus inflatus
Miniopterus schreibersi
Miniopterinae
Molossus rufus
Nyctinomops macrotis
Eumops
Molossops
Tadarida
Mops
Molossidae
Natalus stramineus
Natalus micropus
Natalidae

Figure 4.—Best tree (mean Lnl = -34710.98) from Bayesian analysis (GTR + Γ + I) of ribosomal gene sequences from 62 taxa (*Pipistrellus*-like taxon set). Designated outgroups included representatives of Murininae, Myotinae, and Kerivoulinae. Parameter estimates from Bayesian and Parsimony analyses given in Table 3 and Table 4, respectively. Bayesian posterior probabilities (*P*) if ≥ 0.95 are shown above branches (as symbols) throughout the tree and are averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and 2 different sequence alignments. "*," $P = 1.0$ in all analyses regardless of alignment; "▌," $0.95 \leq P < 1.0$ in all analyses regardless of alignment; "?," $P \geq 0.95$ in all analyses based on 1 alignment, but < 0.95 in all analyses based on other alignment. Bootstrap support from Parsimony analysis if > 50% is shown adjacent to or below branches (as percentage of 200 iterations) and also are averaged conservatively over all analyses. *S.* = *Scotophilus*. Branches leading to *Chalinolobus gouldi* + *C. tuberculatus*, *Tylonycteris*, and *Scotophilus* are drawn half of actual length.

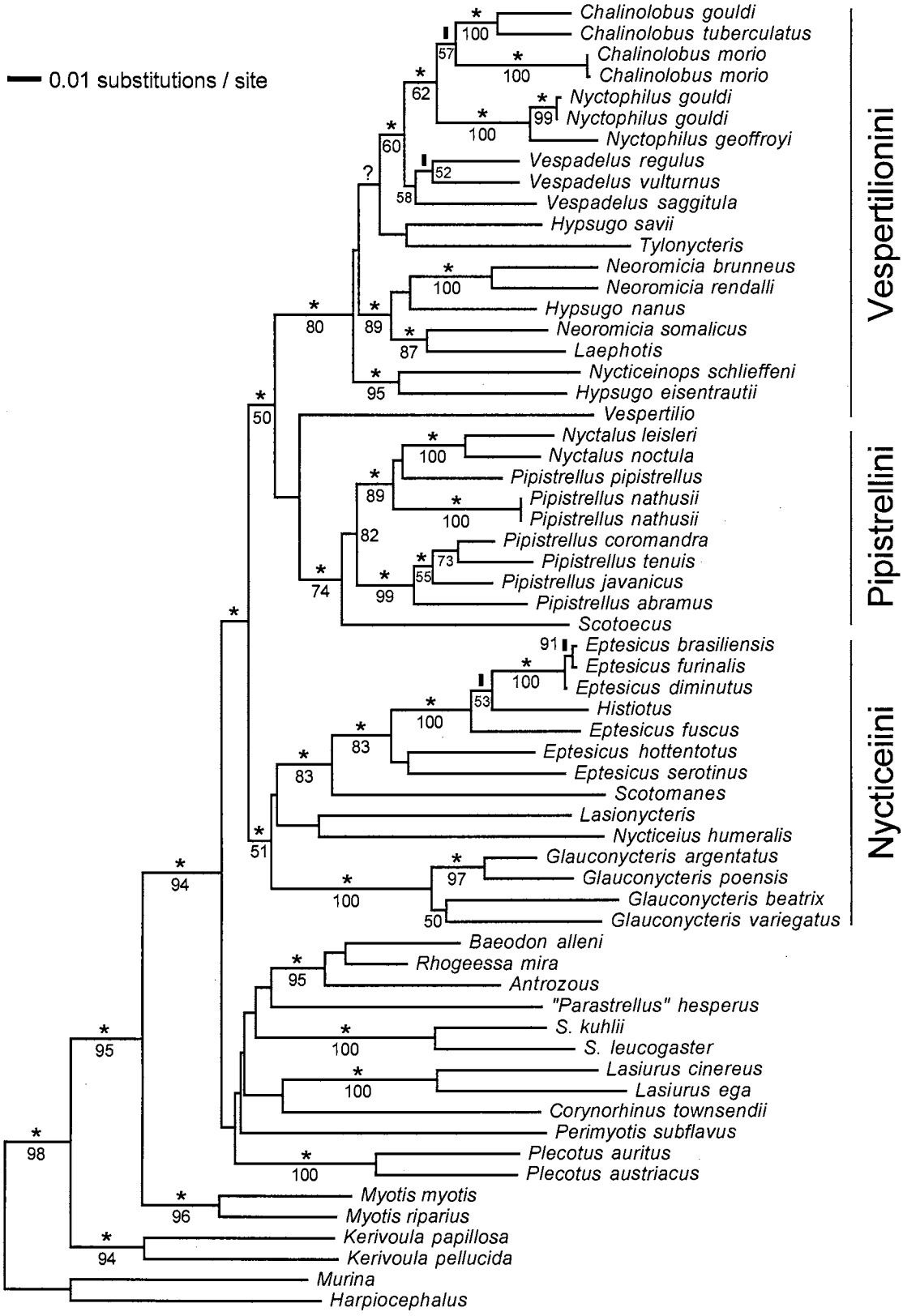

0.01 substitutions / site
Chalinolobus gouldi
Chalinolobus tuberculatus
Chalinolobus morio
Chalinolobus morio
Nyctophilus gouldi
Nyctophilus gouldi
Nyctophilus geoffroyi
Vespadelus regulus
Vespadelus vulturnus
Vespadelus saggitula
Hypsugo savii
Tylonycteris
Neoromicia brunneus
Neoromicia rendalli
Hypsugo nanus
Neoromicia somalicus
Laephotis
Nycticeinops schlieffeni
Hypsugo eisentrautii
Vespertilio
Nyctalus leisleri
Nyctalus noctula
Pipistrellus pipistrellus
Pipistrellus nathusii
Pipistrellus nathusii
Pipistrellus coromandra
Pipistrellus tenuis
Pipistrellus javanicus
Pipistrellus abramus
Scotoecus
Eptesicus brasiliensis
Eptesicus furinalis
Eptesicus diminutus
Histiotus
Eptesicus fuscus
Eptesicus hottentotus
Eptesicus serotinus
Scotomanes
Lasionycteris
Nycticeius humeralis
Glauconycteris argentatus
Glauconycteris poensis
Glauconycteris beatrix
Glauconycteris variegatus
Baeodon alleni
Rhogeessa mira
Antrozous
"Parastrellus" hesperus
S. kuhlii
S. leucogaster
Lasiurus cinereus
Lasiurus ega
Corynorhinus townsendii
Perimyotis subflavus
Plecotus auritus
Plecotus austriacus
Myotis myotis
Myotis riparius
Kerivoula papillosa
Kerivoula pellucida
Murina
Harpiocephalus
Vespertilionini
Pipistrellini
Nycticeiini

Figure 5.— Best tree (mean Lnl = -14052.10) from Bayesian analysis (GTR + Γ + I) of ribosomal gene sequences from 39 taxa (*Myotis* taxon set). Designated outgroups not depicted in tree included members of Kerivoulinae, Murininae, and Vespertilioninae. Parameter estimates from Bayesian and Parsimony analyses given in Table 3 and Table 4, respectively. Bayesian posterior probabilities (*P*) if ≥ 0.95 are shown above branches (as symbols) and are averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and 2 different sequence alignments. "*," $P = 1.0$ in all analyses regardless of alignment; "▌," $0.95 \leq P < 1.0$ in all analyses regardless of alignment; "?," $P \geq 0.95$ in all analyses based on 1 alignment, but < 0.95 in all analyses based on other alignment. Bootstrap support values from Parsimony analysis if > 50% are shown adjacent to or below branches (as percentages of 200 iterations) and also are averaged conservatively over all analyses. Current subgeneric classification is indicated by single letter following each species name: M = *Myotis* (type species *M. myotis*); L = *Leuconoe* (type species *M. daubentoni*); S = *Selysius* (type species *M. mystacinus*, not sampled).

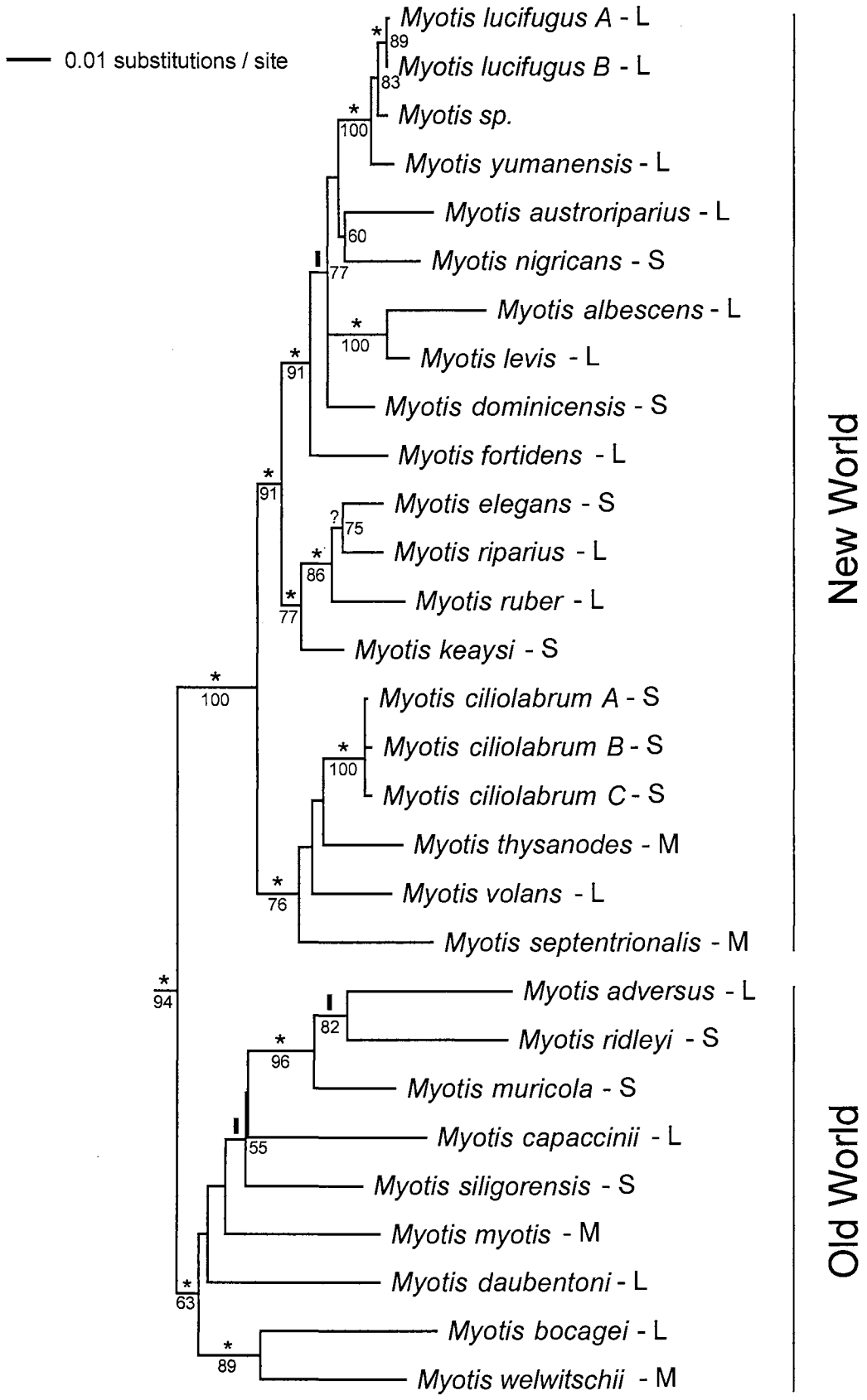
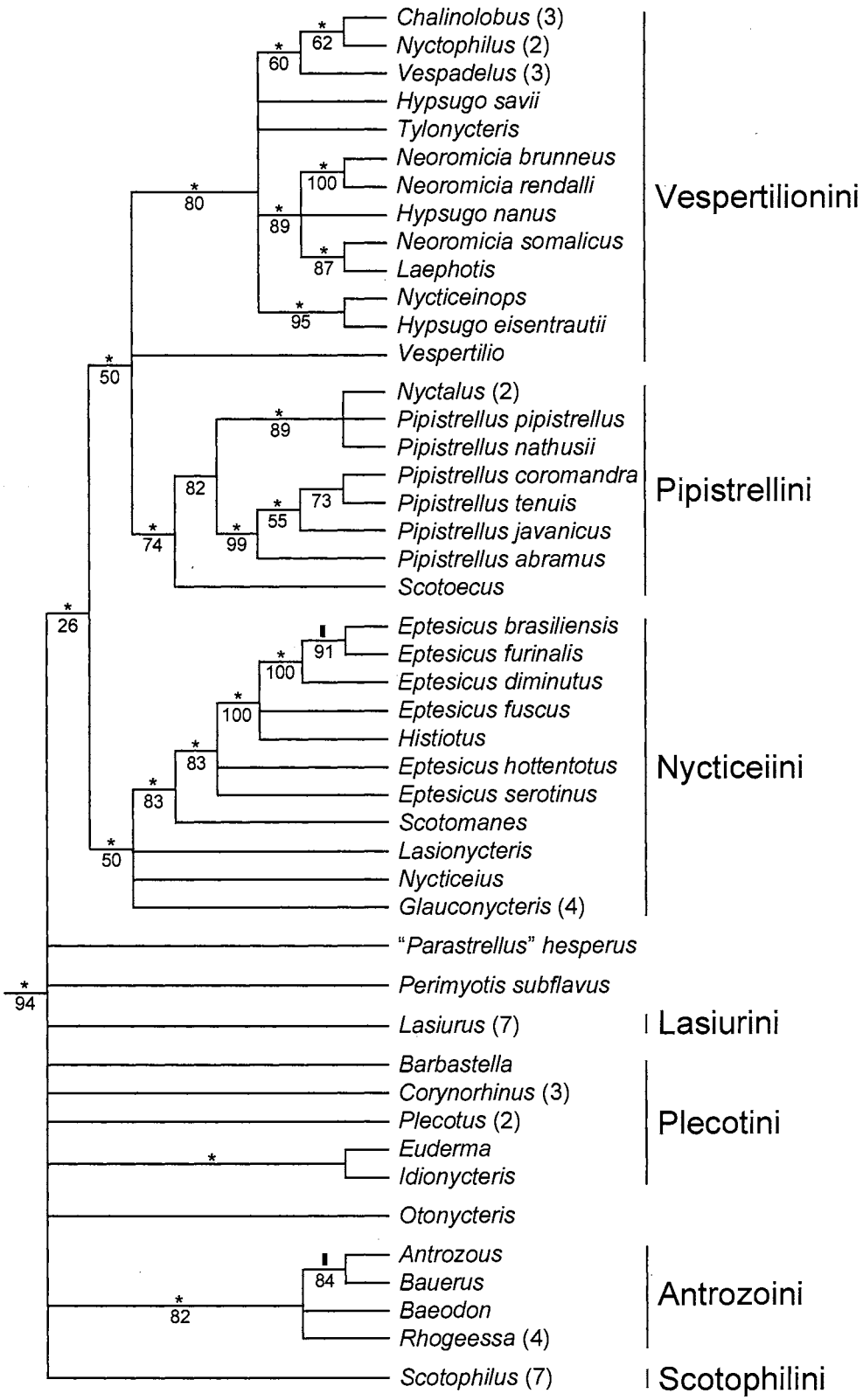

0.01 substitutions / site
Myotis lucifugus A - L
Myotis lucifugus B - L
Myotis sp.
Myotis yumanensis - L
Myotis austroriparius - L
Myotis nigricans - S
Myotis albescens - L
Myotis levis - L
Myotis dominicensis - S
Myotis fortidens - L
Myotis elegans - S
Myotis riparius - L
Myotis ruber - L
Myotis keaysi - S
Myotis ciliolabrum A - S
Myotis ciliolabrum B - S
Myotis ciliolabrum C - S
Myotis thysanodes - M
Myotis volans - L
Myotis septentrionalis - M
Myotis adversus - L
Myotis ridleyi - S
Myotis muricola - S
Myotis capaccinii - L
Myotis siligorensis - S
Myotis myotis - M
Myotis daubentoni - L
Myotis bocagei - L
Myotis welwitschii - M
New World
Old World

Figure 6.—Abbreviated cladogram for subfamily Vespertilioninae summarizing Figs. 3 and 4. Only relationships that were supported strongly by either or both Bayesian and Parsimony analyses are depicted. Symbols above branches indicate Bayesian posterior probabilities (*P*) averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and 2 different sequence alignments. "*," $P = 1.0$ in all analyses regardless of alignment; "▌," $0.95 \leq P < 1.0$ in all analyses regardless of alignment. Numbers below branches are bootstrap support values (percentages of 200 iterations) from Parsimony analysis, also averaged conservatively over all analyses. Numbers following some genera (in parentheses) indicate number of species included in phylogenetic analysis.


Chalinolobus (3)
Nyctophilus (2)
Vespadelus (3)
Hypsugo savii
Tylonycteris
Neoromicia brunneus
Neoromicia rendalli
Hypsugo nanus
Neoromicia somalicus
Laephotis
Nycticeinops
Hypsugo eisentrautii
Vespertilio
Vespertilionini
Nyctalus (2)
Pipistrellus pipistrellus
Pipistrellus nathusii
Pipistrellus coromandra
Pipistrellus tenuis
Pipistrellus javanicus
Pipistrellus abramus
Scotoecus
Pipistrellini
Eptesicus brasiliensis
Eptesicus furinalis
Eptesicus diminutus
Eptesicus fuscus
Histiotus
Eptesicus hottentotus
Eptesicus serotinus
Scotomanes
Lasionycteris
Nycticeius
Glauconycteris (4)
Nycticeiini
"Parastrellus" hesperus
Perimyotis subflavus
Lasiurus (7)
Lasiurini
Barbastella
Corynorhinus (3)
Plecotus (2)
Euderma
Idionycteris
Plecotini
Otonycteris
Antrozous
Bauerus
Baeodon
Rhogeessa (4)
Antrozoini
Scotophilus (7)
Scotophilini

VITA #2

Steven R. Hoofer

Candidate for the Degree of

Doctor of Philosophy

Dissertation: MOLECULAR PHYLOGENETICS OF THE CHIROPTERAN FAMILY VESPERTILIONIDAE

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Oklahoma State University, Ph.D., May 2003

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American Society of Mammalogists, *Life member*
Southwestern Association of Naturalists, 1994–present
Texas Society of Mammalogists, 1998-present
Central Plains Society of Mammalogists, 2000–present
Society of Systematic Biologists, 2002-present