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Molecular phylogenetics of the chiropteran family Vespertilionidae

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Limited information from existing data sets and the tremendous amount of diversity in number and kind within the chiropteran family Vespertilionidae (about one-third of all bat species) have hampered efforts to provide adequate assessments of long-standing genealogic hypotheses (e.g., monophyly of the family and of the five subfamilies). We generated approximately 2.6 kilobase pairs of mitochondrial DNA (mtDNA) sequence ecompassing three adjacent genes (12S rRNA, $tRNA^{Val}$, 16S rRNA) for 120 vespertilionids representing 110 species, 37 of 44 genera, and all subfamilies. We assessed monophyly of Vespertilionidae in initial analyses of 171 taxa including representatives of all bat families (except the monotypic Craseonycteridae), and assessed lower-level relationships by analysis of several truncated taxon sets. Phylogenetic analysis of ribosomal gene sequences provides well-supported resolution for vespertilionid relationships across taxonomic levels. Furthermore, the resolution is not heavily burdened by alignment of ambiguous regions of the ribosomal gene sequences, and topologies and levels of support produced by two phylogenetic methods (Bayesian and Parsimony) agreed markedly. Our analyses suggest relationships that support many parts of the traditional classification but which also support several changes. The majority of these changes also receives support from other data sources, particularly bacular and karyotypic data. We make more than 20 taxonomic conclusions or recommendations and construct a working classification for vespertilionoid bats. Highlights include: *Miniopterus* (subfamily Miniopterinae) is recognized in its own family, Miniopteridae, as it represents an extremely divergent lineage relative to other vespertilionids, and in some analyses is sister to the molossids and natalids; all other vespertilionids examined form a well-supported clade; two 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*; *Myotis* is sister to a clade containing Kerivoulinae and Murininae and is recognized in its own subfamily, Myotinae; *Myotis* subgenera *Leuconoe*, *Selysius*, and *Myotis* are polyphyletic, and 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 (*Aeorestes* Fitzinger, 1870); Vespertilioninae (excluding *Myotis*) is monophyletic; *Pipistrellus*-like bats (i.e., the traditional tribe Vespertilionini) are divided into three tribes (Nycticeiini; Pipistrellini; Vespertilionini); and support for three tribes of *Pipistrellus*-like bats has several implications at the genus level. Overall, this study offers a robust working hypothesis for vespertilionid relationships and provides a good starting point for new investigations into the evolutionary history of Vespertilionidae.

Key words: Bayesian, Chiroptera, classification, phylogenetics, systematics, Vespertilionidae, Miniopteridae

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 (Rosevear, 1965; Koopman, 1970). 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 one 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 (Miller, 1907; Koopman, 1994). 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, 1941*b*, 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; Sudman *et al*., 1994; Simmons, 1998; Simmons and Geisler, 1998). 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 based on ill-defined criteria: nondescript 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 six 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 (Simpson, 1945; Kuzjakin, 1950; Koopman and Cockrum, 1967; Hill and Smith, 1984; Koopman, 1984, 1985, 1993, 1994; Corbet and Hill, 1991; McKenna and Bell, 1997; Nowak, 1999). With minor alterations, arrangements of Miller (1907) and Tate (1941*b*, 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., Topál, 1970; Hill and Topál, 1973; Zima

and Horáček, 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 (1994*b*; stemming from Volleth's 1989 dissertation). They examined banded karyotypes from 50 species representing 23 genera and all subfamilies of Vespertilionidae, but sampled only one 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 three tribes (Eptesicini, Pipistrellini, Vespertilionini) and *Pipistrellus* into four 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* and *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 (Wilson *et al*., 1985; Avise, 1986; Moritz *et al*., 1987; Simon *et al*., 1994), 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., Orti and Meyer, 1997; Whiting *al*., 1997; Leaché and Reeder, 2002). 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. We inferred relationships among 171 taxa by phylogenetic analysis of mtDNA characters (about 2.6 kilobases) encompassing three adjacent genes (12S rRNA, $tRNA^{Val}$, 16S rRNA).

Materials and Methods

Taxon Sampling

We set out to sample about one-third of all vespertilionid species to represent taxonomic, morphologic, ecologic, behavioral, and geographic diversity equally within each subfamily, tribe, and (when appropriate) genus. Aquiring 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 (Corbet and Hill, 1991; Koopman, 1994; McKenna and Bell, 1997: Kerivoulinae, three of 22 species, one of two genera; Miniopterinae, six of 11 species, one of one genus; Murininae, two of 16 species, two of two genera; Nyctophylinae, two of nine species, one of two genera; Vespertilioninae, 97 of 293 species, 32 of 38 genera (Appendix I). We also sampled 51 bats representing all

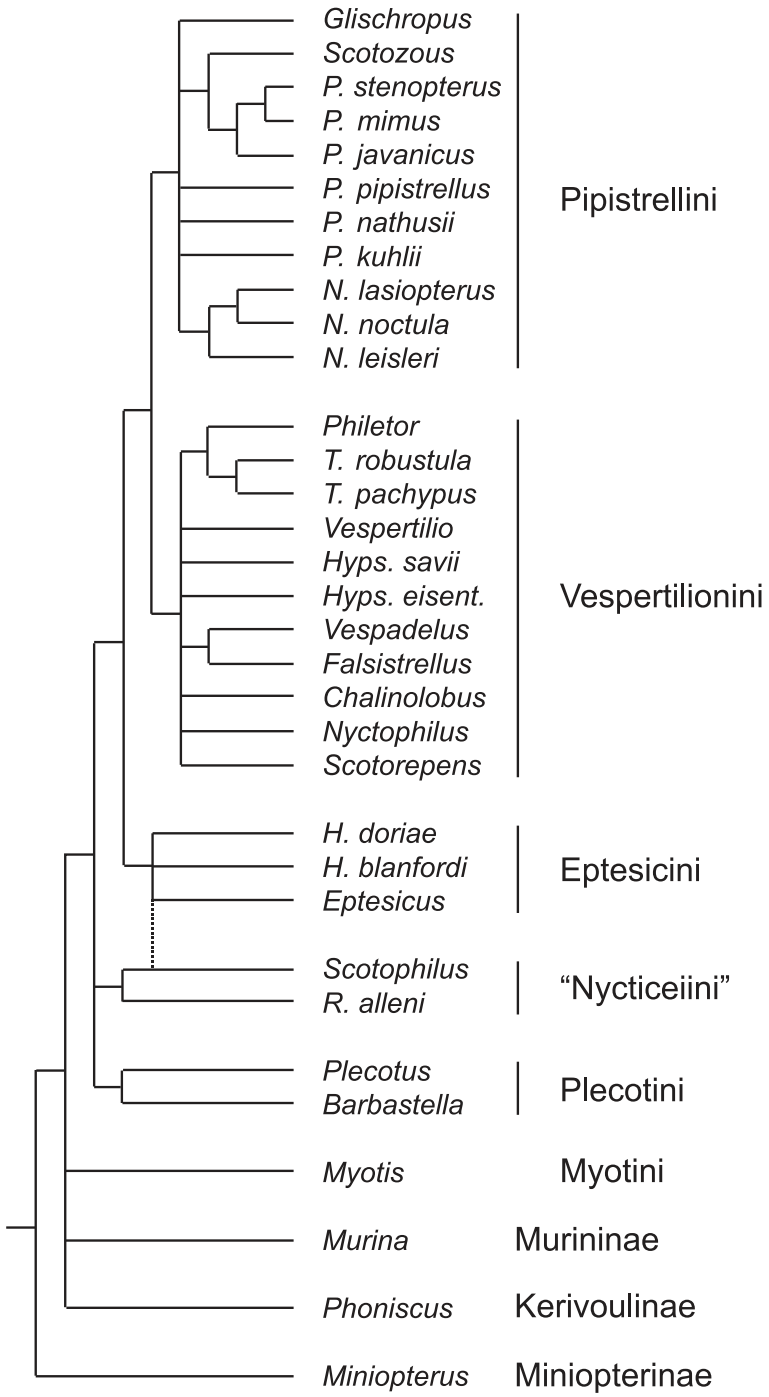


FIG. 1. Volleth and Heller's (1994*b*) cladogram of Vespertilionidae based on parsimony analysis of karyologic features. Topology shown is based on one of three sets of assumptions for ancestral character states. Under this set of assumptions, dotted line indicates another possibility for relationship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

other families (except Craseonycteridae; Appendix I). We 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).

We relied on species identifications made by institutional collections; although many were verified or, for a few, changed by the first author based on examination of voucher specimens. A voucher specimen for nearly all samples (Ruedas *et al*., 2000) is deposited in one 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, Muséum d'Histoire Naturelle de Genève, Museum of Southwestern Biology at the University of New Mexico, Museum of Texas Tech University, 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, United States National Museum of Natural History (USNM), Universidad Autónoma Metropolitana-Iztapalapa, Universidad Nacional Autónoma de Mexico City, University of Memphis, Mammal Collection, and University of Wisconsin Zoological Museum (Appendix I). We were unable to locate voucher information for 14 samples, seven of which were vespertilionids. There also was limited voucher information (e.g., sampling locality) for all six sequences obtained from GenBank, two of which were vespertilionids (Appendix I).

Molecular Methods

We extracted genomic DNA from skeletal muscle or organ tissue samples with standard phenol methods (Longmire *et al*., 1997). We 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, we sequenced all three genes entirely in both directions with an assortment of external and internal primers (Van Den Bussche and Hoofer, 2000). We generated data for several taxa that had been included in previous studies, and sequences were submitted to GenBank prior to completion of this study.

Multiple Sequence Alignment

We aligned sequences in CLUSTAL X software (Thompson *et al*., 1997) following methods of Hoofer *et al*. (2003), who used 15.00:6.66 (default) and 5:4 values for gap-cost ratio (Hickson *et al*., 2000). We refined both alignments by eye according to secondary structural models (Anderson *et al*., 1982; De Rijk *et al*., 1994; Springer and Douzery, 1996). We also identified regions of alignment where positional homology was uncertain by using the 'gap-sliding' method (Lutzoni *et al*., 2000: 634–635, criteria 1–3, and 7; see Appendix II). We were concerned primarily with large regions (e.g., up to 200 sites long) with multiple insertion/deletion events. We 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 one sequence contains one inserted nucleotide (or vice versa) within a highly conserved or constant region of nucleotides. In such cases, placement of one gap in all but one taxon (or one gap in one taxon) allowed assignment of positional homology among neighboring nucleotides. Alignment and phylogenetic analysis of two cost ratios nonetheless provides objectivity for gap placement in the relatively few, unambiguous, and small gapped-regions (Hickson *et al*., 2000).

Taxon Sets

We analyzed four separate sets of taxa to assess relationships at different taxonomic levels (Table 1). We 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 while assuming little about relationships within Chiroptera. We subsequently analyzed three 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). We selected each taxon set, especially the outgroups (see Table 1), based on results from overall analyses and other studies (Volleth and Heller, 1994*b*; Simmons and Geisler, 1998; Teeling *et al*., 2000, 2002; Van Den Bussche and Hoofer, 2001; Volleth *et al*., 2001; Hoofer *et al*., 2003). For each taxon set, we performed new sequence alignments (with two 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, we analyzed six different alignments (two

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 five 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	Nyctophylinae	Vespertilioninae
Mops	*Nyctophilus* (3)	Lasiurini*
Nyctinomops	Vespertilioninae	*Lasiurus*
Tadarida	Antrozoini	Myotini
Vespertilionidae	*Antrozous*	*Lasionycteris*
Kerivoulinae	Lasiurini	*Myotis* (29)
Kerivoula (3)	*Lasiurus* (2)	Nycticeiini*
Miniopterinae	Myotini	*Rhogeessa*
Miniopterus (6)	*Lasionycteris*	*Scotophilus* (2)
Murininae	*Myotis* (2)	
Harpiocephalus	Nycticeiini	
Murina	*Baeodon*	
Nyctophylinae	*Nycticeinops*	
Nyctophilus (4)	*Nycticeius*	
Vespertilioninae	*Rhogeessa*	
Antrozoini	*Scotoecus*	
Antrozous	*Scotomanes*	
Bauerus	*Scotophilus* (2)	
Lasiurini	Plecotini	
Lasiurus (8)	*Corynorhinus*	
Myotini	*Plecotus* (2)	
Lasionycteris	Vespertilionini	
Myotis (29)	*Chalinolobus* (4)	
Nycticeiini	*Eptesicus* (6)	
Baeodon	*Glauconycteris* (4)	
Nycticeinops	*Histiotus*	
Nycticeius	*Hypsugo* (3)	
Otonycteris	*Laephotis*	
Rhogeessa (5)	*Neoromicia* (3)	
Scotoecus	*Nyctalus* (2)	
Scotomanes	'*Parastrellus*'	
Scotophilus (7)	*Perimyotis*	
Plecotini	*Pipistrellus* (7)	
Barbastella	*Tylonycteris*	
Corynorhinus (3)	*Vespadelus* (3)	
Euderma	*Vespertilio*	
Idionycteris		
Plecotus (2)		
Vespertilionini		
Chalinolobus (4)		
Eptesicus (6)		

TABLE 1.Continued

	Taxon sets	
Vespertilionidae	*Pipistrellus*-like	*Myotis*
Glauconycteris (4)		
Histiotus		
Hypsugo (3)		
Laephotis		
Neoromicia (3)		
Nyctalus (2)		
'*Parastrellus*'		
Perimyotis		
Pipistrellus (9)		
Tylonycteris		
Vespadelus (3)		
Vespertilio		

per taxon set), and eight total, including the overall taxon set.

Phylogenetic Inference

We coded nucleotides as unordered, discrete characters (G, A, T, C), multiple states as polymorphisms, and gaps as missing. We analyzed complete sequences for all three 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.

We inferred phylogenetic relationships by using two optimality criteria: Bayesian Likelihood (Li, 1996; Mau, 1996; Rannala and Yang, 1996) and Parsimony. We ran Bayesian analyses in MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) at least 1×10^6 generations with one cold and three incrementally heated Markov chains, random starting trees for each chain, and trees sampled (saved) every 10 generations. For both alignments within each taxon set, we ran a minimum of nine independent analyses (sets of three analyses for each of the three 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. We also ran several other analyses using other outgroup species (but not sets of three analyses) to further assess affects of outgroup choice on topology and posterior probability distribution. We 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). We 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).

We 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). We 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%). We

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 three 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 (see Figs. 2–6). 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 one alignment but not the other. We treated these nodes as unresolved (denoted '?' in Figs. 2–6).

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. 2–6). 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-

TABLE 2. Number of characters (=sites) for each taxon set based on two separate alignments; one 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 four sets of taxa. Estimated parameters are -Ln likelihoods (-Lnl), rates (R) of six 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

Parameter	All taxa	Vespertilionidae	*Pipistrellus*-like	*Myotis*
Burn-in	2,000	2,000	2,000	1,500
-Lnl	42,608.14	34,710.98	22,072.97	14,052.10
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

significant posterior probabilities ($P < 0.95$; Figs. 2–6).

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. 2–6). 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. 3–6). Bootstrap topologies and support were essentially identical between analyses of alternative alignments within taxon sets, with only slight variation in specific lengths of most-parsimonious trees, exact bootstrap proportions, and consistency and retention indices (Table 4). They also were essentially identical between analyses based on different taxon sets (Figs. 2–6). There were no supported conflicts between analyses based on Parsimony and Bayesian methods, and nearly all nodes receiving support from one phylogenetic method also were supported by the other.

DISCUSSION

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 vesperti lionid 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 many 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

TABLE 4. Number of most-parsimonious (MP) trees and lengths and consistency (CI) and retention (RI) indexes for Parsimony analyses of four sets of taxa. All values are based on alignments with default settings for gap-cost ratio

Parameter	All taxa	Vespertilionidae	*Pipistrellus*-like	*Myotis*-like
MP trees	2,566	18	4	1
Length	9,020	7,179	4,425	2,373
CI	0.17	0.20	0.28	0.43
RI	0.60	0.61	0.49	0.55

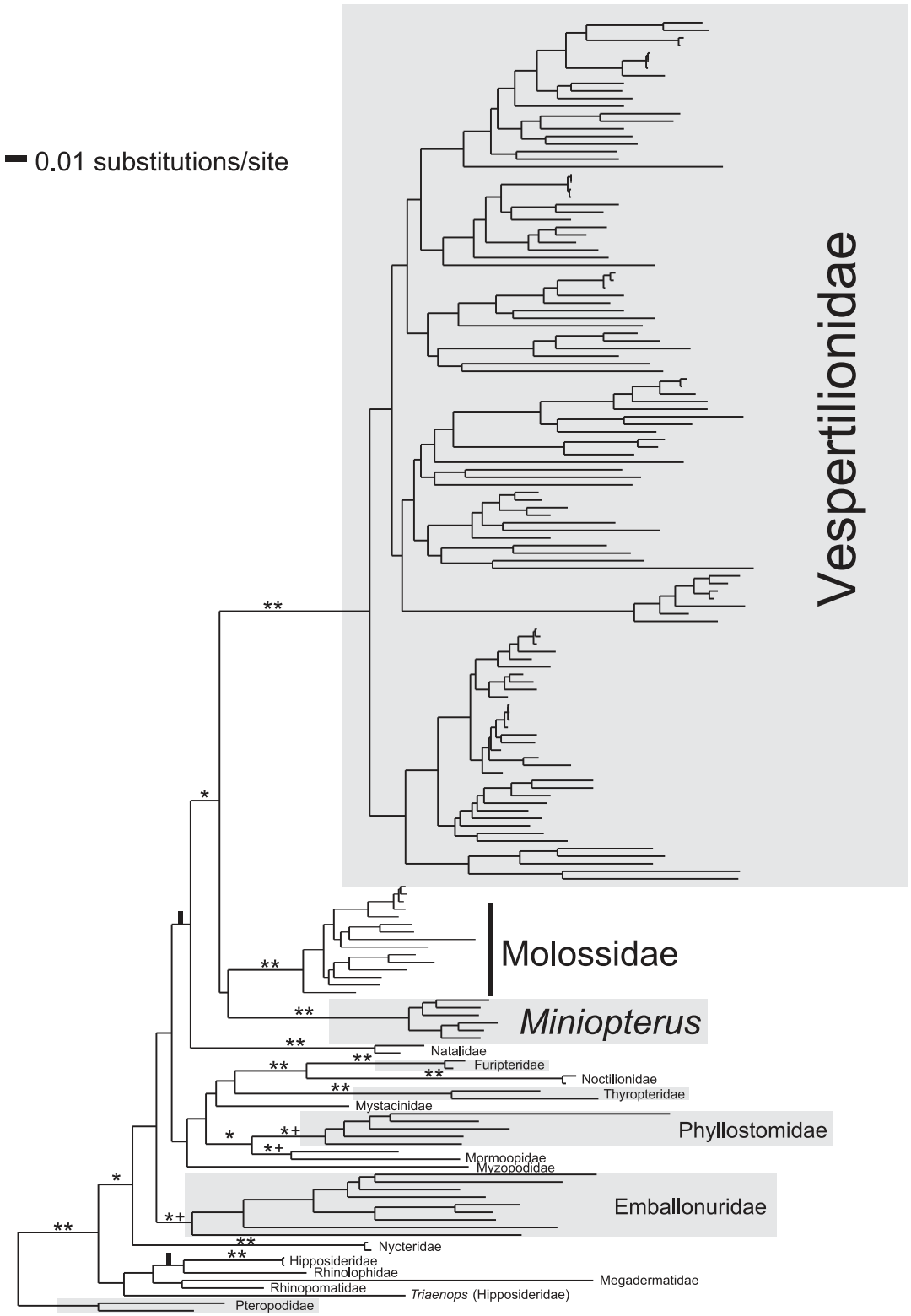
variation in rate of evolution along the length of RNA molecules (Appendix II). At the same time, such characteristics complicate multiple sequence alignment. We implemented two-tier approach to help avoid complications: independent analysis of three sets of taxa truncated from the overall taxon set; 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 four sets of taxa that employed two 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 two methods of phylogenetic inference (Bayesian and Parsimony) also agreed markedly (Appendix III). 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 the 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.

Superfamily Vespertilionoidea

With one exception, all traditional families (other than Vespertilionidae) for which we examined ≥ 2 representatives were supported as monophyletic assemblages. The exception was Hipposideridae relative to

FIG. 2. Maximum posterior probability tree (mean Lnl = -42,608.14) 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 are given in Tables 3 and 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 two 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%; 'I,' $0.95 \leq P < 1.0$ in all analyses regardless of alignment, but BS < 70%. Intermittent shading is only for help visually distinguishing family-level clades



Triaenops (Fig. 2). The position of *Triaenops* may have been spurious, however, resulting from inadequate sampling of taxa within Hipposideridae and closely related families; three hipposiderids (including *Triaenops*), one megadermatid, one rhinolophid, and one 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 (Swofford *et al*., 1996; Graybeal, 1998; Hillis, 1998; Poe, 1998). 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; Miller, 1907; Koopman and Jones, 1970; Smith, 1976; Koopman, 1984; Volleth *et al*., 2002; Teeling *et al*., 2003). Traditional classification of Vespertilionoidea, which is heavily weighted by characters of the wing and shoulder joint, includes four other families (Furipteridae, Mystacinidae, Myzopodidae, Thyropteridae), but there is no consensus for their affinities (Miller, 1907; Smith, 1976, 1980; Koopman, 1984, 1993). Recent studies of morphologic and molecular data contradict this traditional classification, and suggest that all four families share greater affinities with noctilionoid families, or at least that they did not share a recent common ancestry with Molossidae, Natalidae, and Vespertilionidae (Pierson, 1986; Kirsch *et al*., 1998; Kennedy *et al*., 1999; Van Den Bussche and Hoofer, 2000, 2001; Simmons and Conway, 2001; Teeling *et al*., 2002, 2003; Hoofer *et al*., 2003).

The present study supports the revision by Hoofer *et al*. (2003) and Teeling *et al*. (2003) for superfamily Vespertilionoidea to include Molossidae, Natalidae, and Vespertilionidae, with Natalidae representing the basal lineage (Fig. 2). Although those studies of mitochondrial and nuclear DNA sequences support monophyly of Vespertilionidae, both included relatively few taxa, and in particular did not include *Miniopterus*. Thus, the present study supports Hoofer *et al*. (2003) and Teeling *et al*. (2003) but, as discussed at length below, also recognizes a fourth family, Miniopteridae, within Vespertilionoidea.

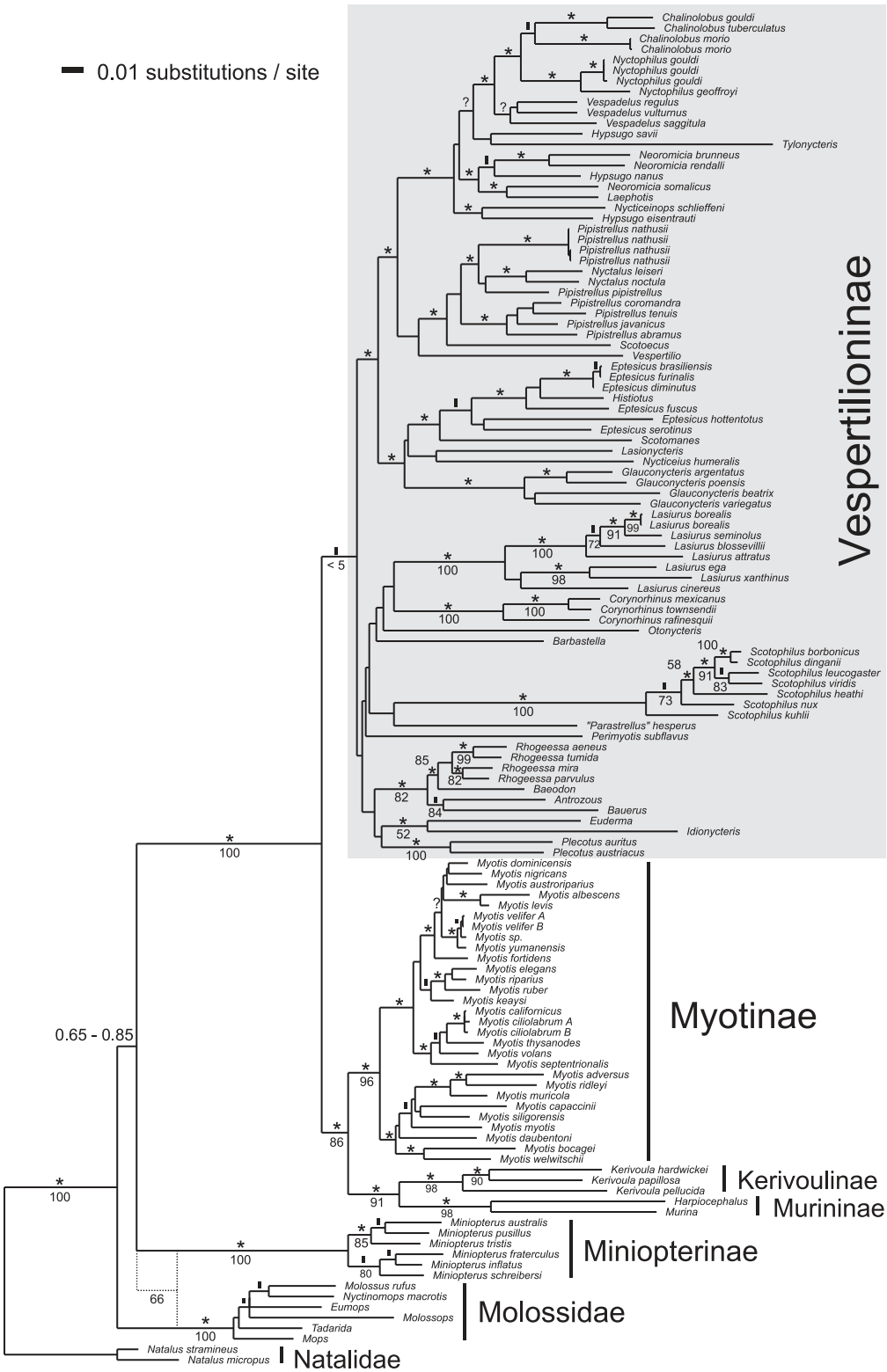
Family Vespertilionidae

This study supports a clade including all traditional vespertilionids examined except *Miniopterus* (Figs. 2 and 3). Thus, this study contradicts previous suggestions for removing Kerivoulinae (Sigé, 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 (two of three 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). We explored the possibility that our biased sampling of vespertilionids relative to other families somehow affected divergence estimates for *Miniopterus* or its phylogenetic placement and level of support. We performed several analyses that included only about 20 representatives of Vespertilionidae and all six *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*. We 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; six of 11 recognized species and four of five 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 first 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 our knowledge, all syntheses of chiropteran systematics have favored Miller's (1907) arrangement (excepting Tomopeatinae), relegating Miniopteridae to subfamily rank within Vespertilionidae (e.g., Koopman, 1984, 1993, 1994; Yoshiyuki, 1989; Corbet and Hill, 1991, 1992; McKenna and Bell, 1997). 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., Topál, 1970; Hill and Topál, 1973; 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 (1994*b*) analysis of banded karyotypes ostensibly supported monophyly of Vespertilionidae including *Miniopterus* (Fig. 1). On one 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*a*, 1979*b*; Zima and Horáček, 1985; Volleth and Heller, 1994*b*). Accordingly, others also have pointed to the study as positive evidence for including *Miniopterus* within Vespertilionidae (e.g., Simmons, 2000: 33–34). On the other hand, however, Volleth and Heller's (1994*b*) 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 (1994*b*) 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 (1994*b*) did not cite the outgroup comparison method explicitly, but described the same procedure nonetheless. They inferred an hypothetical ancestor (or hypothetical ancestral states for each character) from multiple outgroups (one molossid, *Molossus ater*; two natalids, *Natalus stramineus* and *N. tumidirostris*) to polarize each character and maximize global parsimony relative to the ingroup. The inferred ancestor represented one 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

FIG. 3. Maximum posterior probability 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 are given in Tables 3 and 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 two different sequence alignments. '*,' $P = 1.0$ in all analyses regardless of alignment; 'I,' $0.95 \leq P < 1.0$ in all analyses regardless of alignment; '?,' $P \geq 0.95$ in all analyses based on one 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%)

TABLE 5. Apomorphies distinguishing *Miniopterus* from all other vespertilionids

Character	*Miniopterus*	Vespertilionidae	Source
		Anatomy	
Hair structure	Long, entire coronal scales alternating between extremely short hastate scales	Generally hastate scales	Benedict (1957)
Dental formula	Supplementary vestigial tooth present between upper canine and first premolar	No tooth between upper canine and first premolar	Mein and Tupinier, (1977); van der Merwe (1985)
Tongue (papillae)	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	Tiunov (1989)
Second phalanx of third finger	About three times as long as first	Usually about as long as first (always << 3 times as long)	Miller (1907)
Tendon locking mechanism	Absent	Present	Simmons (1998)
Rostral and sylvian sulci	Prominent	Slight	Reep and Bhatnagar (2000)
Baculum	Absent	Present	Mathews (1942)
Sperm head	Long (9 mm), filled with nucleus and massive acrosome	Short (4–5.5 μm), filled with nucleus and capped with small acrosome	Mori and Uchida (1982); Breed and Inns (1985)
Urethral glands	Present	Absent	Tiunov (1989)
Cowper's glands	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)	Tiunov (1989)
		Embryology	
Delayed development	Blastocyst remains free	Blastocyst implants, but development is retarded	Gopalakrishna and Karim (1980); Gopalakrishna and Chari (1983); Karim and Bhatnagar (2000)
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	Gopalakrishna and Karim (1980); Gopalakrishna and Chari (1983); Karim and Bhatnagar (2000)
Roof of amniotic cavity	Developed by uterine endometrial layer (no cavitation)	Developed by cavitation (trophoblastic layer)	Gopalakrishna and Karim (1980); Gopalakrishna and Chari (1983); Karim and Bhatnagar (2000)
Abembryonic yolk	Remains in contact with uterine wall	Remains hanging in persistent uterine lumen	Gopalakrishna and Karim (1980); Gopalakrishna and Chari (1983); Karim and Bhatnagar (2000)
Chorioallantoic placenta	Three types (primary, secondary, tertiary)	One or two types	Gopalakrishna and Karim (1980); Gopalakrishna and Chari (1983); Karim and Bhatnagar (2000)
		Immunology	
MC'F transferrin distances	Closest to anti-*Tadarida*	Closest to anti-*Antrozous*	Pierson (1986)

by six autosomes and the X-chromosome (Volleth and Heller, 1994*b*).

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 Teeling *et al*., 2000, 2002; Murphy *et al*., 2001; Van Den Bussche and Hoofer, In press).

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; Table 5). Evidence available in the literature for phylogenetic affinities of *Miniopterus* is in fact without consensus, pointing toward two 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 one of Miller's (1907) 17 families is no longer recognized (Desmodontidae), and only two families have been added (Craseonycteridae and Mormoopidae). Craseonycteridae represents an addendum to Miller's arrangement, as it contains only one species (*Craseonycteris thonglongyai*) unknown to science until the 1970s (Hill, 1974); it differs markedly from all other morphologic families (Hill and Smith, 1981; see also Hulva and Horáček, 2002).

Justification for reclassifying the other two 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, 2003; Van Den Bussche and Hoofer,

2000, 2001; Simmons and Conway, 2001; Van Den Bussche *et al*., 2002; Hoofer *et al*., 2003). 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, we suggest Miniopteridae be placed incertae sedis within Vespertilionoidea (sensu Hoofer *et al*., 2003 and Teeling *et al*., 2003), specifically within the clade containing Molossidae and Vespertilionidae. This nomenclatural arrangement facilitates recognition of both similarities and differences among the vespertilionoid groups (Natalidae, Molossidae, Miniopteridae, Vespertilionidae).

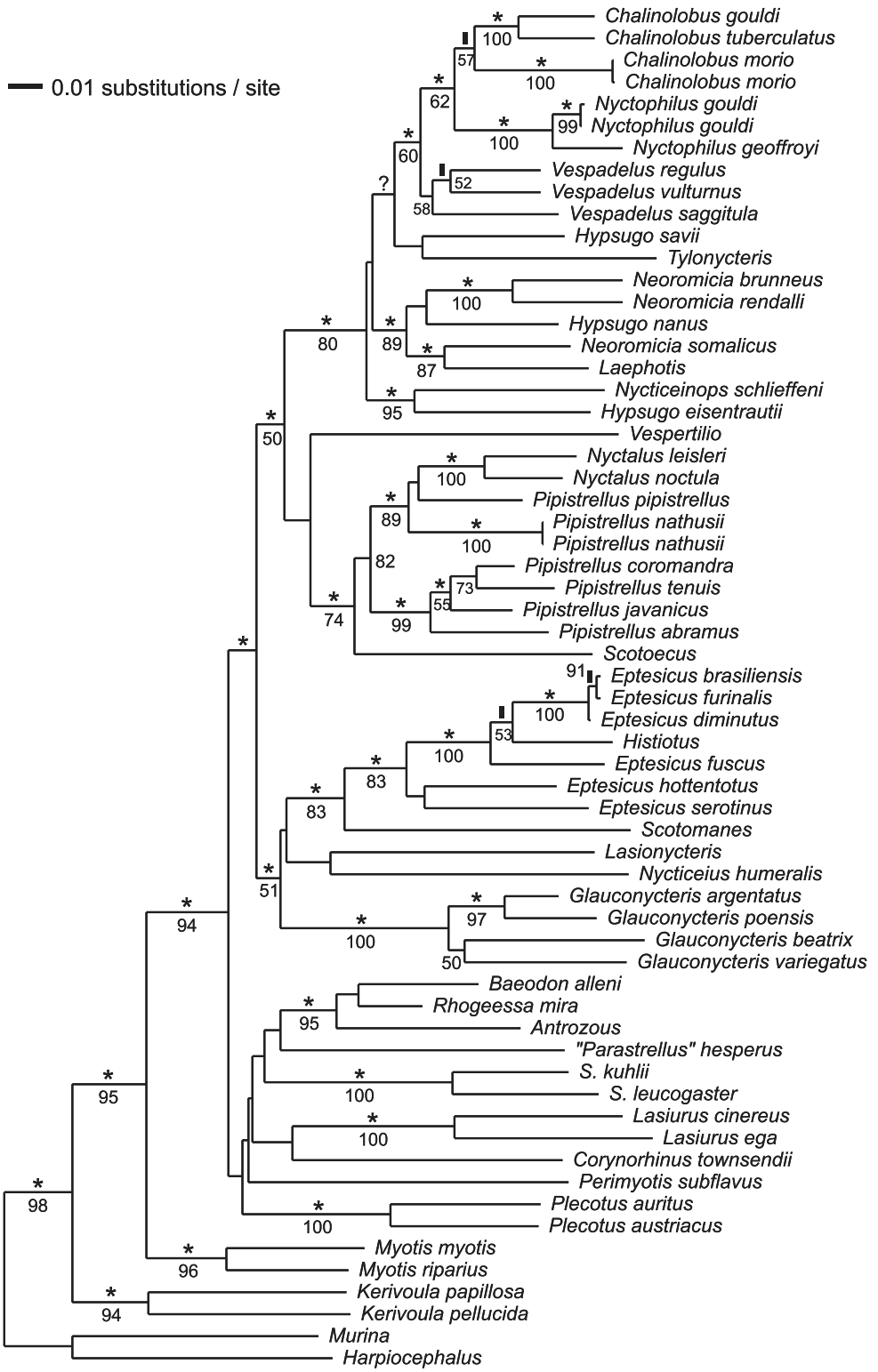
Subfamilies of Vespertilionidae

This study supports monophyly of only two of the traditional subfamilies within Vespertilionidae (sensu stricto), Murininae and Kerivoulinae (sensu Miller, 1907). Nyctophilinae (sensu Miller, 1907; Hill and Harrison, 1987; Corbet and Hill, 1991) clearly "has no real validity" (Koopman, 1985: 27). For mtDNA, *Nyctophilus* nestled deeply within a clade of *Pipistrellus*-like bats. Furthermore, Vespertilioninae (sensu Miller, 1907; Koopman, 1994; McKenna and Bell, 1997) 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% (see 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

FIG. 4. Maximum posterior probability tree (mean Lnl = -34,710.98) 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 are given in Tables 3 and 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 two different sequence alignments. '*,' $P = 1.0$ in all analyses regardless of alignment; 'I,' $0.95 \leq P < 1.0$ in all analyses regardless of alignment; '?,' $P \geq 0.95$ in all analyses based on one 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 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



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, and generally performs poorly under various simulated conditions compared to Bayesian methods (Alfaro *et al*., 2003). On the other hand, Bayesian analysis, which employs complex models of sequence evolution (Whelan *et al*., 2001; Huelsenbeck *et al*., 2002), 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, 1994*b*). Based on the mtDNA tree (Figs. 2 and 3), there is more than one 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, we follow Volleth and Heller's (1994*b*) 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 first 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; Tate, 1942; Horáček, 2001). All vespertilionids apparently exhibit a generalized cranial and dental constitution that, unlike other family groups, essentially was unaffected by any specific rearrangements (Horáček, 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 three 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 (Horáček, 2001; Czaplewski *et*

al., In press). 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 two lineages: early on in the evolution of Vespertilioninae; and subsequently during the evolution of Murininae.

Volleth and Heller's (1994*b*) 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 third set left relationships of all subfamilies unresolved (their figure 6, p. 23). Volleth and Heller (1994*b*) chose to use the third set of assumptions when constructing an overall tree for the family (which is shown in our 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.

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*., 2003). 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 three 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; Tate, 1942; McKenna and Bell, 1997). 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 Horáček, 1985). There has not been, until now, an explicit test of 'Myotini' monophyly. Neither Volleth and Heller (1994*b*) nor Simmons (1998) 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: 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 one of the most karyotypically conservative genera within Vespertilionidae (2N = 44, FN = 50–52; Bickham 1979*a*, 1979*b*; Bickham *et al*., 1986; McBee *et al*., 1986).

Current systematics of *Myotis*, chartered by Miller and Allen (1928) and Tate (1941*a*), essentially follows Findley (1972), who undertook a numerical taxonomic analysis of nearly all species known at that time. The analysis distinguished three phenetic groups, corresponding more or less to three 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 two rare South African species in a fourth subgenus, *Cistugo*; however, morphologically and karyotypically (2N = 50, FN = 48) *Cistugo* probably warrants full generic rank (Rautenbach *et al*., 1993).

mtDNA analysis of nearly one-third of all recognized extant species of *Myotis*, including representatives from all zoogeographic regions and all subgenera except *Cistugo* (Appendix I), 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). Several mtDNA clades contain members of two or all three of the examined subgenera. Thus, based on mtDNA data, morphologic and ecologic similarity generally do not reflect close relationship; rather, morphologic and ecologic similarities defining each of the three 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 three groups: one containing only Nearctic species (*californicus*, *ciliolabrum*, *septentrionalis*, *thysanodes*, *volans*); another containing only Neotropical species (*elegans*, *keaysi*, *riparius*, *ruber*); and a third containing both Nearctic (*austroriparius*, *velifer*, *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 two Palearctic species sampled (*daubentonii*, *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 the phylogenetic history of 13 American, 11 Palaearctic, and six other Old World species of *Myotis* based on DNA sequence data from two other mitochondrial genes (cytochrome *b* and NADH dehydrogenase subunit 1). Their separate and combined analyses of the mitochondrial protein-coding genes provided no support for monophyly of any of the three subgenera (*Leuconoe*, *Myotis*, *Selysius*). The results supported two primary clades, one comprising all New World *Myotis* plus the Old World species *brandtii*, and one 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 based on traditional systematics. For example, they sampled *M. thysanodes* and *M. nattereri*, which represent the

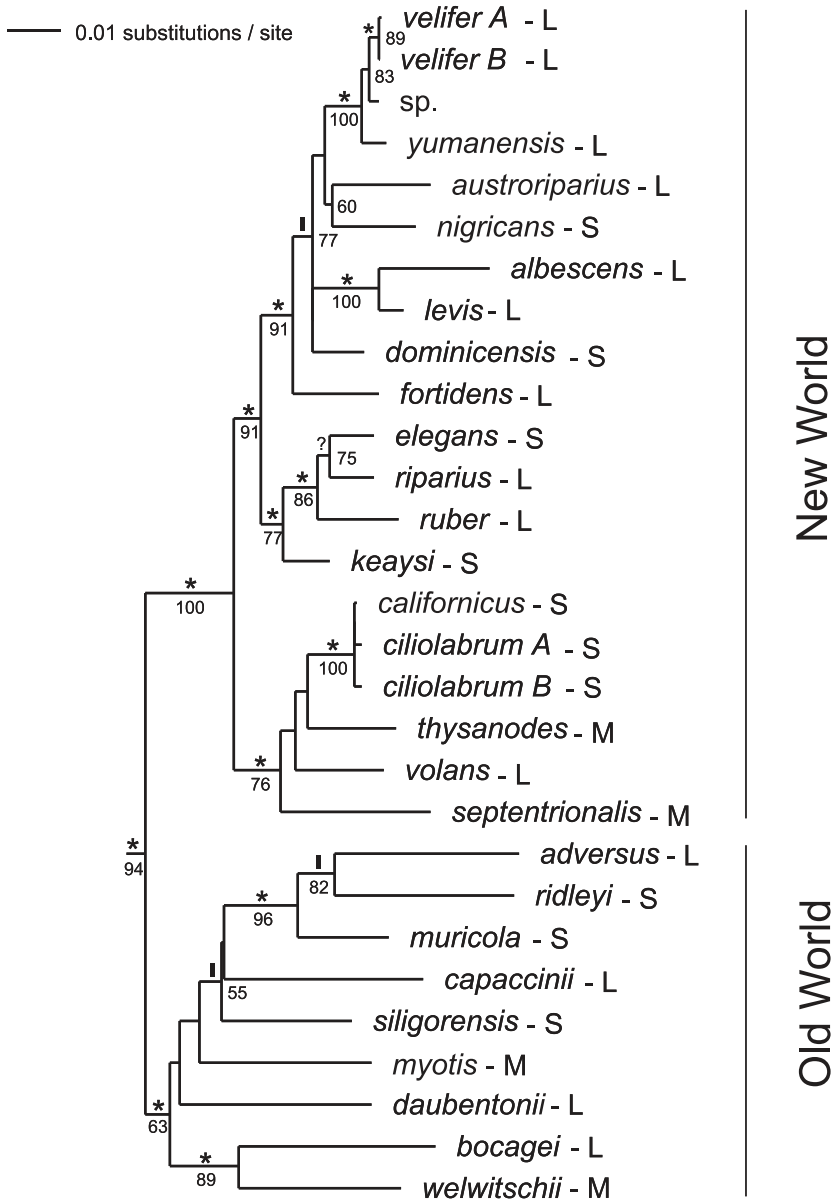


FIG. 5. Maximum posterior probability tree (mean Lnl = -14,052.10) from Bayesian analysis (GTR + Γ + I) of ribosomal gene sequences from 39 taxa (*Myotis* taxon set). Designated outgroups not depicted in tree included members of Kerivoulinae, Murininae, and Vespertilioninae. Parameter estimates from Bayesian and Parsimony analyses are given in Tables 3 and 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 two different sequence alignments. '*,' $P = 1.0$ in all analyses regardless of alignment; 'I,' $0.95 \leq P < 1.0$ in all analyses regardless of alignment; '?,' $P \geq 0.95$ in all analyses based on one 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. daubentonii*); S = *Selysius* (type species *M. mystacinus*, not sampled)

Nearctic and Palearctic members of 'fringed bats,' respectively, and sometimes are recognized in a distinct subgenus, *Isotus* (Tate, 1941*a*; Corbet and Hill, 1991). Ruedi and Mayer (2001) also sampled *M. lucifugus* (Nearctic) and *M. daubentonii* (Palearctic), two small species with short ears that typically forage over water surfaces, morphologic and ecologic equivalents (Fenton and Barclay, 1980; Jones and Rayner, 1988). All of their analyses contradicted monophyly of *Isotus* and close affinities between *M. lucifugus* and *M. daubentonii* by placing the two species of each pair in widely divergent clades, suggesting that remarkable similarities in morphology and ecology are the result of convergent evolution.

Kawai *et al*. (2003) subsequently added to Ruedi and Mayer's (2001) data set by including several Japanese and East Asian species of *Myotis*. Their analyses affirm results of Ruedi and Mayer (2001), and further suggest relationships contrary to the current taxonomy. For example, specimens of *M. daubentonii* (type species of *Leuconoe*) from Japan and Russia and from Europe are related distantly, and *M. mystacinus* from Japan is sister to *M. brandtii* within the New World clade. Kawai *et al*. (2003) point out that future study may reveal other cryptic species of *Myotis* throughout the world (see also Mayer and von Helversen, 2001) and species in addition to *M. brandtii* and *M. mystacinus* that migrated through the Beringean Bridge, subsequently distributing in Asia and Europe.

Overall, relationships supported in this study and that of Ruedi and Mayer (2001) and Kawai *et al*. (2003) require reassessment of the evolutionary history of *Myotis*. Current classification suggests that three 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 (see also Fenton and Bogdanowicz, 2002). "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: 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 (Hollar and Springer, 1997; Alvarez *et al*., 1999), 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 (Horáček, 2001; Czaplewski *et al*., In press).

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. We 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 four 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. brandtii*, Ruedi and Mayer, 2001; *M. mystacinus*, Kawai *et al*., 2003), 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 are needed to provide insight into the tempo and mode of the *Myotis* radiation.

Subfamily Vespertilioninae

The 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., Koopman, 1984, 1985, 1993, 1994; Corbet and Hill, 1991; 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: 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 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; Kearney *et al*., 2002). 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 'nonclassical' studies (Bickham, 1979*b*; Heller and Volleth, 1984; Menu, 1984, 1985, 1987; McBee *et al*., 1986, 1987; Hill and Harrison, 1987; Horáček, 1991; Morales *et al*., 1991; Ruedi and Arlettaz, 1991; Volleth and Tidemann, 1991; Volleth and Heller, 1994*a*, 1994*b*; Volleth *et al*., 2001).

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: 229) wrote, "The Lasiurini may be regarded as having diverged farthest of all from the early vespertilionine bats." Karyology (Bickham, 1979*b*, 1987) and biochemical data (Baker *et al*., 1988) support this view. The mtDNA analysis likewise distinguishes Lasiurini, but provides no supported resolution of its relationship among vespertilionines (Fig. 6).

Within *Lasiurus*, mtDNA analysis gives further support for monophyly of two recognized species groups (red bats, represented by *attratus*, *borealis*, *blossevillii*, *seminolus*; and yellow bats, represented by *ega* and *xanthinus*) and for distinction of a third 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*b*, 1987), allozymes (Baker *et al*., 1988), and restriction sites (Morales and Bickham, 1995) favor recognition of only one 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 two 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

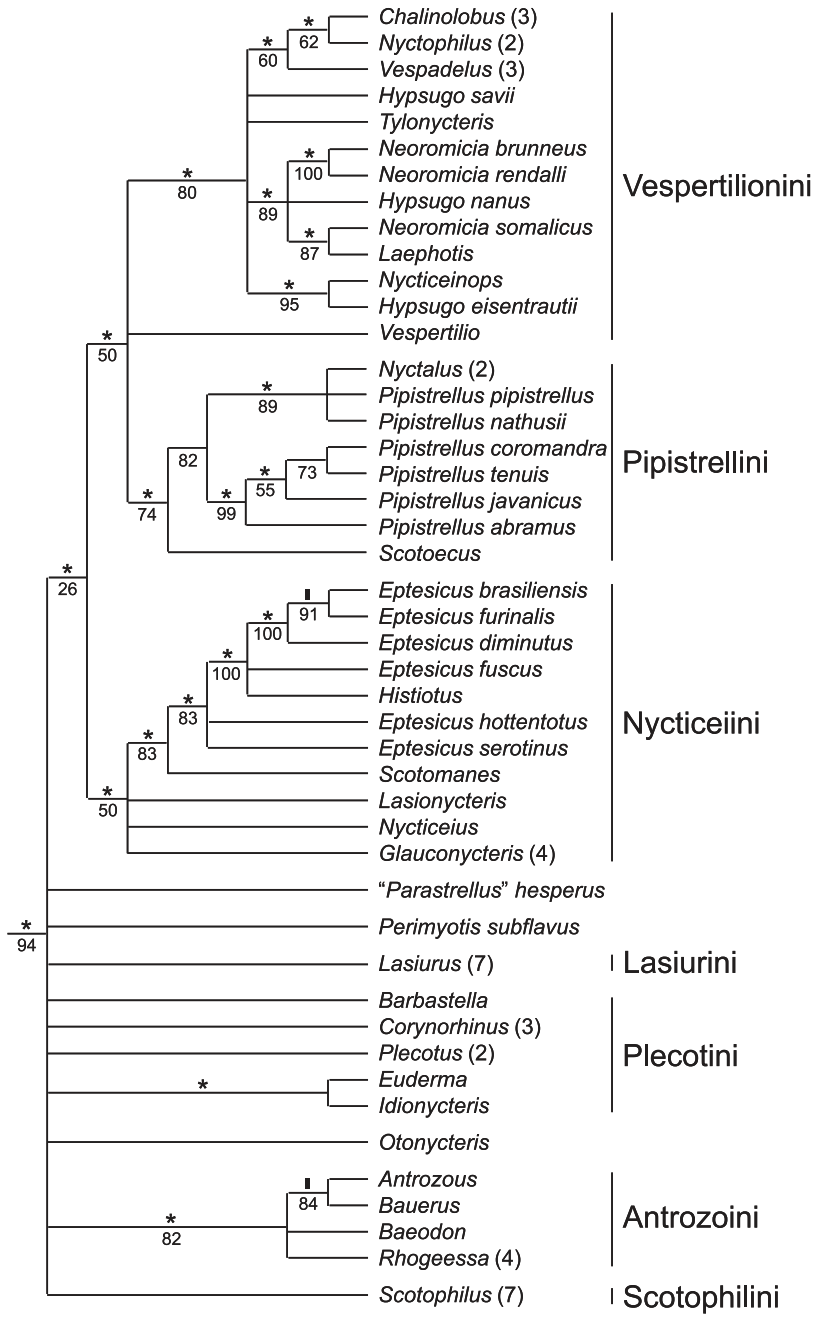


FIG. 6. Abbreviated cladogram for subfamily Vespertilioninae summarizing Figs. 3 and 4. Only relationships that were supported strongly by either or both Bayesian and Parsimony analyses are depicted. Symbols above branches indicate Bayesian posterior probabilities (*P*) averaged conservatively over all multiple, independent analyses that employed various outgroup taxa and two different sequence alignments. '*,' $P = 1.0$ in all analyses regardless of alignment; 'I,' $0.95 \leq P < 1.0$ 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

(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., Pine *et al*., 1971; Bickham, 1979*b*; Breed and Inns, 1985; Hill and Harrison, 1987; Freeman, 1998).

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* (Miller, 1906, 1907; Tate, 1942; Hill and Harrison, 1987; Corbet and Hill, 1991); 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 (Bickham, 1979*b*; Baker *et al*. 1985; see also Volleth and Heller, 1994*b*). We 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 two 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* (Bickham, 1979*b;* Baker *et al*., 1985; Hill and Harrison, 1987; Volleth and Heller, 1994*a*). 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 also provides resolution to relationships among species of

Scotophilus, suggesting a distant relationship between the two Indomalayan forms (*heathi* and *kuhlii*) and close relationship among four Ethiopian forms (*borbonicus*, *dinganii*, *leucogaster*, *nux*; Fig. 3). However, we reserve making conclusions about relationships within *Scotophilus* because taxonomy of *Scotophilus*, especially Ethiopian forms, is unreliable or confused and at times controversial, with little consensus for definition of species and application of some species names (e.g., *borbonicus*, *nux*, *viridis*; Hayman and Hill, 1971; Robbins *et al*., 1985; Koopman, 1994). There is great need for a full review of the Ethiopian forms at the molecular level, in combination with the morphometric revision of Robbins *et al*. (1985). Our analysis of mtDNA minimally suggests that Ethiopian and Indomalayan forms of *Scotophilus* together 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 (Tate, 1942; Handley, 1959; Leniec *et al*., 1987; Frost and Timm, 1992), as demonstrated in a recent consensus analysis of published trees (dubbed '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 we 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., Tumlison and Douglas, 1992; Bogdanowicz *et al*., 1998).

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 five 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., Handley, 1959; Hill and Harrison, 1987; Frost and Timm, 1992; Tumlison and Douglas, 1992; Bogdanowicz *et al*., 1998).

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 (Horáček, 1991), and karyotypic data (Bickham, 1979*b*; Baker *et al*., 1985; see Volleth and Heller, 1994*a*) indicate a possible close association between *Otonycteris* and *Antrozous* + *Bauerus*, as well as some traditional 'nycticeiines.' Other studies of karyotypes (Zima *et al*., 1992; Qumsiyeh and Bickham, 1993), 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, we suggest incertae sedis placement for *Otonycteris* within Vespertilioninae.

These results differ somewhat from our earlier work (Hoofer and Van Den Bussche, 2001), in which we 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, our earlier 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 the present study and that of Hoofer and Van Den Bussche (2001). First, the earlier study examined a much smaller taxonomic sample, which undoubtedly reduced overall homoplasy. Second, the earlier study employed differential weighting schemes under parsimony analysis. Without such weighting schemes, particularly successive weighting (Farris, 1969), the majority of relationships in the tree, including position of *Otonycteris,* was unresolved. Third, the earlier study 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, results from our earlier study (Hoofer and Van Den Bussche, 2001) 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 (Handley, 1959; Koopman, 1984, 1985; Hill and Harrison, 1987; McKenna and Bell, 1997; Bogdanowicz *et al*., 1998).

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 two tribes, formally recognizing a distinction between *Pipistrellus*-types (Pipistrellini) and *Eptesicus*-types (Vespertilionini). Their classification also recognized seven subgenera within *Pipistrellus* (*Pipistrellus*, *Hypsugo*, *Falsistrellus*, *Perimyotis*, *Arielulus*, *Vespadelus*, *Neoromicia* — the latter two formerly classified within *Eptesicus*); some of which were given full generic rank after detailed morphologic or biochemical analyses (*Hypsugo*, Horáček and Hanák, 1985, 1986; Ruedi and Arlettaz, 1991; *Falsistrellus*, Kitchener *et al*., 1986; Adams *et al*., 1987*a*,

1987*b*; *Perimyotis*, Menu, 1984, 1987; *Arielulus*, Csorba and Lee, 1999).

Karyotypic studies also have helped elucidate relationships among *Pipistrellus*-like bats (Volleth, 1987, 1989; Volleth and Tidemann, 1989, 1991; Volleth and Heller, 1994*b*; Volleth *et al*., 2001). 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 two 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 four former subgenera (*Falsistrellus*, *Hypsugo*, *Neoromicia*, *Vespadelus*) and *Chalinolobus*, *Nyctophilus*, *Philetor*, *Scotorepens*, *Tylonycteris*, and *Vespertilio*. *Eptesicus*, together with *Hesperoptenus*, formed a third, 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 two New World '*Pipistrellus*' (*hesperus* and *subflavus*); however, none of these New World taxa were studied by Volleth and Heller (1994*b*), or by any other comprehensive phylogenetic analysis. The present study also supports classification of *Pipistrellus*-like bats into three tribes (Nycticeiini, Pipistrellini, Vespertilionini), corresponding closely with Volleth and Heller's (1994*b*) arrangement and further documenting a sister relationship between Pipistrellini and Vespertilionini.

There are only two principle differences between mtDNA and karyotypic results (Volleth and Heller, 1994*b*). First, the position of *Vespertilio* was unresolved within the clade containing Pipistrellini and Vespertilionini rather than supported within Vespertilionini (sensu Volleth and Heller, 1994*b*). This unresolved placement, although not contradictory to monophyly of Vespertilionini (sensu Volleth and Heller, 1994*b*), suggests further study is needed to assess certain affinities of *Vespertilio*. The second 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 (1994*b*) Eptesicini included only *Eptesicus* and *Hesperoptenus*. If only three 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 1994, respectively.

Thus, mtDNA analysis agrees markedly with karyotypic data in supporting three 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*'

The 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, 1994*b*; Fig. 1). Such revision is beyond the scope of this study and more thorough examinations will be necessary to resolve the situation. We suggest provisionally treating *Nyctalus* as a member of *Pipistrellus* (as proposed by Simpson, 1945).

The 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 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; Kearney *et al*., 2002). Although not well-supported, mtDNA analysis does not refute monophyly of *Vespadelus* (see Fig. 3), and supports karyotypic data for close affinities between *Vespadelus* and other Australian genera (*Chalinolobus*, *Nyctophilus*; Volleth and Tidemann, 1991; Volleth and Heller, 1994*b*; Volleth *et al*., 2001).

Within Vespertilionini, however, mitochondiral DNA 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 three 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. eisentrauti* supported sister to *Nycticeinops*.

Thus, as with *Pipistrellus* (sensu stricto) the definitions of *Hypsugo* and *Neoromicia* are questionable. Volleth and Heller (1994*b*) 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 *eisentrauti* 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, we recommend retaining the genus *Neoromicia* (i.e., not lumping it within *Laephotis*), but restricting it to the type species *N. somalicus*. We further suggest provisional allocation of (*Hypsugo*) *nanus* and (*Neoromicia*) *brunneus* and *rendalli* to a separate, as yet unnamed genus. This allocation corresponds with Kearney *et al*. (2002), who transferred (*Hypsugo*) *nanus* to the genus *Neoromicia* based on GTG-banded chromosomes; however, the type species of *Neoromicia* (*somalicus*) was not included in their study. Our allocation of *nanus*, *brunnues*, and *rendalli* to an unnamed genus seems the best alternative pending further study, especially of karyotypes, of additional putative members of *Hypsugo* (sensu lato), *Laephotis*, and *Neoromicia* (sensu lato).

The mtDNA analysis reveals no support for including the two New World '*Pipistrellus*' (*hesperus* and *subflavus*) within any of the three 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*), leading him to suggest "generic, or at least subgeneric differences" for the two 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 two 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. Horáček and Hanák (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 a nomen nudum in these publications and not properly available under the rules of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999). We use '*Parastrellus*' in this paper to facilitate discussion of this taxon. We intend, in an appropriate publication, to make the existing nomen nudum, '*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 first 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., Tate, 1942; Hill and Harrison, 1987), 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. We 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 (Dobson, 1875, 1878; Miller, 1907; Ryan, 1966; Hayman and Hill, 1971; Koopman, 1971, 1993; Peterson and Smith, 1973; Peterson, 1982; Skinner and Smithers, 1990; Corbet and Hill, 1991; McKenna and Bell, 1997). The present study provides further justification for generic distinction between *Chalinolobus* and *Glauconycteris* (see also Eger and Schlitter, 2001). 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 (1994*b*) 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 two species, the Nearctic *humeralis* and Ethiopian *schlieffeni* (Kitchener and Caputi, 1985; Corbet and Hill, 1986; 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*b*; 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 *eisentrauti* from *Hypsugo* to *Nycticeinops* (along with *schlieffeni*).

Histiotus and Laephotis

The genera *Histiotus* and *Laephotis* are two 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 two 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: 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, first 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 rather unique karyotype uniting the two groups (2N = 50, FN = 48, with acrocentric autosomes only; Williams and Mares, 1978; McBee *et al*., 1987; Rautenbach *et al*., 1993); although, *Myotis* (*Cistugo*) *seabrai* and *lesueuri* also possess this karyotype (Rautenbach *et al*., 1993; unbanded chromosomes only).

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, we 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 and Perspectives

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) *Miniopterus* (subfamily Miniopterinae) is recognized in its own family, Miniopteridae, as it represents an extremely divergent lineage relative to other vespertilionids, and in some analyses is sister to the molossids and natalids. All other vespertilionids examined form a well-supported clade (pp. 12–19);
2) Only two 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. 18–21);
3) *Myotis* is sister to a clade containing Kerivoulinae and Murininae and is recognized in its own subfamily, Myotinae (pp. 18–21);
4) Myotini (*Myotis* + *Lasionycteris*) does not represent a natural assemblage (pp. 18–21);
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. 21–25);
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. 18–21, 25–26);
7) Lasiurini, including only *Lasiurus*, is monophyletic. Within *Lasiurus*, three traditional species groups (red bats, yellow bats, hoary bats) are each monophyletic (p. 26);
(8) Antrozoini, including *Antrozous* and *Bauerus*, is monophyletic, and closely allied with *Baeodon* and *Rhogeessa*. The latter two genera are allocated to tribe Antrozoini (pp. 26–28);
9) Scotophilini, including *Scotophilus*, is monophyletic and distinguished as the most divergent tribe (genus) within Vespertilioninae (pp. 28–29);
10) Monophyly of traditional Plecotini (i.e., excluding *Otonycteris*) is neither supported nor refuted. Recognition of five plecotine genera (*Barbastella*, *Corynorhinus*, *Euderma*, *Idionycteris*, *Plecotus*) is supported (p. 29);
11) Position of *Otonycteris* is unresolved, and the genus is placed incertae sedis within Vespertilioninae (pp. 29–30);
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 three tribes: Nycticeiini; Pipistrellini; and Vespertilionini (pp. 30–31);
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. 32–34);
15) *Hypsugo*, *Neoromicia*, and *Vespadelus* are valid genera distinct from *Pipistrellus*, as each belongs to the tribe Vespertilionini (not Pipistrellini) (pp. 32–34);
16) Definitions of *Hypsugo* and *Neoromicia* 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.*) *eisentrauti* is transferred to *Nycticeinops*, and (*H.*) *nanus* and (*N.*) *brunneus* and

rendalli are allocated to a separate, as yet unnamed genus (pp. 32–34);
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 nudum, but will be made available as the valid name for this genus in an appropriate publication (pp. 33–34);
18) *Chalinolobus* (tribe Vespertilionini) and *Glauconycteris* (tribe Nycticeiini) are distinct genera and do not form a monophyletic group (p. 34);
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*, and also *eisentrauti* (transferred from *Hypsugo*) (p. 34);
20) The genus *Eptesicus*, subgenus *Eptesicus*, and *serotinus* subgroup within *Eptesicus* are paraphyletic relative to position of *Histiotus*. *Histiotus* is relegated to 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. 35).

Overall, the present study offers a robust working hypothesis for vespertilionid systematics (Table 6). 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 two-thirds of the family waits to be analyzed.

At the onset of this study, we 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 two-thirds 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 three 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; Volleth and Heller, 1994*b*) 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 resembling 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 traditional *Nycticeius*, traditional *Eptesicus*, *Chalinolobus* and *Glauconycteris*, *Histiotus* and *Laephotis*, and New World '*Pipistrellus*.'

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 vespertilionids. As shown for other vertebrate groups, the zoogeographic history of vesper bats may have been far less

TABLE 6. Classification for vespertilionoid bats examined in this study

Superfamily Vespertilionoidea
- Family Natalidae
- Family Molossidae
- Family Miniopteridae
  - Genus *Miniopterus*
- Family Vespertilionidae
  - Subfamily Vespertilioninae
    - Genus *Otonycteris*[a]
    - Genus '*Parastrellus*'[a, b]
    - Genus *Perimyotis*[a, c]
    - Tribe Antrozoini[d]
      - Genus *Antrozous*
      - Genus *Bauerus*
      - Genus *Baeodon*
      - Genus *Rhogeessa*
    - Tribe Lasiurini[d]
      - Genus *Lasiurus*
    - Tribe Plecotini[d]
      - Genus *Barbastella*
      - Genus *Corynorhinus*
      - Genus *Euderma*
      - Genus *Idionycteris*
      - Genus *Plecotus*
    - Tribe Scotophilini[d]
      - Genus *Scotophilus*
    - Tribe Nycticeiini
      - Genus *Glauconycteris*
      - Genus *Lasionycteris*
      - Genus *Nycticeius*[e]
      - Genus *Scotomanes*
      - Genus *Eptesicus*
        - Subgenus *Cnephaeus*[f]
        - Subgenus *Eptesicus*[g]
        - Subgenus *Histiotus*
    - Tribe Pipistrellini
      - Genus *Pipistrellus*[h]
      - Genus *Scotoecus*
    - Tribe Vespertilionini
      - Genus *Vespertilio*
      - Unnamed Genus[i]
      - Genus *Neoromicia*[j]
      - Genus *Laephotis*
      - Genus *Nycticeinops*[k]
      - Genus *Hypsugo*[l]
      - Genus *Tylonycteris*
      - Genus *Vespadelus*
      - Genus *Chalinolobus*
      - Genus *Nyctophilus*
  - Subfamily Myotinae
    - Genus *Myotis*
      - Subgenus *Aeorestes*[m]
      - Subgenus *Myotis*[n]
  - Subfamily Kerivoulinae
    - Genus *Kerivoula*
  - Subfamily Murininae
    - Genus *Harpiocephalus*
    - Genus *Murina*

[a] — Placed incertae sedis within Vespertilioninae
[b] — '*Parastrellus*' includes only *P. hesperus* and currently is a nomen nudum
[c] — *Perimyotis* includes only *P. subflavus*
[d] — Tribes Antrozoini, Lasiurini, Plecotini, and Scotophilini are sedis mutabilis
[e] — *Nycticeius* includes only *N. humeralis*
[f] — Subgenus *Cnephaeus* includes *E. hottentotus* and *E. serotinus*
[g] — Subgenus *Eptesicus* includes *E. brasiliensis*, *E. diminutus*, *E. furinalis*, and *E. fuscus*
[h] — *Pipistrellus* includes *Nyctalus*
[i] — We allocate (*Hypsugo*) *nanus* and (*Neoromicia*) *brunneus* and *rendalli* to a separate, as yet unnamed genus
[j] — *Neoromicia* includes only *N. somalicus*
[k] — *Nycticeinops* includes *N. eisentrauti* and *N. schlieffeni*
[l] — *Hypsugo* includes only *H. savii*
[m] — Subgenus *Aeorestes* includes all sampled New World species of *Myotis*
[n] — Subgenus *Myotis* includes all sampled Old World species of *Myotis*

complex than traditionally thought, especially regarding New World/Old World disperal events, 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 different areas, like the

anoles of the Caribbean, but on a larger, world-wide scale. Future study with greater taxonomic sampling and additional phylogenetic markers will be necessary for meaningful assessments of these and other evolutionary and zoogeographic hypotheses for the Vespertilionidae.

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Appendix I

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), Musèum d'Histoire Naturelle de Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB), Museum of Texas Tech University (TTU), 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), United States National Museum of Natural History (USNM), 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, seven of which were vespertilionids. Additionally, voucher information was undertermined for all six sequences that we did not generate [denoted by (*)].

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
Pteropodidae				
*Nyctimene robinsoni**				U 93061, AF 069536
*Pteropus hypomelanus**				U 93073, AF 069537
Rhinopomatidae				
Rhinopoma hardwickei	TK 25643	TTU 40639	Palestine: West Bank	AF 263231
Megadermatidae				
Macroderma gigas	ECT-1	***		AY 395854
Hipposideridae				
Hipposideros abae	AMNH 268375	AMNH 268375	Central African Republic	AY 395855
H. cyclops	AMNH 268380	AMNH 268380	Central African Republic	AY 395857
Triaenops furculus	ECT-2	***		AY 495453
Rhinolophidae				
Rhinolophus alcyone	AMNH 268373	AMNH 268373	Central African Republic	AY 395858
Nycteridae				
Nycteris argae	AMNH 268371	AMNH 268371	French Guiana: Paracou	AY 395860
Nycteris sp.	TK 21558	CM 90794	Gabon: Estuaire Prov.	AY 395861
Emballonuridae				
Balantiopteryx plicata	ECT	***		AY 395847
Cormura brevirostris	AMNH 267822	AMNH 267822	French Guiana: Paracou	AY 395848
Diclidurus scutatus	AMNH 267832	AMNH 267832	French Guiana: Paracou	AY 141036
*Emballonura atrata**				AF 203773
Peropteryx macrotis	TK 70465	***	PERU — Probably	AY 395850

APPENDIX I. Continued

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
Rhynchonycteris naso	AMNH 267373	AMNH 267373	French Guiana: Paracou	AY 395851
Saccopteryx bilineata	AMNH 267842	AMNH 267842	French Guiana: Paracou	AY 263213
S. leptura	TK 70480	***	PERU — Probably	AY 395852
Taphozous nudiventris	TK 16602	CM 62342	Egypt: Giza	AY 395853
MYZOPODIDAE				
Myzopoda aurita	OK 4246	USNM 448885	Madagascar: Fianarantsoa	AF 345926
MYSTACINIDAE				
Mystacina tuberculata	UWZM M27027	UWZM M27027	New Zealand: North Island	AF 263222
FURIPTERIDAE				
Furipterus horrens	AMNH 272837	AMNH 272837	French Guiana: Paracou	AF 345921
F. horrens	F 34443	ROM 100202	Guyana: East Berbice-Corentyne Prov.	AF 345922
NOCTILIONIDAE				
Noctilio albiventris	TK 86633	***	Guyana: Berbice Dist.	AF 263223
N. leporinus	TK 10224	CM 63552	Suriname: Saramacca	AF 263224
MORMOOPIDAE				
Mormoops megalophylla	TK 19311	CM 78267	Venezuela: Barinas	AF 407173
Pteronotus parnellii	TK 17953	CM 77083	Suriname: Marowijne	AF 407180
PHYLLOSTOMIDAE				
Centurio senex	TK 13110	CM 55731	Mexico: Veracruz	AF 263227
Diphylla ecaudata	TK 13514	***	Mexico: Yucatán	AF 411533
Lophostoma brasiliense	TK 18834	AMNH 267103	French Guyana: Paracou	AF 411544
Trachops cirrhosus	TK 18829	AMNH 267129	French Guyana: Paracou	AF 411539
Vampyrum spectrum	TK 40370	TTU 61071	Hondurus: Atlantida	AF 411537
THYROPTERIDAE				
Thyroptera discifera	TK 17210	CM 68440	Suriname: Saramacca	AF 345923
T. tricolor	AMNH 268577	AMNH 268577	French Guiana: Paracou	AF 263233
NATALIDAE				
Natalus stramineus	TK 15660	TTU 31457	Dominica: St John	AF 345924
N. micropus	TK 9454	CM 44578	Jamaica	AF 345925
MOLOSSIDAE				
Eumops auripendula	AMNH 268594	AMNH 268594	French Guiana: Paracou	AF 263214
Chaerephon pumila	FMNH 137634	FMNH 137634	Uganda: South Buganda	AY 495454

APPENDIX I. Continued

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
Molossops abrasus	AMNH 267534	AMNH 267534	French Guiana: Paracou	AY 395862
Molossus molossus	AMNH 269102	AMNH 269102	French Guiana: Paracou	AF 263215
M. molossus	AMNH 269105	AMNH 269105	French Guiana: Paracou	AY 495455
M. rufus	AMNH 268595	AMNH 268595	French Guiana: Paracou	AF 263216
*M. sinaloe**				U 93053, AF 203739
Mops condylurus	FMNH 151943	FMNH 151943	Madagascar: Toliara Prov.	AY 495456
Mormopterus planiceps	RLH 63	TCWC	Australia	AY 495457
Nyctinomops femorosaccus	TK 19552	TTU 37731	Mexico: Jalisco	AT 495458
N. macrotis	TK 78908	TTU 79570	USA: Texas	AF 263217
Otomops martiensseni	FMNH 137633	FMNH 137633	Burundi: Muramuya	AT 495459
Promops centralis	AMNH 269114	AMNH 269114	French Guiana: Paracou	AF 263218
Sauromys petrophilus	SP 7791	CM 105758	South Africa: Transvaal Prov.	AY 495460
Tadarida brasiliensis	OK 430	OSU 12794	USA: New Mexico	AF 263219
VESPERTILIONIDAE				
Antrozous pallidus	TK 49646	TTU 71101	USA: Texas	AF 326088
Baeodon alleni	TK 45023	UNAM	Mexico: Michoacan	AF 326108
Barbastella barbastellus	IZEA 3590	MHNG 1804.094	Switzerland: Valais Prov.	AF 326089
Bauerus dubiaquercus	FN 33200	ROM 97719	Mexico: Campeche	AY 395863
Chalinolobus gouldi	RLH 27	TCWC	Australia	AY 495461
C. morio	05M3	TCWC	Australia	AY 495462
C. morio	05M4	TCWC	Australia	AY 495463
*C. tuberculatus**			New Zealand	AF 321051
Corynorhinus mexicanus	TK 45849	UAM-I	Mexico: Michoacan	AF 326090
C. rafinesquii	TK 5959	TTU 45380	USA: Arkansas	AF 326091
C. townsendii	TK 83182	TTU 78531	USA: Texas	AF 263238
Eptesicus brasiliensis	TK 17809	CM 76812	Suriname: Nickerie	AY 495464
E. diminutus	TK 15033	TTU 48154	Venezuela: Guarico	AY 495465
E. furinalis	AMNH 268583	AMNH 268583	French Guiana: Paracou	AF 263234
E. fuscus	SP 844	CM 102826	USA: West Virginia	AF 326092
E. hottentotus	TK 33013	CM 89000 (type)	Kenya: Rift Valley Prov.	AY 495466
E. serotinus	TK 40897	TTU 70947	Tunisia: Sidi Bou Zid Government	AY 495467
Euderma maculatum	NK 36260	MSB 121373	USA: Utah	AF 326093
Glauconycteris argentatus	FMNH 15119	FMNH 15119	Tanzania: Kilimanjaro Region	AY 495468

APPENDIX I. Continued

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
G. beatrix	FMNH 149417	FMNH 149417	Zaire: Haute Zaire	AY 495469
G. poensis	AMNH 268381	AMNH 268381	Central African Republic	AY 495470
G. variegatus	TK 33545	CM 97983	Kenya: Western Prov.	AY 495471
Harpiocephalus harpia	TK 21258	CM 88159	Thailand: Uthai Thani Prov.	AF 263235
Histiotus macrotus	FMNH 129207	FMNH 129207	Peru: Ancash	AY 495472
'*Hypsugo*' *eisentrauti*	F 34348	ROM 100532	Ivory Coast	AY 495473
'*Hypsugo*' *nanus*	TK 33378	CM 98003	Kenya: Eastern Prov.	AY 495474
Hypsugo savii	IZEA 3586	MHNG 1804.100	Switzerland: Valais Prov.	AY 495475
Idionycteris phyllotis	NK 36122	MSB 120921	USA: Utah	AF 326094
Kerivoula hardwickei	F 44154	ROM 110829	Vietnam: Dong Nai	AF 345928
K. papillosa	F 44175	ROM 110850	Vietnam: Dong Nai	AF 345927
K. pellucida	F 35987	ROM 102177	Indonesia: East Kalimantan	AY 495476
Laephotis namibiensis	SP 4097	TM 37547	Namibia: Luderitz Dist.	AY 495477
Lasionycteris noctivagans	TK 24216	TTU 56255	USA: Texas	AF 326095
Lasiurus attratus	F 39221	ROM 107228	Guyana: Potaro-Siparuni	AY 495478
L. blossevillii	F 38133	ROM 104285	Panama: Chiriqui	AY 495479
L. borealis	TK 49732	TTU 71170	USA: Texas	AY 495480
L. borealis	TK 84510	TTU 80739	USA: Texas	AY 495481
L. cinereus	TK 78926	TTU	USA: Texas	AY 495482
L. ega	TK 43132	UNAM	Mexico: Michoacan	AY 495483
L. seminolus	TK 90686	***	USA	AY 495484
L. xanthinus	TK 78704	TTU 78296	USA: Texas	AY 495485
Miniopterus australis	TK 20330	***	Papua New Guinea: Central Prov.	AY 395864
M. fraterculus	TK 33132	CM 98058	Kenya: Rift Valley Prov.	AY 495486
M. inflatus	TK 33539	CM 98079	Kenya: Western Prov.	AY 495487
M. pusillus	F44196	ROM 110871	Vietnam: Lam Dong	AY 495488
M. schreibersi	TK 40910	TTU 70985	Tunisia: Beja Government	AY 395865
M. tristis	TK 20337	TTU 36281	Papua New Guinea: Central Prov.	AY 495489
Murina huttoni	F 42722	ROM 107739	Vietnam: Dak Lak	AY 495490
Myotis adversus	RLH 62	TCWC	Australia	AY 495491
M. albescens	TK 17932	CM 77691	Suriname: Marowijne	AY 495492
M. austroriparius	MLK 4079	UM 16629	USA: Tennessee	AY 495493
M. bocagei	FMNH 150075	FMNH 150075	Tanzania: Tanga Region	AF 326096

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Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
M. capaccinii	TK 25610	TTU 40554	Jordan: Northern Prov.	AY 495494
M. californicus	TK 78797	TTU 79325	USA: Texas	AY 495495
M. ciliolabrum A	TK 24872	TTU 40680	USA: Oklahoma	AY 495496
M. ciliolabrum B	TK 83155	TTU 78520	USA: Texas	AY 495497
M. daubentonii	IZEA 2692	MHNG 1805.054	Switzerland: Vaud Prov.	AY 495498
M. dominincensis	TK 15613	***	Dominica: St. Joseph Parish	AY 495500
M. elegans	F 35471	ROM 101293	El Salvador: Ahuachapan	AY 495501
M. fortidens	TK 43186	***	Mexico: Michoacan	AY 495502
M. keaysi	TK 13532	***	Mexico: Yucatan	AY 495503
M. levis	FMNH 141600	FMNH 141600	Brazil: Sao Paulo	AF 326097
M. muricola	FMNH 147067	FMNH 147067	Philippine Islands: Mindanao Island	AY 495504
M. myotis	IZEA 3790	MHNG 1805.062	Switzerland: Bern Prov.	AF 326098
M. nigricans	FMNH 129210	FMNH 129210	Peru: Amazonas	AF 326099
M. ridleyi	F 44086	ROM 110767	Vietnam: Dong Nai	AY 495505
M. riparius	AMNH 268591	AMNH 268591	French Guiana: Paracou	AF 263236
M. ruber	F 44409	ROM 111110	Brazil: Sao Paulo	AY 495506
M. septentrionalis	DWS 608	ISUV 6454	USA: Indiana	AY 495507
M. siligorensis	F 42629	ROM 107649	Vietnam: Tuyen Quang	AY 495508
M. thysanodes	TK 78800	TTU 79328	USA: Texas	AF 326100
M. velifer A	TK 11929	TTU 46405	USA: Texas	AY 495509
M. velifer B	TK 79170	TTU 78599	USA: Texas	AF 263237
M. volans	TK 78980	TTU 79545	USA: Texas	AY 495510
M. welwitschii	FMNH 144313	FMNH 144313	Uganda: Kasese Dist.	AY 495511
M. yumanensis	TK 28753	TTU 43200	USA: Oklahoma	AY 495512
Myotis sp.	TK 48587	***	North America	AY 495513
'Neoromicia' brunneus	TK 21501	CM 90802	Gabon: Estuaire Prov.	AY 495514
'Neoromicia' rendalli	TK 33238	CM 97977	Kenya: Coastal Prov.	AY 495515
Neoromicia somalicus	TK 33214	CM 97978	Kenya: Coastal Prov.	AY 495516
'*Nyctalus*' *leisleri*	FMNH 140374	FMNH 140374	Pakistan: Malakand Div.	AY 495517
'*Nyctalus*' *noctula*	NHMB 209/87	NHMB 209/87	Switzerland: Bern Prov.	AY 495518
Nycticeius humeralis	TK 26380	TTU 49536	USA: Texas	AF 326102
Nycticeinops schlieffeni	TK 33373	CM 97998	Kenya: Eastern Prov.	AF 326101

APPENDIX I. Continued

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
Nyctophilus geofroyii	RLH 23	TCWC	Australia	AY 495519
N. gouldi	09M1	TCWC	Australia	AY 395868
N. gouldi	1804	SMF 64967	Australia: Australian Capital Terr.	AY 495520
N. gouldi	RLH 29	TCWC	Australia	AY 495521
Otonycteris hemprichii	SP 7882	CM	Jordan: Maan Government	AF 326103
'Parastrellus' hesperus	TK 78703	TTU 79269	USA: Texas	AY 495522
Perimyotis subflavus	TK 90671	TTU 80684	USA: Texas	AY 495523
*Pipistrellus abramus**				AB 061528
P. coromandra	FMNH 140377	FMNH 140377	Pakistan: Malakand Div.	AY 495524
P. javanicus	FMNH 147069	FMNH 147069	Philippine Islands: Mindanao Isl.	AY 495525
P. nathusii	IZEA 2830	MHNG 1806.003	Switzerland: Vaud	AF 326104
P. nathusii	IZEA 3406	MHNG 1806.001	Sswitzerland: Vaud	AY 495526
P. nathusii	TK 81167	TTU	Ukraine: Chornobyl Dist.	AY 495527
P. nathusii	TK 81169	TTU	Ukraine: Chornobyl Dist.	AY 495528
P. pipistrellus	IZEA 3403	MHNG 1806.032	Spain: Barcelone Prov.	AF 326105
P. tenuis	FMNH 137021	FMNH 137021	Philippine Islands: Sibuyan Isl.	AY 495529
Plecotus auritus	IZEA 2694	MHNG 1806.047	Switzerland: Valais Prov.	AF 326106
P. austriacus	IZEA 3722	MHNG 1806.042	Switzerland: Vaud Prov.	AF 326107
Rhogeessa aeneus	TK 20712	TTU 40012	Belize: Belize Dist.	AY 495530
R. mira	TK 45014	UNAM	Mexico: Michoacan	AY 495531
R. parvula	TK 20653	TTU 36633	Mexico: Sonora	AF 326109
R. tumida	TK 40186	TTU 61231	Honduras: Valle	AF 326110
Scotophilus borbonicus	TK 33267	CM 98041	Kenya: Coastal Prov.	AY 495532
S. dinganii	FMNH 147235	FMNH 147235	Tanzania: Tanga Region	AY 495533
S. heathi	F 42769	ROM 107786	Vietnam: Dak Lak	AY 495534
S. kuhlii	FMNH 145684	FMNH 145684	Philippine Islands: Sibuyan Isl.	AF 326111
S. leucogaster	TK 33359	CM 98054	Kenya: Eastern Prov.	AY 395867
S. nux	K 33484	***	Kenya: Western Prov.	AY 495535
S. viridis	FMNH 150084	FMNH 150084	Tanzania: Tanga Region	AF 326112
Scotoecus hirundo	FMNH 151204	FMNH 151204	Tanzania: Kilimanjaro Region	AY 495536
Scotomanes ornatus	F 42568	ROM 107594	Vietnam: Tuyen Quang	AY 495537
Tylonycteris pachypus	F 38442	ROM 106164	Vietnam: Tuyen Quang	AY 495538
Vespadelus regulus	RLH 30	TCWC	Australia	AY 495539

Appendix I. Continued

Taxon	Tissue Collection No.	Museum Catalog No.	Locality	GenBank Accession Number
V. sagittula	RLH 20	TCWC	Australia	AY 495540
V. vulturnus	RLH 16	TCWC	Australia	AY 495499
Vespertilio murinus	IZEA 3599	MHNG 1808.017	Switzerland: Valais Prov.	AY 395866

Appendix II. Phylogenetic utility and alignment of ribosomal gene sequences

Bayesian and Parsimony analyses of mtDNA sequences from 12S rRNA, tRNAVal, 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. 4–6), 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 (Van Den Bussche and Hoofer, 2000, 2001; Hoofer and Van Den Bussche, 2001; Lee *et al*., 2002; Van Den Bussche *et al*., 2002; Hoofer *et al*., 2003). 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 four sets of taxa and use of two phylogenetic methods, two independent alignments, multiple independent runs, and > 30 designated outgroups allowed assessment of repeatability (Figs. 2–6). 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 our motivation for analyzing smaller sets of taxa.

Accordingly, the greatest difference between taxon sets involved the two 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 two-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 one of several computer programs, yet different optimal alignments may be favored by different programs and by different parameter values (Fitch and Smith, 1983; Lake, 1991; Mindell, 1991; Wheeler and Gladstein, 1991; Gatesy *et al*., 1993; DeSalle *et al*., 1994; Wheeler, 1995; Morrison and Ellis, 1997; Hickson *et al*., 2000; Lutzoni *et al*., 2000). 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*. (2003) 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., Bruns *et al*., 1992; Turbeville *et al*., 1992; Berbee, 1996; Springer, 1997; Lutzoni, 1995; Hoofer and Van Den Bussche, 2001; 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 fifth 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 (Lake, 1991; Waterman *et al*., 1992; Gatesy *et al*., 1993); 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, we applied a slightly modified version of the 'gap-sliding' approach of Lutzoni *et al*. (2000: 634–635). We used their criteria 1–3, and 7:

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 nine-point font on a 15-inch monitor, only about 40 taxa at a time can be visualized, requiring about five complete page scrolls between the first and 171st taxon, not to mention the approximately 100 page scrolls separating the beginning and end of a 2.6 kb alignment. We therefore relied on criterion #7 almost exclusively, defining boundaries of ambiguous regions by conserved, invariant flanking regions, such that the first 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.

Appendix III. Methods of inference

We employed two 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 Larget and Simon, 1999; Hall, 2001; Lewis, 2001;

Huelsenbeck *et al*., 2002). 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., Murphy *et al*., 2001*b*; Buckley *et al*., 2002; Leaché and Reeder, 2002; Hoofer *et al*., 2003); although Guindon and Gascuel (2003) have introduced an algorithm for Maximum Likelihood analysis that apparently reduces computer time dramatically. 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 (Hillis and Bull, 1993; Efron *et al*., 1996). Moreover, recent simulation studies suggest that Bayesian methods perform equally-well or better than bootstrapping with Parsimony or Maximum Likelihood across a variety of conditions (Wilcox *et al*., 2002; Alfaro *et al*., 2003; Douady *et al*., 2003).

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 two independent alignments (= 8 different sets of data) and multiple independent runs of at least 1×10^6 generations with one cold and three incrementally heated Markov chains, random starting trees for each chain, and > 30 designated outgroups (Figs. 2–6).

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 two methods, none affected inferences of relationship. All taxonomic recommendations in this study are supported by ≥ 0.95 Bayesian probabilities and in ≥ 50% of the bootstrap proportions under Parsimony.

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SPECIES AND SUBSPECIES

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