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Source: Journal of Mammalogy, 91(5):1073-1092. 2010.
Published By: American Society of Mammalogists
DOI: http://dx.doi.org/10.1644/09-MAMM-A-325.1
URL: http://www.bioone.org/doi/full/10.1644/09-MAMM-A-325.1

Journal of Mammalogy, 91(5):1073–1092, 2010

Tribal phylogenetic relationships within Vespertilioninae (Chiroptera: Vespertilionidae) based on mitochondrial and nuclear sequence data

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A paucity of useful characters, morphological convergence, and potential rapid radiation has hindered systematists in elucidating evolutionary relationships within Vespertilioninae. In this study >8,500 base pairs of digenomic DNA for 111 taxa were sequenced and analyzed using maximum-parsimony and Bayesian phylogenetic methods to construct trees and reexamine hypotheses of supergeneric evolutionary relationships in Vespertilioninae. Results of these analyses validate monophyly of Vespertilioninae with the exclusion of *Myotis* and support recognition of 6 tribes: Antrozoini, Lasiurini, Scotophilini, Vespertilionini, and 2 new unnamed tribal clades, the perimyotine group and the hypsugine group. Tree topologies indicate a Nycticeiini–Eptesicini group, but this clade is not supported. The heuristically pleasing tribe Plecotini also is unresolved in these analyses. These results provided further support and greater resolution for previously proposed hypotheses of Vespertilioninae evolution based on mitochondrial DNA, and although deep branching patterns are not fully resolved, these data increase our understanding of the evolution of this ecologically important and diverse group of bats. DOI: 10.1644/09-MAMM-A-325.1.

Key words: Antrozoini, digenomic sequence data, Eptesicini–Nycticeiini, Lasiurini, mitochondrial DNA, nuclear DNA, Scotophilini, systematics, Vespertilioninae, Vespertilionini

Understanding the evolutionary relationships within the subfamily Vespertilioninae (Mammalia: Chiroptera: Vespertilionidae) has been difficult for systematists because of the evolutionary and ecological success (in terms of species richness and biogeography) and constrained circumscription (in terms of morphological diversification) of this subfamily. Approximately 240 species have been described and placed in this subfamily (Simmons 2005). However, few useful synapomorphic morphologic character states exist that unambiguously define taxa belonging to Vespertilioninae (Hill and Harrison 1987; Koopman 1994; Miller 1907; Simmons 1998; Tate 1942; Wallin 1969). The significance any 1 of these characters receives in relation to the divergence of these taxa is in debate (lumper or splitter—Ellerman and Morrison-Scott 1951; Hill and Harrison 1987; Simpson 1945; Zima and Horáček 1985). Furthermore, it seems likely that parallel or convergent evolution of some of these characters (e.g., number of incisors, cusp pattern, and I2 size; number of anterior upper premolars; and pelage color) has led to classifications incongruent with evolutionary history within Vespertilioninae (Ärnbäck-Christie-Linde 1909; Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill 1966; Hill and Harrison 1987; Hill and Topál 1973; Horáček and Zima 1978; Koopman 1975; Rosevear 1962; Tate 1942; Volleth and Heller 1994b; Zima and Horáček 1985). These limitations have led to ambiguity in our understanding of evolutionary relationships within this diverse subfamily, which has hindered development of a generally agreed-upon classification.

Of particular interest in this study are supergeneric relationships of bats within Vespertilioninae. Although Miller (1907) set the foundation for our modern classification of these bats (without downplaying work of his predecessors—Dobson 1875, 1878; Gill 1885; Gray 1821, 1866) and drew attention to similarities between genera (e.g., ''*Eptesicus*-like'' or ''*Pipistrellus*-like''), he did not formally elucidate evolutionary relationships or provide taxonomic names to any rank above genus within Vespertilioninae. It was not until the work of Tate (1942) that a testable hypothesis for classification of bats within Vespertilioninae was described (Table 1). This is in stark contrast to Simpson (1945), who rejected a tribal classification rank for Vespertilioninae and synonomized many genera. Most authors since these classic works have followed the classification of Tate (1942), using a tribal rank, but followed Simpson (1945) in identifying fewer genera for their classifications (Koopman 1984, 1994; Koopman and Jones 1970; McKenna and Bell 1997). Although more recent studies based on bacular morphology and cytogenetics have provided insight into evolutionary relationships of Vesperti-

Table 1.—Historic classifications of Vespertilioninae. Taxa marked with an asterisk (*) are currently recognized taxa that would have been synonyms in authors' taxonomic system. A dagger (†) denotes these taxa as incertae sedis.

Tate (1942)	Simpson (1945)	Hill and Harrison (1987)	Koopman[a]	Volleth[b]	McKenna and Bell (1997)	Hoofer and Van Den Bussche (2003)	Simmons (2005)
Vespertilioninae	Vespertilioninae	Vespertilioninae	Vespertilioninae		Vespertilioninae		
Myotini							
Myotini†		Myotini	Myotini	Myotinae	Myotini	Myotinae	Myotinae
Lasionycteris	*Lasionycteris*	*Lasionycteris*	*Lasionycteris*	*Myotis*	*Lasionycteris*	*Myotis*	*Lasionycteris*
Cistugo	*Cistugo*	*Myotis*	*Myotis*		*Myotis*	Vespertilioninae	*Cistugo*
Myotis	*Myotis*	*Cistugo	*Cistugo		*Cistugo	*Otonycteris†	*Myotis*
Pizonyx	*Pizonyx	*Pizonyx	*Pizonyx		*Pizonyx	*Parastrellus†	
				Vespertilioninae		*Perimyotis†	Vespertilioninae
Plecotini†		Plecotini	Plecotini	Plecotini	Plecotini	Plecotini†	Plecotini
Corynorhinus	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*
Euderma	*Euderma*	*Euderma*	*Euderma*	*Euderma*	*Euderma*	*Corynorhinus*	*Corynorhinus*
Idionycteris	*Idionycteris*	*Idionycteris*	*Plecotus*	*Idionycteris*	*Idionycteris*	*Euderma*	*Euderma*
Plecotus	*Plecotus*	*Plecotus*	*Corynorhinus	*Plecotus	*Plecotus	*Idionycteris	*Idionycteris
	*Corynorhinus	*Corynorhinus	*Idionycteris	*Corynorhinus	*Corynorhinus	*Plecotus	*Otonycteris
		*Otonycteris		*Otonycteris			*Plecotus
		*Baeodon		*Rhogeessa			
		*Rhogeessa		*Baeodon			
		*Nycticeius					
Lasiurini		Lasiurini	Lasiurini		Lasiurini	Lasiurini†	Lasiurini
Dasypterus	*Lasiurus*	*Lasiurus*	*Lasiurus*		*Lasiurus*	*Lasiurus*	*Lasiurus*
Lasiurus	*Dasypterus	*Dasypterus*	*Dasypterus		*Dasypterus		
Nycticeiini		Scotophilini	Nycticeiini	Scotophilini	Nycticeiini	Scotophilini†	Nycticeiini
Otonycteris	*Otonycteris*	*Scotomanes*	*Otonycteris*	*Scotophilus*	*Otonycteris*	*Scotophilus*	*Rhogeessa*
Baeodon	*Rhogeessa*	*Scoteinus	*Rhogeessa		*Rhogeessa		*Baeodon
Rhogeessa	*Baeodon	*Scotophilus*	*Baeodon		*Baeodon		*Nycticeinops
Nycticeius	*Nycticeius		*Nycticeius		*Nycticeius		*Nycticeius
Scoteinus	*Scoteinus		*Nycticeinops		*Nycticeinops		*Scoteanax
*Scoteanax	*Scoteanax		*Scoteanax		*Scoteanax		*Scotoecus
*Scotorepens	*Scotorepens		*Scotorepens		*Scotorepens		*Scotomanes
*Scotoecus	*Scotoecus		*Scotoecus		*Scotoecus		*Scoteinus
*Scotomanes	*Scotomanes		*Scotomanes		*Scotomanes		*Scotophilus
*Scotophilus	*Scotophilus		*Scotophilus		*Scoteinus		*Scotorepens
					*Scotophilus		
Pipistrellini		Vespertilionini	Vespertilionini		Vespertilionini		
Eudiscopus	*Eudiscopus*		*Chalinolobus*		*Chalinolobus*		
Eptesicoid			*Glauconycteris	Eptesicini	*Glauconycteris	Nycticeiini	Eptesicini
Eptesicus	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Arielulus*
Hypsugo	*Hesperoptenus	*Glauconycteris	*Eudiscopus	*Arielulus	*Eudiscopus	*Histiotus	*Eptesicus
*Vespadelus	*Histiotus	*Histiotus	*Glischropus	*Hesperoptenus	*Glischropus	*Glauconycteris	*Hesperoptenus
*Histiotus	*Laephotis	*Ia	*Hesperoptenus	*Histiotus	*Hesperoptenus	*Lasionycteris	
*Laephotis	*Mimetillus	*Mimetillus	*Histiotus		*Histiotus	*Nycticeius	
*Rhinopterus	*Philetor	*Tylonycteris	*Ia		*Ia	*Scotomanes	
*Vespertilio	*Rhinopterus	*Vespertilio	*Laephotis		*Laephotis		
	*Tylonycteris		*Mimetillus		*Mimetillus		

TABLE 1—Continued.

Tate (1942)	Simpson (1945)	Hill and Harrison (1987)	Koopman[a]	Volleth[b]	McKenna and Bell (1997)	Hoofer and Van Den Bussche (2003)	Simmons (2005)
Pipistrelloid		Pipistrellini	*Nyctalus*	Pipistrellini	*Nyctalus*	Pipistrellini	Pipistrellini
Barbastella	*Chalinolobus*	*Chalinolobus*	*Philetor*	*Glischropus*	*Nycticeinops*	*Pipistrellus*	*Glischropus*
Chalinolobus	**Glauconycteris*	*Eudiscopus*	*Pipistrellus*	*Nyctalus*	*Philetor*	**Nyctalus*	*Nyctalus*
Glauconycteris	*Pipistrellus*	*Glischropus*	**Arielulus*	*Pipistrellus*	*Pipistrellus*	*Scotoecus*	*Pipistrellus*
Glischropus	**Glischropus*	*Hesperoptenus*	**Falsistrellus*	**Parastrellus*	**Arielulus*		**Perimyotis*
Hesperoptenus	**Ia*	*Laephotis*	**Hypsugo*	**Perimyotis*	**Falsistrellus*		**Parastrellus*
Ia	**Nyctalus*	*Nyctalus*	**Neoromicia*	*Scotozous*	**Hypsugo*		*Scotozous*
Mimetillus	**Scotozous*	*Nycticeinops*	**Perimyotis*	Vespertilionini	**Neoromicia*	Vespertilionini	Vespertilionini
Nyctalus	*Vespertilio*	*Philetor*	**Parastrellus*	*Chalinolobus*	**Perimyotis*	*Chalinolobus*	*Chalinolobus*
Philetor		*Pipistrellus*	**Scotozous*	*Falsistrellus*	**Parastrellus*	*Hypsugo*	*Eudiscopus*
Pipistrellus		**Arielulus*	**Vespadelus*	*Hypsugo*	**Scotozous*	*Laephotis*	*Falsistrellus*
**Arielulus*		**Falsistrellus*	*Tylonycteris*	*Laephotis*	**Vespadelus*	*Neoromicia*	*Glauconycteris*
**Falsistrellus*		**Hypsugo*	*Vespertilio*	*Neoromicia*	*Tylonycteris*	*Nycticeinops*	*Histiotus*
**Hypsugo*		**Neoromicia*		*Nyctophilus*	*Vespertilio*	*Nyctophilus*	*Hypsugo*
**Parastrellus*		**Perimyotis*		*Philetor*		*Tylonycteris*	*Ia*
**Perimyotis*		**Parastrellus*		*Scotorepens*		Unnamed genus	*Laephotis*
**Vespadelus*		**Vespadelus*		*Tylonycteris*		*Vespadelus*	*Mimetillus*
Scotozous		*Scoteanax*		*Vespadelus*		*Vespertilio*	*Neoromicia*
Tylonycteris		*Scotoecus*		*Vespertilio*			*Philetor*
		Scotorepens					*Tylonycteris*
		Scotozous					*Vespadelus*
							Vespertilio
Nyctophilinae	Nyctophilinae	Nyctophilinae	Nyctophilini		Nyctophilini		Nyctophilini
Antrozous	*Antrozous*	*Nyctophilus*	*Nyctophilus*		*Nyctophilus*		*Nyctophilus*
Nyctophilus	*Nyctophilus*	*Pharotis*	*Pharotis*		*Pharotis*		*Pharotis*
Pharotis		Antrozoini	Antrozoini		Antrozoini	Antrozoini†	Antrozoinae
		Antrozous	*Antrozous*		*Antrozous*	*Antrozous*	*Antrozous*
		Bauerus	*Bauerus*		*Bauerus*	*Bauerus*	*Bauerus*
						Baeodon	
						Rhogeessa	

[a] This arrangement can be found in Koopman and Jones (1970), Koopman (1984), and Koopman (1994), but the latter provides the most information and is the basis for depicted classification.

[b] This is a combination of results taken from Heller and Volleth (1984), Kearney et al. (2002), Volleth and Tidemann (1991), Volleth and Heller (1994b), and Volleth et al. (2001), with most-recent papers taking precedence.

lioninae, many relationships remain unresolved, many taxa remain unstudied, and some of these findings contradict previous hypotheses about evolution of Vespertilioninae (Ao et al. 2006; Hill and Harrison 1987; Volleth and Heller 1994a, 1994b; Volleth et al. 2001, 2006). Excluding Myotini, which has been elevated to its own subfamily (Hoofer and Van Den Bussche 2003; Lack et al. 2010; Stadelmann et al. 2004), historically 9 tribes have been proposed in various classifications to organize the systematics of Vespertilioninae, including Antrozoini (Miller 1897), Eptesicini (Volleth and Heller 1994b), Lasiurini (Tate 1942), Nycticeiini (Gervais 1855), Nyctophilini (Peters 1865), Pipistrellini (Tate 1942), Plecotini (Gray 1866), Scotophilini (Hill and Harrison 1987), and Vespertilionini (Gray 1821). The validity of these tribes has been accepted or discredited to various degrees, and their exact rank, position, circumscription, and composition are subjects of continuing debate (Hill and Harrison 1987; Hoofer and Van Den Bussche 2003; Table 1).

With the development of modern techniques in polymerase chain reaction, DNA sequencing, and molecular data analysis, researchers are reevaluating evolutionary relationships of bats in this family, bringing to bear the advantages of the enormous number of characters provided by molecular data (Bickham et al. 2004; Gu et al. 2008; Hoofer and Van Den Bussche 2001; Hoofer et al. 2003, 2006; Lack et al. 2010; Miller-Butterworth et al. 2007; Ruedi and Mayer 2001; Stadelmann et al. 2004, 2007). Mayer and von Helversen (2001) and Mayer et al. (2007) sequenced the ND1 mitochondrial coding gene of western Palearctic vespertilionids, Kawai et al. (2002) examined ND1, the nuclear exon vWF, and short interspersed elements (SINEs) of mainly eastern Palearctic bats, and Hoofer and Van Den Bussche (2003) used 2.6 kilobases of the ribosomal mitochondrial genome from 120 globally sampled vespertilionids to evaluate evolutionary relationships within Vespertilionidae. However, as in previous studies, results of these studies provided insufficient resolution to explicate the deep branching patterns within Vespertilioninae.

Potentially convergent or uninformative characters, rapid diversification of vespertilionids leading to deep branching patterns, and subsequent lack of genetic resolution have left our understanding of evolutionary relationships relatively ambiguous for the last 100 years. The purpose of this study was to elucidate polygenetic relationships within Vespertilioninae using both coding and noncoding regions of nuclear and mitochondrial genomes with the focus on resolving tribal composition and intertribal systematic relationships. Furthermore, these digenomic data were used to assess the validity of previously proposed tribes (Antrozoini, Eptesicini, Lasiurini, Nycticeiini, Nyctophilini, Pipistrellini, Plecotini, Scotophilini, and Vespertilionini) within Vespertilioninae. Production of a resolved and supported phylogeny for Vespertilioninae would enhance our understanding of the evolution of one of the most taxonomically diverse, geographically widespread, and ecologically successful groups of mammals and would increase our abilities to answer important ecological, evolutionary, and biogeographical questions.

Materials and Methods

Taxonomic sampling.—Included in this study are samples from 31 (70%) of the 44 currently recognized genera, 77 (32%) of the 241 species within Vespertilioninae, and 21 species of Myotinae (Simmons 2005; see Appendix I for list of taxa, general collecting locality, and voucher information). Taxa were included based on availability with the intent of representing distributional and ecological diversities of its members. Representatives of the subfamilies Kerivoulinae and Murininae were included as out-groups to polarize character-state transformations. Tissue samples were provided by several natural history collections, and most tissues are represented by voucher specimens (Ruedas et al. 2000) in the following institutions: Abilene Christian University, American Museum of Natural History, Carnegie Museum of Natural History, Colección Mamíferos Lilto, Universidad Nacional de Tucuman, Durban Natural Science Museum, 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, Sam Noble Oklahoma Museum of Natural History, Texas Cooperative Wildlife Collection at Texas A&M University, Universidad Autónoma Metropolitana–Iztapalapa, Universidad Nacional Autónoma de México, and University of Lausanne, Institut de Zoologie et d'Ecologie Animale (Appendix I). The acquisition of tissues samples and voucher specimens by the authors were conducted following the guidelines of the American Society of Mammalogists (Gannon et al. 2007). However, the majority of tissue samples came from preexisting collections housed in previously mentioned collections. Identifications of many specimens were verified by Steven R. Hoofer (Hoofer and Van Den Bussche 2003) and Manuel Ruedi (Muséum d'Histoire Naturelle de Genève, pers. comm.); otherwise, we relied on the identifications of the above collections.

Extraction, amplification, and sequencing.—Whole genomic DNA was isolated from skeletal muscle or organ tissue samples from 111 individuals following procedures of Longmire et al. (1997) or the DNeasy Tissue Kit (Qiagen, Austen, Texas). Previously designed primers were used to target 3 exons, apolipoprotein B (APOB), dentin matrix acidic phosphoprotein I (DMP1), and recombination activating gene II (RAG2), and intron regions of 3 other genes, protein kinase C, iota (PRKCI), signal transducer and activator of transcription 5A (STAT5A), and thyrotropin (THY—Lack et al. 2010). These nuclear markers were chosen because they have resolved deep branching patterns in Chiroptera and other mammalian taxa (Amrine-Madsen et al. 2003; Baker et al. 2000; Eick et al. 2005; Matthee and Davis 2001; Matthee et al. 2001, 2004, 2007; Van Den Bussche et al. 2003). Polymerase chain reaction amplifications were conducted using 200–500 ng of DNA, 1 unit of Taq polymerase, 0.14 mM of each deoxynucleoside triphosphate, 5 μl of 10× buffer, 3.5 mM of $MgCl_2$, 0.8 mg/ml of bovine serum albumin, and 0.15 μl of

each primer in a 30-μl total volume reaction. The general polymerase chain reaction thermal profile used for these reactions began with an initial 3-min denaturing of 94–95°C, followed by 35–40 cycles of 94–95°C for 30 s, 40–62°C for 1.5 min, and 72°C for 1 min (Lack et al. 2010). Amplification ended with a final elongation at 72° C for 10 min to ensure all reactions were completed. Polymerase chain reaction products were filtered to remove excess reactants using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin). Sequencing reactions were conducted in both directions using Big Dye chain terminator and a 3130 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California).

In addition to the sequence data generated for this study, we included previously published mitochondrial ribosomal DNA (mtDNA; comprising 12S rRNA, $tRNA^{Val}$, and 16S rRNA) for 100 individuals, DMP1 for 3 individuals, and RAG2 for 6 individuals (Hoofer et al. 2003; Hoofer and Van Den Bussche 2001, 2003; Lack et al. 2010; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2003). Amplifications and sequencing of the mtDNA gene regions were conducted for 11 additional individuals using primers and methods outlined in Van Den Bussche and Hoofer (2000). Sequence data for the nuclear DNA (nDNA) also were supplemented for 4 individuals with sequences of PRKCI, STAT5A, and THY published by Eick et al. (2005) and deposited in GenBank (http://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis.—Forward and reverse sequences for each gene region were assembled using the program Geneious 4.5.4 (Biomatters Ltd., Auckland, New Zealand). Alignment of sequence contigs was performed using ClustalW 1.83.XP (Thompson et al. 1994) through Geneious 4.5.4 and then assessed and manually optimized using MacClade 4.05 (Maddison and Maddison 2002). Regions appearing to violate the assumption of positional homology were recognized and excluded from phylogenetic analyses based on the procedures of Lutzoni et al. (2000). The mtDNA and each of the nDNA gene regions were analyzed independently using maximum parsimony in PAUP* version 4.0b10 (Swofford 2002) and Bayesian phylogenetic methods in MRBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001). An unweighted nucleotide substitution model, a heuristic search with 25 random additions of taxa, a tree-bisection-reconnection branch exchanging algorithm, and 1,000 bootstrap replicates were parameters used in maximum-parsimony analysis. Bayesian analysis employed a 4-chain (3 hot and 1 cold) parallel Metropolis-coupled Markov chain Monte Carlo, which was run for 1×10^7 generations, with sampling every 1,000 generations, and a temperature parameter of $T = 0.02$. Data were partitioned by codon for exons (APOB, DMP1, and RAG2), by marker for introns (PRKCI, STAT5A, and THY), and as a separate partition for mtDNA data. ModelTest version 3.06 (Posada and Crandall 1998) was used to identify the most appropriate nucleotide substitution model for Bayesian analysis resulting in the implementation of a General Time Reversible model with gamma-distributed rate variation among sites and inclusion of a proportion of invariable sites (GTR + Γ + I—Rodríguez et al. 1990). Model parameters were not defined a priori in Bayesian analysis but were treated as unknown variables with uniform priors. A random unconstrained starting tree with uniform priors was used for Bayesian analysis, and the burn-in values were determined by plotting likelihood scores per 1,000 generations and locating the region at which model parameters and tree scores reach stationarity. Nodes in the resulting trees were considered supported if they had ≥70% maximum-parsimony bootstrap support and ≥0.95 Bayesian posterior probabilities.

To examine incongruencies between gene regions and evaluate appropriateness of combining gene regions, each was analyzed independently, and resulting gene trees were compared. We used a 90% concordance criterion to evaluate appropriateness of concatenation where resulting gene trees must be in concordance at a minimum of 90% of nodes before concatenation of these data for further analysis (De Queiroz 1993). Based on results of these concordance tests (described in ''Results''), data were concatenated into 3 data sets: mtDNA, nDNA, and combined (mtDNA + nDNA) data sets for maximum-parsimony and Bayesian phylogenetic analysis. The program TREEPUZZLE 5.2 (Schmidt et al. 2002) was used to conduct likelihood-mapping (Strimmer and von Haeseler 1997) with a GTR + Γ + I model of nucleotide substitution to examine the phylogenetic potential of each independent gene region and combined data partitions.

Results

Independent gene regions and concordance.—All sequences generated in this study have been submitted to GenBank (see Appendix I for GenBank accession numbers). The nuclear gene regions analyzed were relatively short, 280–1,240 base pairs (bp; Table 2), and independently contained few phylogenetically informative positions (129–415 bp). Likelihood-mapping demonstrated that for these independent nDNA gene regions the number of positions analyzed is correlated positively to quartet resolution (Strimmer and von Haeseler 1997; Fig. 1). The mtDNA, nDNA, and combined data sets showed the same trend, but the slope was less positive. However, while accounting for this general size-resolvability relationship, the exons (especially DMP1 and RAG2) outperformed introns in their ability to resolve quartets, possibly indicating greater systematic error caused by signal saturation due to a potentially higher substitution rate in these introns. Analysis of each of nDNA gene regions independently and comparison of these gene trees (not shown) provided a high level of topology concordance (>90% concordance in supported topology). The only repeatedly supported incongruencies were in the variable position of *Baeodon* and in a few Vespertilioninae taxa embedded in the *Myotis* clades for APOB and PRKCI. The combined data set was analyzed twice, once excluding APOB and once excluding PRKCI, resulting in no effect to topology and relatively few clades becoming unsupported (posterior probabilities ≥ 0.95; e.g., support for inclusion of *Baeodon* in Antrozoini). Therefore,

TABLE 2.—Characteristics of individual gene regions and combined data partitions. Aligned positions constitute the full aligned length including indel regions. Excluded positions are those that potentially violate positional homogeneity. Analyzed positions are aligned minus excluded positions. Percent resolved and percent unresolved refers to the percent of quartets resolved and unresolved in likelihood-mapping analysis (Strimmer and von Haeseler 1997). APOB = apolipoprotein B; DMP1 = dentin matrix acidic phosphoprotein I; RAG2 = recombination activating gene II; PRKCI = protein kinase C, iota; STAT5A = signal transducer and activator of transcription 5A; THY = thyrotropin; mtDNA = mitochondrial DNA; and nDNA = nuclear DNA.

Marker	No. taxa	Aligned positions	Excluded positions	Analyzed positions	Variable positions	Phylogenetically informative positions	Percent resolved[a]	Percent unresolved[b]
APOB	110	282	0	282	173	129	72	28
DMP1	109	1,023	33	990	520	339	89	11
RAG2	111	1,239	0	1,239	570	415	87	13
PRKCI	111	792	55	737	285	191	64	34
STAT5A	95	1,154	667	487	327	283	78	22
THY	111	1,080	11	1,069	383	308	77	23
mtDNA	111	2,940	968	1,972	861	671	92	8
nDNA	111	5,570	766	4,804	2,232	1,654	94	6
Combined	111	8,510	1,734	6,776	3,093	2,344	96	4

[a] Percent resolved = percent occupancy of P vectors in attraction basins for fully resolved topologies [$A_1 + A_2 + A_3$].
[b] Percent unresolved = percent occupancy of P vectors in attraction basins for unresolved topologies [$A_{13} + A_{12} + A_{23} + A_*$].

the independent nDNA gene regions were concatenated for further analysis.

Mitochondrial DNA sequences.—New ribosomal mtDNA sequence data were generated for 11 individuals of 9 taxa: 3 individuals of *Eptesicus macrotus*, and 1 individual each of *Arielulus aureocollaris*, *E. magellanicus*, *E. serotinus*, *Lasiurus intermedius*, *Pipistrellus hesperidus*, *P. paterculus*, *P. pipistrellus*, and *Tylonycteris robustula*, which supplemented 100 mtDNA sequences previously generated (Hoofer and Van Den Bussche 2003; Lack et al. 2010). These 111 sequences were aligned to provide 2,940 aligned positions, of which 968 were excluded prior to analysis for potential violation of positional homology (Table 2). Of the remaining 1,972 positions, 861 were variable and 671 were phylogenetically informative. Maximum-parsimony analysis resulted in 718 parsimonious trees of 5,713 steps, with 43 supported clades (Fig. 2), a consistency index excluding uninformative characters (CI) of 0.1978, and a retention index (RI) of 0.5727. Bayesian analysis had a burn-in value of 882 generations and resulted in 58 supported clades (Fig. 2).

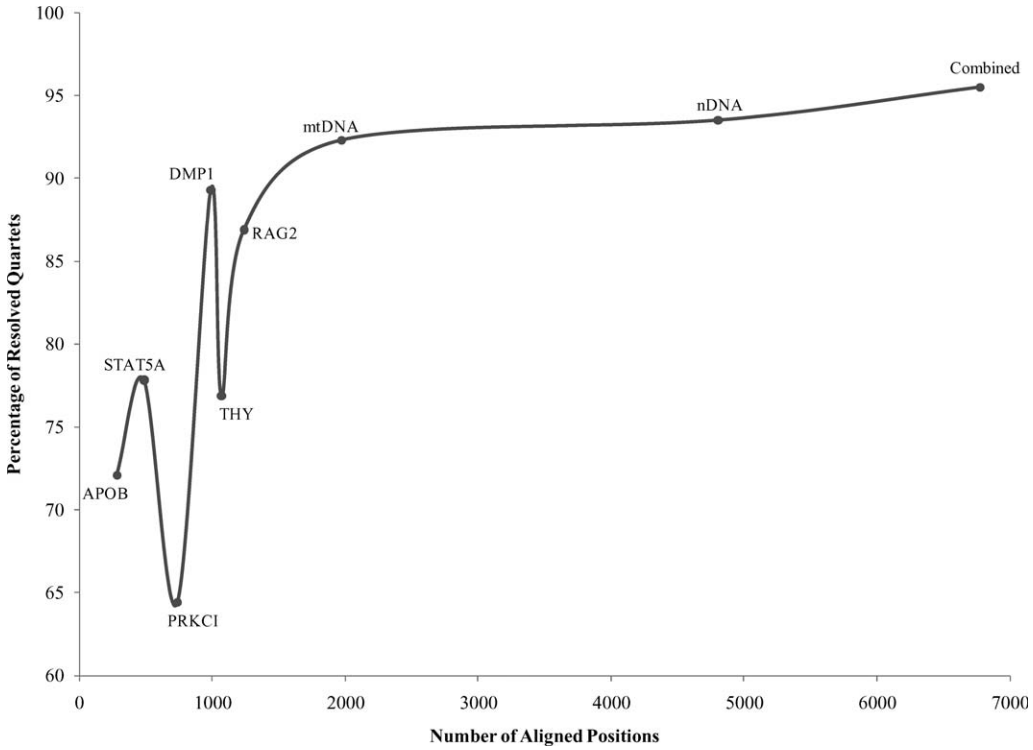

FIG. 1.—Scatter plot of the percentage of resolved quartets from likelihood-mapping by number of analyzed positions for each individual nuclear DNA (nDNA) gene region and the mitochondrial DNA, nDNA, and combined data sets. See Table 2 for data and Strimmer and von Haeseler (1997) for discussion of likelihood-mapping.

Nuclear DNA sequences.—Sequence data for the concatenated nDNA partition were generated for 111 taxa, of which 18 are missing ≥1 gene region (13–23% of nDNA data set). *Vespadelus vulturnus* was missing the most sequence data because we were unable to amplify or sequence the APOB or DMP1 gene regions successfully for this taxon, and *Baeodon alleni* was missing the least with the last 470 bp of RAG2 missing. In most cases missing data were from the STAT5A gene region, which proved to be the most difficult to amplify and was not generated for the following 16 taxa: *Eptesicus magellanicus*, *Glauconycteris beatrix*, *G. egeria*, *Hypsugo cadornae*, *H. savii*, *Nyctalus leisleri*, *N. noctula*, *Pipistrellus coromandra*, *P. hesperidus*, *P. javanicus*, *P. nathusii*, *P. tenuis*, *Scotoecus hirundo*, *Tylonycteris pachypus*, *T. robustula*, and *Vespertilio murinus*. No changes in clade support or topological resolution were observed when the data set was analyzed excluding STAT5A (data not shown).

Concatenated alignment of the nDNA gene regions provided 5,570 aligned positions (Table 2). With the exclusion of 766 positions for possible violations of positional homology prior to analysis, the remaining 4,804 positions included 2,232 variable positions and 1,654 phylogenetically informative positions. The maximum-parsimony analysis resulted in 24 most-parsimonious trees of 7,941 steps, 52 supported clades, and a CI of 0.4574 and an RI of 0.7152, excluding uninformative characters (Fig. 3). The majority of differences between the 24 most-parsimonious trees involved the relationship between the clades comprising the Antrozoini, Plecotini, Lasiurini, Scotophilini, New World pipistrelles, and a clade including the remaining *Pipistrellus*-like bats. Also variable was the position of *Arielulus* and *Lasionycteris* within Nycticeiini (sensu Hoofer and Van Den Bussche 2003, excluding *Nycticeius*) and the intrarelationships of some member of the *Pipistrellus*. Finally, some variation in topologies was attributed to variability within the genus *Scotophilus*. A burn-in value of 1,001 generations was used for the Bayesian analysis, which resulted in a tree with 57 supported clades (Fig. 3).

Combined sequences.—More than 90% of clades were in concordance between mtDNA and nDNA trees, and those data sets were concatenated for the combined analysis. Despite this high level of concordance, 2 areas with supported discrepancies between the mtDNA and nDNA trees were found. These supported discrepancies were found toward clade tips and fell outside the focus of this study. The 1st discrepancy related to the sister taxon of *P. coromandra*, which was *P. tenuis* in the mtDNA tree (Fig. 2) and *P. javanicus* in the nDNA tree (Fig. 3). The 2nd difference involved relationships within *Lasiurus*, which formed a well-supported clade in both analyses. Concatenation of the mtDNA and nDNA data sets resulted in 8,510 aligned positions. Because of possible violation of positional homology, 1,734 positions were excluded prior to analysis leaving 6,776 positions for phylogenetic analysis (Table 2). Of those remaining positions, 3,093 were variable and 2,344 were phylogenetically informative. The maximum-parsimony analysis resulted in 4 most-parsimonious trees, with 13,885 steps and 57 supported clades (Fig. 4). Excluding uninformative characters, the CI was 0.3380 and the RI was 0.6439. Differences among the 4 most-parsimonious trees related to relationships among taxa in the New World *Myotis* and the position of *Otonycteris–Barbastella* basal to either the genus *Plecotus* or *Corynorhinus*. For the Bayesian analysis a burn-in of 1,000 generations was used and resulted in a tree with 69 supported clades (Fig. 4).

Discussion

Elucidating evolutionary relationships within Vespertilioninae historically has been problematic. A paucity of useful characters, possible convergence among these character states, and a rapid radiation of major lineages within this subfamily have hindered efforts to understand evolutionary relationships of these taxa for >100 years (Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill 1966; Hill and Harrison 1987; Hill and Topál 1973; Horáček and Zima 1978; Koopman 1975, 1994; Lack et al. 2010; Miller 1907; Rosevear 1962; Simmons 1998; Tate 1942; Volleth and Heller 1994b; Zima and Horáček 1985). Efforts over the last 20 years provided some refined hypotheses but were incomplete, were incongruent with historic hypotheses, or did not clarify all relationships within Vespertilioninae (Hill and Harrison 1987; Volleth and Heller 1994b; Volleth et al. 2001, 2006). Recent molecular analyses (Hoofer et al. 2003; Hoofer and Van Den Bussche 2001, 2003; Kawai et al. 2002; Mayer et al. 2007; Mayer and von Helversen 2001) have tested previous hypotheses with new informative characters using phylogenetic methods. Using ribosomal mtDNA sequence data, Hoofer and Van Den Bussche (2003) completed the most comprehensive phylogenetic study of Vespertilionidae and provided a sound hypothesis for the evolutionary relationships for many of these bats. However, they were still unable to resolve many of the supergeneric relationships within Vespertilioninae and presented new evolutionary hypotheses that require further testing. To resolve these relationships >5,500 bp of coding and noncoding sequence data from the nDNA genome were analyzed in combination with the previously sequenced mtDNA data to reevaluate hypotheses of the evolutionary relationships within Vespertilioninae. Because of the stochastic nature of lineage sorting the inclusion of data from the nuclear and mitochondrial genomes is important in fully understanding evolutionary relationships (Avise 1994).

Tribes of Vespertilioninae.—This study provides phylogenetic information for 8 of the 10 tribes previously proposed in various classifications of Vespertilioninae (Antrozoini, Eptesicini, Lasiurini, Myotini, Nycticeiini, Nyctophilini, Pipistrellini, Plecotini, Scotophilini, and Vespertilionini). We were unable to obtain tissue samples from either *Nyctophilus* or *Pharotis* (Nyctophilini sensu Koopman 1994; Simmons 2005) and therefore were unable to address phylogenetic affinities of these taxa. Accumulating evidence of the affinities of *Myotis* to Kerivoulinae and Murininae has required removal of

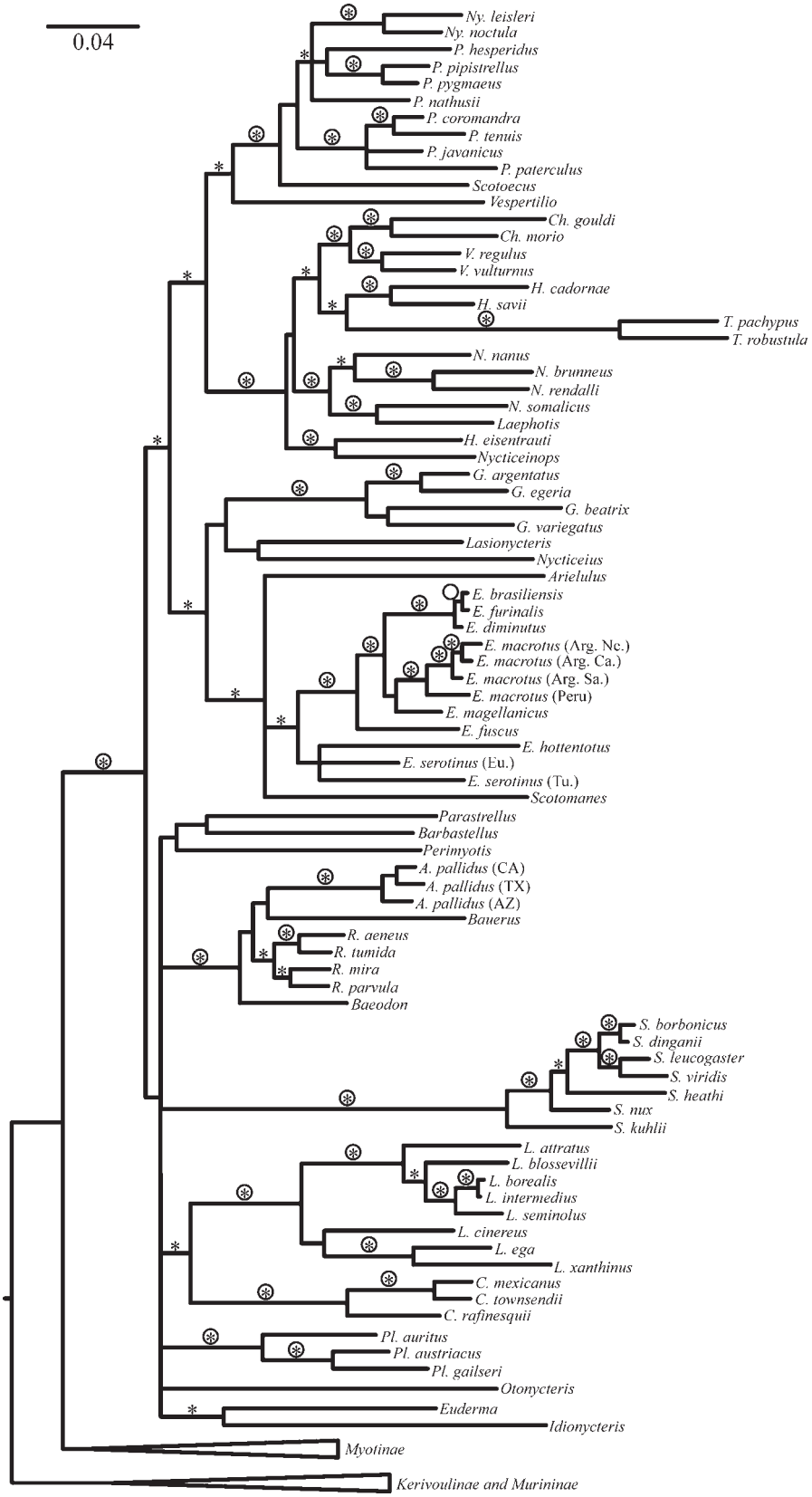

Fig. 2.—Phylogram from Bayesian analysis of the 12S rRNA, $tRNA^{Val}$, and 16S rRNA mitochondrial DNA genes, with supported phylogenetic relationships from both maximum-parsimony and Bayesian analysis depicted. Circles indicate clades supported by maximum parsimony (≥70% bootstrap values), whereas asterisks indicate clades supported by Bayesian analysis (≥0.95 posterior probability). Taxonomic abbreviations include: A. = *Antrozous*, Ch. = *Chalinolobus*, C. = *Corynorhinus*, E. = *Eptesicus*, G. = *Glauconycteris*, H. = *Hypsugo*, L. = *Lasiurus*, N. = *Neoromicia*, Ny. = *Nyctalus*, P. = *Pipistrellus*, Pl. = *Plecotus*, R. = *Rhogeessa*, S. = *Scotophilus*, T. = *Tylonycteris*, and V. =

Myotini (excluding *Lasionycteris*) from Vespertilioninae and elevation of *Myotis* to subfamily rank (Myotinae—Hoofer and Van Den Bussche 2003; Kawai et al. 2002; Lack et al. 2010; Stadelmann et al. 2004; Volleth and Heller 1994b). Although *Myotis* taxa were included in this study and are supported as a monophyletic group, this study was not designed to examine the affinities of the *Myotis*.

With regard to the remaining 8 traditionally recognized tribes, the combined tree provided support for 6 tribes, Antrozoini, Lasiurini, Scotophilini, Vespertilionini, and 2 unnamed tribes hereafter referred to as the hypsugine group and the perimyotine group (Fig. 4). *Lasiurus* has been recognized as a unique group within Vespertilioninae since the genus was 1st described (Gray 1831), and classification of *Lasiurus* into its own tribe by Tate (1942) has not been challenged (Bickham 1979, 1987; Hall and Jones 1961; Handley 1960; Hill and Harrison 1987; Hoofer and Van Den Bussche 2003; Koopman 1994; Miller 1907). Results from the combined analysis also support monophyly of Lasiurini (Fig. 4). The combined tree is not fully resolved with respect to interspecific relationships within *Lasiurus*, but a supported red bat clade (*L. atratus*, *L. seminolus*, *L. blossevillii*, and *L. borealis*) is present. However, without full resolution within *Lasiurus*, previous hypotheses about relationships of red bats to proposed lineages of yellow bat (*Dasypterus*) and hoary bat (*Lasiurus cinereus*) cannot be tested.

Scotophilini was the 2nd tribe supported by the combined analysis (Fig. 4). The genus *Scotophilus* historically has been included in the tribe Nycticeiini (Koopman 1994; McKenna and Bell 1997; Simmons 2005; Tate 1942). This position has been contradicted by bacular morphology (Hill and Harrison 1987), cytogenetics (Volleth et al. 2006), and ribosomal mtDNA (Hoofer and Van Den Bussche 2003) and was rejected in this study by the combined mtDNA and nDNA analysis and by each independently (Fig. 4). These results are congruent with phylogenetic analysis of a combined mtDNA and nDNA supermatrix targeted at assessing Nycticeiini and Scotophilini monophyly (Roehrs 2009).

Antrozoini is the 3rd supported clade in the combined analysis (Fig. 4). The group consisting of *Antrozous* and *Bauerus* (often a synonym of *Antrozous*—cf. Engstrom and Wilson 1981) was 1st described as subfamily Antrozoinae (Miller 1897; Simmons 2005) and has since been unstable in position and rank. Miller (1907) grouped *Antrozous* and *Bauerus* in subfamily Nyctophilinae with *Nyctophilus* and *Pharotis*, a classification supported by Tate (1941) and Simpson (1945). Koopman and Jones (1970) were 1st to place *Antrozous* and *Bauerus* into tribe Antrozoini, but its position remained within Nyctophilinae. This position of Antrozoini within Nyctophilinae was questioned by Koopman (1970) based on zoogeography and Pine et al. (1971) based on bacular morphology. Antrozoini has since been placed within Vespertilioninae by most authors, with various affinities (Hill and Harrison 1987; Koopman 1994; McKenna and Bell 1997). The most divergent exception to this hypothesis is the elevation of Antrozoini to its own family, Antrozoidae, aligned closely to Molossidae (Simmons 1998; Simmons and Geisler 1998). However, this hypothesis has not been supported by phylogenetic analysis of mtDNA (Hoofer and Van Den Bussche 2003) or nDNA (Miller-Butterworth et al. 2007). Hoofer and Van Den Bussche (2003) redefined Antrozoini by including *Rhogeessa* and *Baeodon* into the tribe. Their arrangement is supported largely by the combined tree with a monophyletic *Rhogeessa* sister to an *Antrozous*–*Bauerus* clade; however, the position of *Baeodon* was unresolved (Fig. 4). As in Hoofer and Van Den Bussche (2003), our mtDNA gene tree supports the inclusion of *Baeodon* in Antrozoini (Fig. 2), but results of the nDNA analysis place *Baeodon* basal to the Lasiurini (Fig. 3). This relationship is not supported, and it is possible that the incomplete nDNA data set for *Baeodon* may cause instability at this node resulting in a lack of resolution.

The 4th supported group consisted of New World pipistrelles, *Parastrellus hesperus* and *Perimyotis subflavus* (Fig. 4), and would constitute a new, yet-to-be-named, tribe referred to here as the perimyotine group. These results support Hoofer and Van Den Bussche (2003) placing each species in its own genus, but their phylogeny was unresolved relative to the position of these taxa within Vespertilioninae and their relationship to each other. Although affinities for this perimyotine group are not clear, these 2 taxa are supported in a deeply diverging clade. Furthermore, the combined analysis demonstrates that these taxa are distinct from *Pipistrellus* and fall outside of the other *Pipistrellus*-like bats. The inclusion of *Parastrellus* and *Perimyotis* into their own tribe initially seems counterintuitive based on previous research (Baker and Patton 1967; Hamilton 1949; Hill and Harrison 1987; Tate 1942). However, these taxa were problematic to place within *Pipistrellus* (sensu Koopman 1994), and many other taxa (*Arielulus*, *Falsistrellus*, *Hypsugo*, *Neoromicia*, and *Vespadelus*) previously included in *Pipistrellus* are today considered valid genera with different affinities than to *Pipistrellus*. Furthermore, a single colonization of the Nearctic by the most recent common ancestor of these taxa is more parsimonious than multiple colonization events, and their deep divergence allows for the morphological and chromosomal divergence separating them. Considering New World pipistrelles as a separate tribe preserves their generic and deeply divergent differences (Hamilton 1949) while maintaining their apparent common ancestry (Fig. 4). However, this tribal-level peri-

←

Vespadelus. For species with more than 1 representative, general locality information is provided in parentheses following the species name. Locality abbreviations follow United States postal codes or include: Arg. = Argentina; Ca. = Catamarca Province, Argentina; Eu. = Europe; Ne. = Neuquén Province, Argentina; Sa. = Salta Province, Argentina; and Tu. = Tunisia. Scale is in number of substitutions per site.

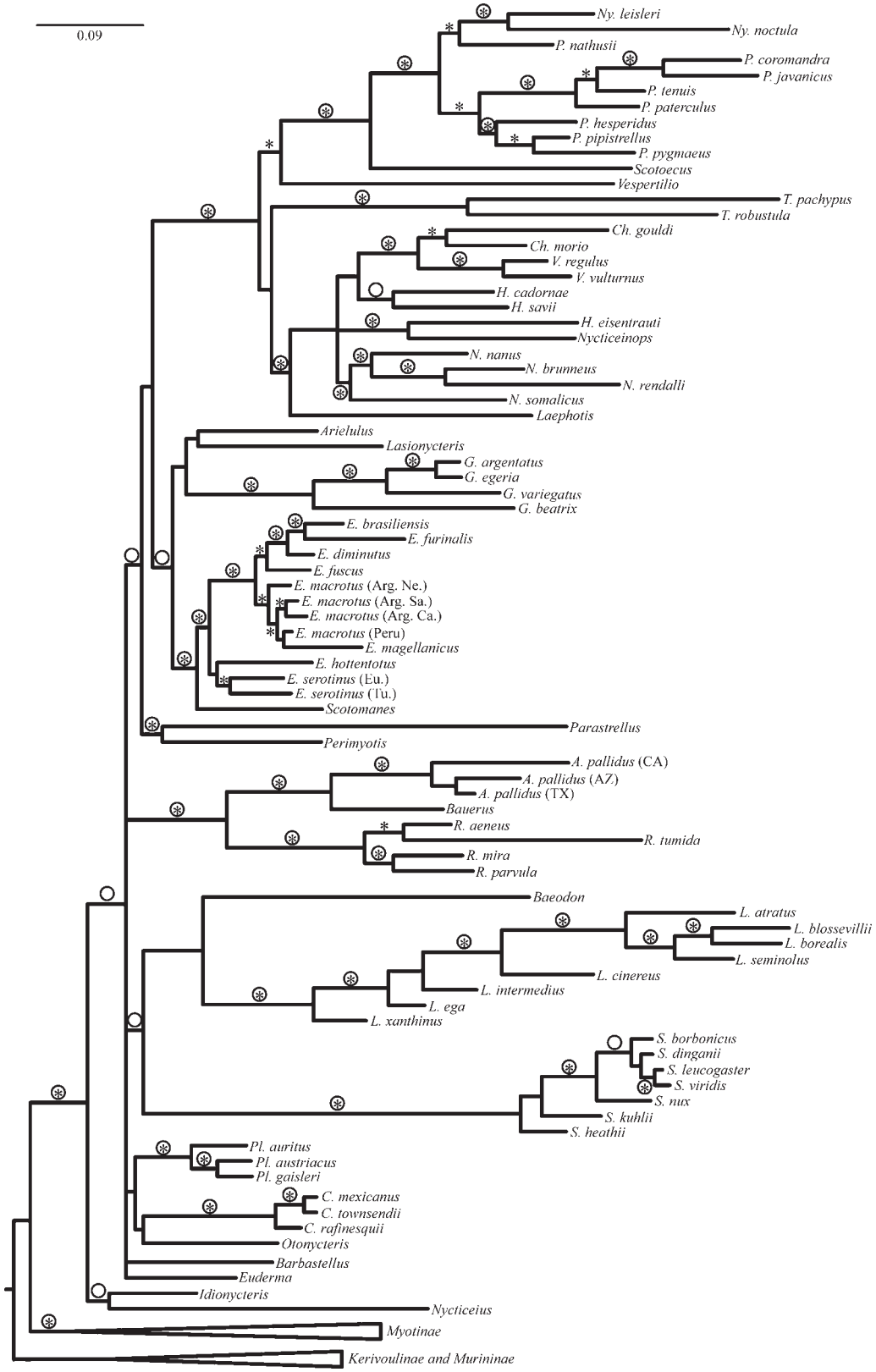

FIG. 3.—Phylogram from Bayesian analysis of the concatenated nuclear DNA gene regions apolipoprotein B (APOB), dentin matrix acidic phosphoprotein I (DMP1), recombination activating gene II (RAG2), protein kinase C, iota (PRKCI), signal transducer and activator of transcription 5A (STAT5A), and thyrotropin (THY), with supported phylogenetic relationships from both maximum-parsimony ($\geq$70% bootstrap values) and Bayesian analysis ($\geq$0.95 posterior probability) depicted. Scale is in number of substitutions per site. Symbols and abbreviations as in Fig. 2.

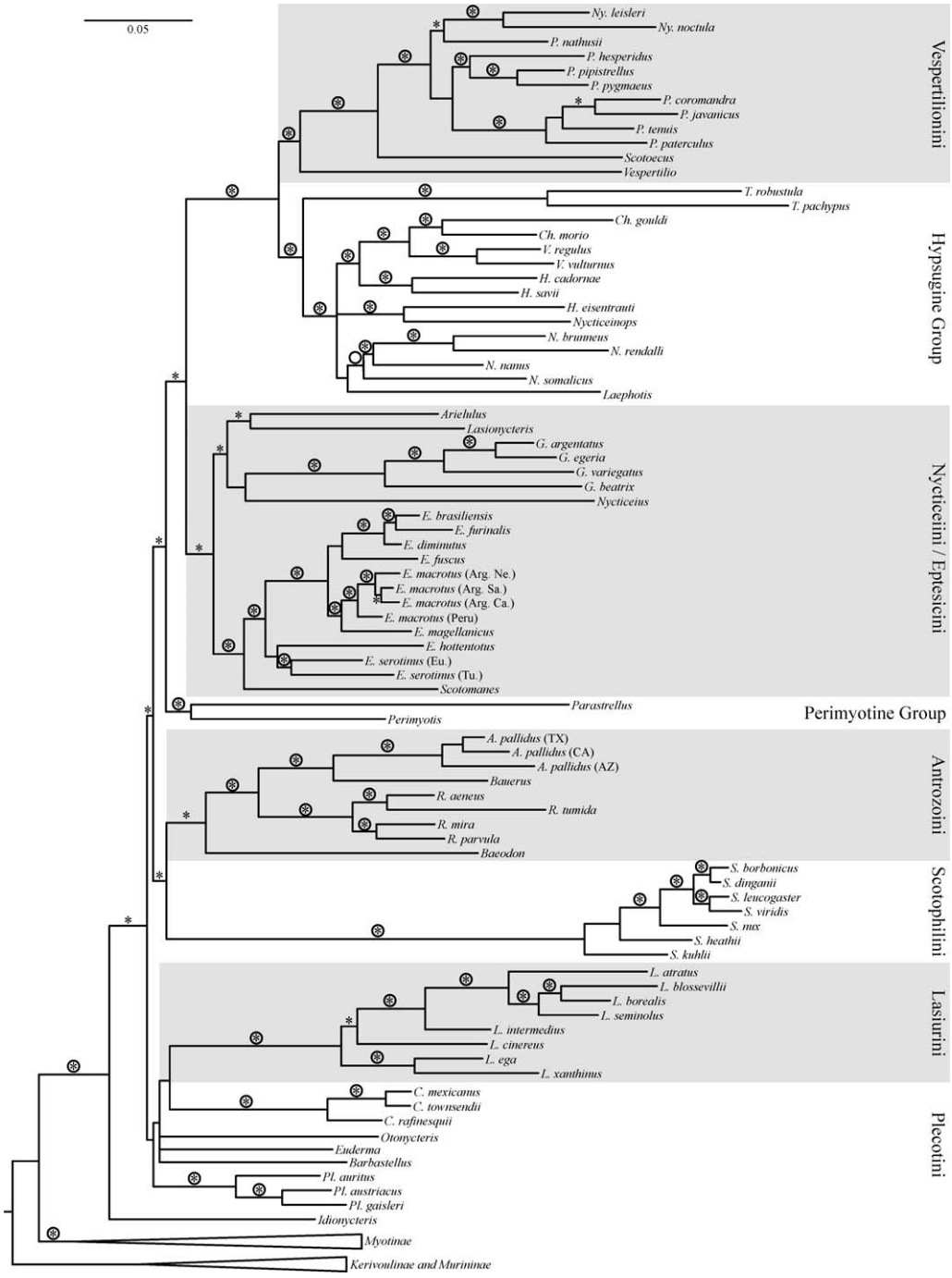

FIG. 4.—Phylogram from Bayesian analysis of the combined ribosomal mitochondrial DNA (12S rRNA, $tRNA^{Val}$, and 16S rRNA) and nuclear DNA (apolipoprotein B [APOB], dentin matrix acidic phosphoprotein I [DMP1], recombination activating gene II [RAG2], protein kinase C, iota [PRKCI], signal transducer and activator of transcription 5A [STAT5A], and thyrotropin [THY]) gene regions, with supported phylogenetic relationships from both maximum-parsimony (≥70% bootstrap values) and Bayesian analysis (≥0.95 posterior probability) depicted. Scale is in number of substitutions per site. Symbols and abbreviations as in Fig. 2.

myotine group should be considered tentative until further research corroborates this relationship and resolves their position within Vespertilioninae.

The last 2 supported tribes form a sister relationship in the combined tree and include most taxa historically considered *Pipistrellus*-like (Fig. 4). The 1st of these tribes is composed of *Nyctalus*, *Pipistrellus*, *Scotoecus*, and *Vespertilio*. Because of inclusion of *Vespertilio* in this tribe and *Vespertilio* having priority, the most appropriate name for this tribe is Vespertilionini. The other tribe consisted of *Chalinolobus*, *Hypsugo*, *Laephotis*, *Neoromicia*, *Nycticeinops*, *Tylonycteris*, and *Vespadelus*. This tribe is currently unnamed, but because *Hypsugo* has priority, this group will be referred to as the hypsugine group. These results are congruent with the results

of Roehrs (2009), who addressed intergeneric relationships of *Pipistrellus*-like bats using a digenomic data set with reduced taxon sampling.

Two other previously documented tribes, Nycticeiini and Plecotini, deserve mention. The combined phylogram presented here (Fig. 4) corroborates recent research (Hill and Harrison 1987; Hoofer and Van Den Bussche 2003; Roehrs 2009; Volleth et al. 2006) in rejecting Nycticeiini (sensu Tate 1942). However, with regard to Nycticeiini (sensu Hoofer and Van Den Bussche 2003), the combined analysis was in congruence topologically, but the clade lacked statistical support (Fig. 4). This lack of support likely stems from a difference between the mtDNA and nDNA tree topologies. The mtDNA gene tree from this study is in agreement with Hoofer and Van Den Bussche (2003) with only the Bayesian analysis supporting Nycticeiini. The nDNA tree includes an unsupported Nycticeiini clade excluding *Nycticeius* making this clade more appropriately named Eptesicini. As discussed by Roehrs (2009), it is apparent that *Arielulus*, *Eptesicus* (including *Histiotus*), *Glauconycteris*, *Lasionycteris*, and *Scotomanes* form a tribal-level clade, but more effort will be required to resolve the position of *Nycticeius* and will have an impact on the nomenclature of this clade.

Although taxa included in Plecotini have not been completely stable, this tribe has been consistently included in Vespertilioninae classification since it was described by Gray (1866) as Plecotina (Table 1). Handley (1959) is responsible for establishing the core Plecotini genera currently recognized: *Barbastella*, *Corynorhinus*, *Euderma*, *Idionycteris*, and *Plecotus*. Other taxa also have been included in Plecotini: *Baeodon*, *Nycticeius*, *Otonycteris*, *Rhogeessa*, *Nyctophilus*, and *Histiotus* (Bogdanowicz et al. 1998; Dobson 1878; Hill and Harrison 1987; Kawai et al. 2002; Pine et al. 1971; Qumsiyeh and Bickham 1993). Although morphologic and cytogenetic data support monophyly of the core Plecotini (Bogdanowicz et al. 1998; Frost and Timm 1992; Handley 1959; Leniec et al. 1987; Tate 1942; Tumlison and Douglas 1992; Volleth and Heller 1994a, 1994b), monophyly of this tribe only recently has been tested explicitly (Hoofer and Van Den Bussche 2001, 2003). Hoofer and Van Den Bussche (2003) were unable to unambiguously support monophyly of the core Plecotini or their relationship to other previously proposed closely related genera. The combined analysis of this study, and the mtDNA and nDNA trees independently, also were unable to resolve Plecotini, leaving this tribe neither supported nor rejected (Fig. 4). These taxa could be extant members of one of the earliest radiations from Vespertilioninae ancestral stock and appear to have rapidly diverged, not allowing time for these gene regions to accumulate sufficient synapomorphic characters to clarify their evolutionary histories. Finally, despite a general lack of resolution of deep phylogenetic relationships within Vespertilioninae, the subfamily is supported as a monophyletic group to the exclusion of *Myotis*, which is congruent with current hypotheses.

Usefulness of nDNA and combined data.—The nuclear gene regions included in this study were individually relatively short (averaging ~800 bp), had few variable positions (173–570 bp), and included even few potential phylogenetically informative positions (129–415 bp; Table 2). For any 1 nDNA gene region relatively few potentially informative positions per taxon were found, resulting in topologies that were not fully resolved and less informative of true evolutionary relationships. Results of likelihood-mapping tended to support this supposition, with most independent nDNA gene regions resolving <80% of quartets and all independent nDNA gene regions resolving <90% of quartets (Fig. 1). Furthermore, because it is difficult to predict whether a particular gene tree reflects true evolutionary relationships, most studies currently use a suite of gene regions from multiple genomes to overcome potential problems with nonphylogenetic signal within any 1 particular gene region (Philippe and Telford 2006; Rodríguez-Ezpeleta et al. 2007). Gene regions included in this study have been used successfully in various combinations in previous studies of bats and other mammals (Amrine-Madsen et al. 2003; Baker et al. 2003; Eick et al. 2005; Matthee and Davis 2001; Matthee et al. 2001, 2004, 2007; Murphy et al. 2001; Van Den Bussche et al. 2003), and all of these markers have been included in a recent study of the phylogenetic relationships of Miniopteridae, *Cistugo*, Myotinae, Kerivoulinae, Murininae, and Vespertilioninae (Lack et al. 2010).

Although results presented here provide a more resolved hypothesis of evolutionary relationships of Vespertilioninae than previous phylogenetic studies, it appears that more sequence data and more taxa will be necessary to overcome stochastic error and fully resolve deep evolutionary patterns within this subfamily. However, these studies will need to overcome potential systematic errors that tend to increase with increasing amounts of sequence data by excluding taxa, genes, and possibly even codon positions that exhibit relatively rapid rates of evolution (Baurain et al. 2007; Brinkmann and Philippe 2008; Philippe and Telford 2006; Rodríguez-Ezpeleta et al. 2007).

Acknowledgments

This project would not have been possible without the support and loan of tissues from individuals and institutions including: R. J. Baker, Museum of Texas Tech University; N. B. Simmons, American Museum of Natural History; B. D. Patterson, L. R. Heaney, and W. T. Stanley, Field Museum of Natural History; S. B. McLaren, Carnegie Museum of Natural History; M. D. Engstrom and B. Lim, Royal Ontario Museum; M. Ruedi, Muséum d'Histoire Naturelle de Genève and the University of Lausanne, Institut de Zoologie et d'Ecologie Animale; P. J. Taylor, Durban Natural Science Museum; J. K. Braun and M. A. Mares, Sam Noble Oklahoma Museum of Natural History; T. L. Yates, Museum of Southwestern Biology at the University of New Mexico; R. L. Honeycutt and D. Schlitter, Texas Cooperative Wildlife Collection at Texas A&M University; J. O. Whitaker, Jr., and D. S. Sparks, Indiana State University Vertebrate Collection; and T. E. Lee, Jr., Abilene Christian University. We thank C. E. Stanley, Jr., for generating a portion of the sequence data included in this project. G. Eick was helpful in providing primers and advice on polymerase chain reaction profiles for the nuclear introns used in this

study. Thanks go to the Oklahoma State University Recombinant DNA/Protein Core Facility for use of its equipment and assistance in troubleshooting. J. K. Braun, M. J. Hamilton, D. M. Leslie, M. A. Magnuson, R. J. Tyrl, and 2 anonymous reviewers reviewed drafts of this document. National Science Foundation grants DEB-9873657 and DEB-0610844 to RAVDB funded this research. Any opinions, findings, and conclusions or recommendations expressed in this paper are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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Submitted 3 October 2009. Accepted 9 April 2010.

Associate Editor was David L. Reed.

Appendix I

Taxonomic samples included in this study with tissue collection number, voucher specimen catalog number, general locality, and GenBank accession numbers (http://www.ncbi.nlm.nih.gov/). Specimens and tissue samples are housed in the following institutions: Abilene Christian University (ACU), American Museum of Natural History (AMNH), Carnegie Museum of Natural History (CM, SP), Colección Mamíferos Lillo, Universidad Nacional de Tucumán (CML), Durban Natural Science Museum (DM), Field Museum of Natural History (FMNH), Indiana State University Vertebrate Collection (ISUV), Muséum d'Histoire Naturelle de Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB, NK), Museum of Texas Tech University (TTU, TK), Natural History Museum of Bern (NHMB), Oklahoma State University Collection of Vertebrates (OSU, OK), Royal Ontario Museum (ROM, F), Sam Noble Oklahoma Museum of Natural History (OMNH, OCGR), Texas Cooperative Wildlife Collection at Texas A&M University (TCWC), Universidad Autónoma Metropolitana–Iztapalapa (UAMI), Universidad Nacional Autónoma de México (UNAM), and University of Lausanne, Institut de Zoologie et d'Ecologie Animale (IZEA). Mitochondrial DNA (mtDNA) for a specific taxon with GenBank accession number starting in AF or AY may not have been amplified from the specific specimen indicated. A dash (—) denotes information unavailable and therefore missing. A few specimens came from the personal collections of Dale W. Sparks (DWS), Manuel Ruedi (M), and Rodney L. Honeycutt (RLH and 05M3). GenBank accession numbers for sequences generated in this study are indicated in boldface type; all others were published previously (Eick et al. 2005; Hoofer and Van Den Bussche 2003; Lack et al. 2010). APOB = apolipoprotein B; DMP1 = dentin matrix acidic phosphoprotein I; RAG2 = recombination activating gene II; PRKCI = protein kinase C, iota; STAT5A = signal transducer and activator of transcription 5A; and THY = thyrotropin.

Taxon	Tissue collection no.	Museum catalog no.	Locality	GenBank accession no.						
				mtDNA	APOB	DMP1	RAG2	PRKC1	STAT5A	THY
Kerivoulinae										
Kerivoula hardwickii	F44154	ROM110829	Đồng Nai Province, Vietnam	AF345928	GU328143	AY141893	AY141034	GU328304	GU328378	GU328447
Kerivoula lenis (analyzed previously as *K. papillosa*)	F44175	ROM110850	Đồng Nai Province, Vietnam	AF345927	GU328144	GU328229	AY141035	GU328305	GU328379	GU328448
Kerivoula pellucida	F35987	ROM102177	East Kalimantan Province, Indonesia	AY495476	GU328145	GU328230	GU328064	GU328306	GU328380	GU328449
Murininae										
Harpiocephalus harpia	TK21258	CM88159	Uthai Thani Province, Thailand	AF263235	GU328139	AY141892	AY141031	GU328300	GU328375	GU328443
Murina cyclotis	M1209	MHNG1826.033	Phôngsaly Province, Lao People's Democratic Republic	GU952767	GU328155	GU328238	GU328072	GU328313	GU328386	GU328456
Murina huttoni	F42722	ROM107739	Đắk Lắk Province, Vietnam	AY495490	GU328156	GU328239	GU328073	GU328314	GU328387	GU328457
Murina tubinaris	M1179	MHNG1926.034	Phôngsaly Province, Lao People's Democratic Republic	GU952768	GU328157	GU328240	GU328074	GU328315	GU328388	GU328458
Myotinae										
Myotis albescens	TK17932	CM77691	Marowijne District, Suriname	AY495492	GU328159	GU328241	GU328076	GU328317	GU328390	GU328460
Myotis bocagii	FMNH150075	FMNH150075	Tanga Region, Tanzania	AF326096	GU328160	GU328242	GU328077	GU328318	GU328391	GU328461
Myotis cf. *browni* (analyzed previously as *M. muricola*)	FMNH147067	FMNH147067	Mindanao Island, Philippine Islands	AY495504	GU328169	GU328251	GU328086	GU328327	GU328400	GU328470
Myotis californicus	TK78797	TTU79325	Texas	AY495495	GU328161	GU328243	GU328078	GU328319	GU328392	GU328462
Myotis capaccinii	TK25610	TTU40554	Northern Province, Jordan	AY495494	GU328162	GU328244	GU328079	GU328320	GU328393	GU328463
Myotis ciliolabrum	TK83155	TTU78520	Texas	AY495497	GU328163	GU328245	GU328080	GU328321	GU328394	GU328464
Myotis dominicensis	TK15613	TTU31503	St. Joseph Parish, Dominica	AY495500	GU328164	GU328246	GU328081	GU328322	GU328395	GU328465
Myotis fortidens	TK43186	UAMI	Michoacán, Mexico	AY495502	GU328165	GU328247	GU328082	GU328323	GU328396	GU328466
Myotis keaysi	TK13532	—	Yucatán, Mexico	AY495503	GU328166	GU328248	GU328083	GU328324	GU328397	GU328467
Myotis latirostris	M606	MHNG	Miao-Li County, Taiwan	GU952769	GU328167	GU328249	GU328084	GU328325	GU328398	GU328468
Myotis levis	FMNH141600	FMNH141600	São Paulo, Brazil	AF326097	GU328168	GU328250	GU328085	GU328326	GU328399	GU328469
Myotis moluccarum (analyzed previously as *M. adversus*)	RLH62	TCWC	Australia	AY495491	GU328158	(not sequenced)	GU328075	GU328316	GU328389	GU328459
Myotis myotis	IZEA3790	MHNG1805.062	Canton of Bern, Switzerland	AF326098	GU328170	GU328252	GU328087	GU328328	GU328401	GU328471
Myotis nigricans	FMNH129210	FMNH129210	Amazonas, Peru	AF326099	GU328171	GU328253	GU328088	GU328329	GU328402	GU328472

Appendix I.—Continued.

Taxon	Tissue collection no.	Museum catalog no.	Locality	GenBank accession no.						
				mtDNA	APOB	DMP1	RAG2	PRKC1	STAT5A	THY
Myotis riparius	AMNH268591	AMNH268591	Paracou, French Guiana	AF263236	GU328172	GU328254	GU328089	GU328330	GU328403	GU328473
Myotis septentrionalis	DWS609	ISUV6454	Indiana	AY495507	GU328173	GU328255	GU328090	GU328331	GU328404	GU328474
Myotis thysanodes	TTU79327	TK78796	Texas	(not sequenced)	(not sequenced)	(not sequenced)	GU328091	(not sequenced)	(not sequenced)	GU328475
Myotis thysanodes	TK78802	TTU79330	Texas	AF326100	GU328174	GU328256	(not sequenced)	GU328332	GU328405	(not sequenced)
Myotis velifer	TK79170	TTU78599	Texas	AF263237	GU328175	GU328257	AY141033	GU328333	GU328406	GU328476
Myotis volans	TK78980	TTU79545	Texas	AY495510	GU328176	GU328258	GU328092	GU328334	GU328407	GU328477
Myotis welwitschii	FMNH144313	FMNH144313	Kasese District, Uganda	AY495511	GU328177	GU328259	GU328093	GU328335	GU328408	GU328478
Myotis yumanensis	TK28753	TTU43200	Oklahoma	AY495512	GU328178	GU328260	GU328094	GU328336	GU328409	GU328479
Vespertilioninae										
Antrozoini										
Antrozous pallidus	NK506	MSB40576	California	GU328037	GU328120	GU328209	GU328045	GU328285	GU328360	GU328428
Antrozous pallidus	NK39195	MSB	Arizona	GU328038	GU328121	GU328210	GU328046	GU328286	GU328361	GU328429
Antrozous pallidus	TK49646	TTU71101	Texas	AF326088	GU328122	GU328211	GU328047	GU328287	GU328362	GU328430
Baeodon alleni	TK45023	UNAM	Michoacán, Mexico	AF326108	**HM561577**	**HM561677**	**HM561632**	**HM568371**	**HM568338**	**HM593057**
Bauerus dubiaquercus	F33200	ROM97719	Campeche, Mexico	AY395863	GU328125	GU328214	GU328050	GU328289	GU328364	GU328432
Rhogeessa aeneus	TK20712	TTU40012	Belize District, Belize	AY495530	**HM561578**	**HM561678**	**HM561633**	**HM568372**	**HM568339**	**HM593058**
Rhogeessa mira	TK45014	UNAM	Michoacán, Mexico	AY495531	**HM561579**	**HM561679**	**HM561634**	**HM568373**	**HM568340**	**HM593059**
Rhogeessa parvula	TK20653	TTU36633	Sonora, Mexico	AF326109	GU328196	GU328274	GU328108	GU328350	GU328419	GU328492
Rhogeessa tumida	TK40186	TTU61231	Valle Department, Honduras	AF326110	GU328197	GU328275	GU328109	GU328351	GU328420	GU328493
Hypsugine group										
Chalinolobus gouldii	RLH27	TCWC	Australia	AY495461	**HM561610**	**HM561710**	**HM561665**	**HM568404**	**HM568363**	**HM593090**
Chalinolobus morio	05M3	TCWC	Australia	AY495462	GU328129	GU328218	GU328054	GU328292	GU328367	GU328435
Hypsugo cadornae	M1183	MHNG1926.050	Phôngsaly Province, Lao People's Democratic Republic	GU328041	GU328140	GU328226	GU328061	GU328301	(not sequenced)	GU328444
Hypsugo eisentrauti	F34348	ROM100532	Côte d'Ivoire	AY495473	**HM561611**	**HM561711**	**HM561666**	**HM568405**	**HM568364**	**HM593091**
Hypsugo savii	IZEA3586	MHNG1804.100	Canton of Valais, Switzerland	AY495475	**HM561612**	**HM561712**	**HM561667**	**HM568406**	(not sequenced)	**HM593092**
Laephotis namibensis	SP4160	CM93187	Maltahöhe District, Namibia	AY495477	**HM561613**	**HM561713**	**HM561668**	**HM568407**	**HM568365**	**HM593093**
Neoromicia brunnea	TK21501	CM90802	Estuaire Province, Gabon	AY495514	**HM561614**	**HM561714**	**HM561669**	**HM568408**	**HM568366**	**HM593094**
Neoromicia nana	DM7542	DM7542	KwaZulu-Natal Province, South Africa	AY495474	GU328141	GU328227	GU328062	GU328302	GU328376	GU328445
Neoromicia rendalli	TK33238	CM97977	Coastal Province, Kenya	AY495515	**HM561615**	**HM561715**	**HM561670**	**HM568409**	**HM568367**	**HM593095**
Neoromicia somalica	TK33214	CM97978	Coastal Province, Kenya	AY495516	**HM561616**	**HM561716**	**HM561671**	**HM568410**	**HM568368**	**HM593096**
Nycticeinops schlieffeni	TK33373	CM97998	Eastern Province, Kenya	AF326101	GU328183	GU328261	GU328095	AJ866330	AJ865440	AJ865685
Tylonycteris pachypus	F38442	ROM106164	Tuyen Quang Province, Vietnam	AY495538	**HM561617**	**HM561717**	**HM561672**	**HM568411**	(not sequenced)	**HM593097**
Tylonycteris robustula	M1203	MHNG1926.059	Phôngsaly Province, Lao People's Democratic Republic	**HM561631**	**HM561618**	**HM561718**	**HM561673**	**HM568412**	(not sequenced)	**HM593098**
Vespadelus regulus	RLH30	TCWC	Australia	AY495539	**HM561619**	**HM561719**	**HM561674**	**HM568413**	**HM568369**	**HM593099**
Vespadelus vulturnus	RLH16	TCWC	Australia	AY495499	(not sequenced)	(not sequenced)	**HM561675**	**HM568414**	**HM568370**	**HM593100**

Appendix I.—Continued.

Taxon	Tissue collection no.	Museum catalog no.	Locality	GenBank accession no.						
				mtDNA	APOB	DMP1	RAG2	PRKC1	STAT5A	THY
Lasiurini										
Lasiurus atratus	F39221	ROM107228	Potaro-Siparuni, Guyana	AY495478	**HM561580**	**HM561680**	**HM561635**	**HM568374**	**HM568341**	**HM593060**
Lasiurus blossevillii	F38133	ROM104285	Chiriquí Province, Panama	AY495479	**HM561581**	**HM561681**	**HM561636**	**HM568375**	**HM568342**	**HM593061**
Lasiurus borealis	TK49732	TTU71170	Texas	AY495480	**HM561582**	**HM561682**	**HM561637**	**HM568376**	**HM568343**	**HM593062**
Lasiurus cinereus	TK78926	TTU	Texas	AY495482	**HM561583**	**HM561683**	**HM561638**	**HM568377**	**HM568344**	**HM593063**
Lasiurus ega	TK43132	UNAM	Michoacán, Mexico	AY495483	**HM561584**	**HM561684**	**HM561639**	**HM568378**	**HM568345**	**HM593064**
Lasiurus intermedius	TK20513	TTU36631	Oaxaca, Mexico	(not sequenced)	**HM561585**	(not sequenced)	**HM561640**	**HM568379**	**HM568346**	**HM593065**
Lasiurus intermedius	TK84510	TTU80739	Texas	**HM561627**	(not sequenced)	**HM561685**	(not sequenced)	(not sequenced)	(not sequenced)	(not sequenced)
Lasiurus seminolus	TK90686	TTU80699	Texas	AY495484	**HM561586**	**HM561686**	**HM561641**	**HM568380**	**HM568347**	**HM593066**
Lasiurus xanthinus	TK78704	TTU78296	Texas	AY495485	**HM561587**	**HM561687**	**HM561642**	**HM568381**	**HM568348**	**HM593067**
Nycticeiini										
Arielulus aureocollaris	F38447	ROM106169	Tuyen Quang Province, Vietnam	**HM561621**	**HM561588**	**HM561688**	**HM561643**	**HM568382**	**HM568349**	**HM593068**
Eptesicus brasiliensis	TK17809	CM76812	Nickerie District, Suriname	AY495464	**HM561589**	**HM561689**	**HM561644**	**HM568383**	**HM568350**	**HM593069**
Eptesicus diminutus	TK15033	TTU48154	Guarico, Venezuela	AY495465	GU328133	GU328220	GU328056	GU328295	GU328370	GU328438
Eptesicus furinalis	AMNH268583	AMNH268583	Paracou, French Guiana	AF263234	GU328135	GU328222	AY141030	GU328297	GU328372	GU328440
Eptesicus fuscus	SP844	CM102826	West Virginia	AF326092	GU328136	GU328223	GU328058	GU328298	GU328373	GU328441
Eptesicus hottentotus	TK33013	CM89000	Rift Valley Province, Kenya	AY495466	GU328137	GU328224	GU328059	AJ866329	AJ865438	AJ865683
Eptesicus macrotus	OCGR2301	CML3230	Neuquén Province, Argentina	**HM561622**	**HM561590**	**HM561690**	**HM561645**	**HM568384**	**HM568351**	**HM593070**
Eptesicus macrotus	FMNH129207	FMNH129207	Ancash Region, Peru	AY495472	**HM561591**	**HM561691**	**HM561646**	**HM568385**	**HM568352**	**HM593071**
Eptesicus macrotus	OCGR4227	OMNH27925	Salta Province, Argentina	**HM561623**	**HM561592**	**HM561692**	**HM561647**	**HM568386**	**HM568353**	**HM593072**
Eptesicus macrotus	OCGR3806	OMNH32879	Catamarca Province, Argentina	**HM561624**	**HM561593**	**HM561693**	**HM561648**	**HM568387**	**HM568354**	**HM593073**
Eptesicus magellanicus	OCGR2303	OMNH23500	Neuquén Province, Argentina	**HM561625**	**HM561594**	**HM561694**	**HM561649**	**HM568388**	(not se quenced)	**HM593074**
Eptesicus serotinus	M816	MHNG1807.065	Greece	**HM561626**	**HM561595**	**HM561695**	**HM561650**	**HM568389**	**HM568355**	**HM593075**
Eptesicus serotinus	TK40897	TTU70947	Sidi Bou Zid Governorate, Tunisia	AY495467	**HM561596**	**HM561696**	**HM561651**	**HM568390**	**HM568356**	**HM593076**
Glauconycteris argentata	FMNH15119	FMNH15119	Kilimanjaro Region, Tanzania	AY495468	**HM561597**	**HM561697**	**HM561652**	**HM568391**	**HM568357**	**HM593077**
Glauconycteris beatrix	FMNH149417	FMNH149417	Haute Zaire, Zaire	AY495469	**HM561598**	**HM561698**	**HM561653**	**HM568392**	(not sequenced)	**HM593078**
Glauconycteris egeria	AMNH268381	AMNH268381	Central African Republic	AY495470	**HM561599**	**HM561699**	**HM561654**	**HM568393**	(not sequenced)	**HM593079**
Glauconycteris variegata	TK33545	CM97983	Western Province, Kenya	AY495471	**HM561600**	**HM561700**	**HM561655**	**HM568394**	**HM568358**	**HM593080**
Lasionycteris noctivagans	TK24216	TTU56255	Texas	AF326095	GU328146	(not sequenced)	GU328065	(not sequenced)	GU328381	(not sequenced)
Lasionycteris noctivagans	—	TK24889	Oklahoma	(not sequenced)	(not sequenced)	GU328231	(not sequenced)	GU328307	(not sequenced)	GU328450
Nycticeius humeralis	TK26380	TTU49536	Texas	AF326102	(not sequenced)	(not sequenced)	GU328096	(not sequenced)	(not sequenced)	(not sequenced)
Nycticeius humeralis	TK90649	TTU80664	Texas	(not sequenced)	GU328184	GU328262	(not sequenced)	GU328338	GU328411	GU328481
Scotomanes ornatus	F42568	ROM107594	Tuyen Quang Province, Vietnam	AY495537	**HM561601**	**HM561701**	**HM561656**	**HM568395**	**HM568359**	**HM593081**

Appendix I.—Continued.

Taxon	Tissue collection no.	Museum catalog no.	Locality	GenBank accession no.						
				mtDNA	APOB	DMP1	RAG2	PRKC1	STAT5A	THY
Scotophilini										
Scotophilus borbonicus	TK33267	CM98041	Coastal Province, Kenya	AY495532	GU328199	GU328276	GU328110	GU328352	GU328421	GU328494
Scotophilus dinganii	FMNH147235	FMNH147235	Tanga Region, Tanzania	AY495533	GU328200	GU328277	GU328111	AJ866332	AJ865441	AJ865686
Scotophilus heathii	F42769	ROM107786	Đắk Lắk Province, Vietnam	AY495534	GU328201	GU328278	GU328112	GU328353	GU328422	GU328495
Scotophilus kuhlii	FMNH145684	FMNH145684	Sibuyan Island, Philippine Islands	AF326111	GU328202	GU328279	GU328113	GU328354	GU328423	GU328496
Scotophilus leucogaster	TK33359	CM90854	Eastern Province, Kenya	AY395867	GU328203	GU328280	GU328114	GU328355	GU328424	GU328497
Scotophilus nux	TK33484	—	Western Province, Kenya	AY495535	GU328204	GU328281	GU328115	GU328356	GU328425	GU328498
Scotophilus viridis	FMNH150084	FMNH150084	Tanga Region, Tanzania	AF326112	GU328206	GU328283	GU328117	GU328357	GU328426	GU328499
Perimyotine group										
Parastrellus hesperus	TK78703	TTU79269	Texas	AY495522	GU328187	GU328265	GU328099	GU328341	GU328413	GU328483
Perimyotis subflavus	TK90671	TTU80684	Texas	AY495523	GU328191	GU328269	GU328103	GU328345	GU328416	GU328487
Plecotini										
Barbastella barbastellus	IZEA3590	MHNG1804.094	Canton of Valais, Switzerland	AF326089	GU328124	GU328213	GU328049	GU328288	GU328363	GU328431
Corynorhinus mexicanus	TK45849	UAMI	Michoacán, Mexico	AF326090	GU328128	GU328217	GU328053	GU328291	GU328366	GU328434
Corynorhinus rafinesquii	TK5959	TTU45380	Arkansas	AF326091	GU328130	GU328219	GU328055	GU328293	GU328368	GU328436
Corynorhinus townsendii	OK11530	OSU13099	Oklahoma	(not sequenced)	GU328131	(not sequenced)	(not sequenced)	GU328294	GU328369	GU328437
Corynorhinus townsendii	TK83182	TTU78531	Texas	AF263238	(not sequenced)	AY141891	AY141029	(not sequenced)	(not sequenced)	(not sequenced)
Euderma maculatum	NK36260	MSB121373	Utah	AF326093	GU328138	GU328225	GU328060	GU328299	GU328374	GU328442
Idionycteris phyllotis	ACU736	ACU736	—	(not sequenced)	GU328142	GU328228	(not sequenced)	GU328303	GU328377	(not sequenced)
Idionycteris phyllotis	NK36122	MSB120921	Utah	AF326094	(not sequenced)	(not sequenced)	GU328063	(not sequenced)	(not sequenced)	GU328446
Otonycteris hemprichii	SP7882	—	Maan Government, Jordan	AF326103	GU328186	GU328264	GU328098	GU328340	GU328412	GU328482
Plecotus auritus	IZEA2693	—	—	(not sequenced)	(not sequenced)	GU328266	(not sequenced)	GU328342	GU328414	(not sequenced)
Plecotus auritus	IZEA2694	MHNG1806.047	Canton of Valais, Switzerland	AF326106	GU328188	(not sequenced)	GU328100	(not sequenced)	(not sequenced)	GU328484
Plecotus austriacus	IZEA3722	MHNG1806.042	Canton of Valais, Switzerland	AF326107	GU328189	GU328267	GU328101	GU328343	GU328415	GU328485
Plecotus gaisleri	IZEA4780	MHNG1806.051	Meknés-tafilalet, Morocco	GU328043	GU328192	GU328270	GU328104	GU328346	GU328417	GU328488
Vespertilionini										
Nyctalus leisleri	FMNH140374	FMNH140374	Malakand Division, Pakistan	AY495517	**HM561602**	**HM561702**	**HM561657**	**HM568396**	(not sequenced)	**HM593082**
Nyctalus noctula	NHMB 209/87	NHMB 209/87	Canton of Berne, Switzerland	AY495518	**HM561603**	**HM561703**	**HM561658**	**HM568397**	(not sequenced)	**HM593083**
Pipistrellus coromandra	FMNH140377	FMNH140377	Malakand Division, Pakistan	AY495524	GU328190	GU328268	GU328102	GU328344	(not sequenced)	GU328486
Pipistrellus hesperidus	DM8013	DM8013	KwaZulu-Natal, South Africa	**HM561628**	**HM561604**	**HM561704**	**HM561659**	**HM568398**	(not sequenced)	**HM593084**
Pipistrellus javanicus	FMMNH147069	FMMNH147069	Mindanao Island, Philippines	AY495525	GU328193	GU328271	GU328105	GU328347	(not sequenced)	GU328489
Pipistrellus nathusii	IZEA2830	MHNG1806.003	Canton of Vaud, Switzerland	AF326104	**HM561605**	(not sequenced)	**HM561660**	(not sequenced)	(not sequenced)	(not sequenced)

Appendix I.—Continued.

Taxon	Tissue collection no.	Museum catalog no.	Locality	GenBank accession no.						
				mtDNA	APOB	DMP1	RAG2	PRKC1	STAT5A	THY
Pipistrellus nathusii	IZEA3406	MHNG1806.001	Canton of Vaud, Switzerland	(not sequenced)	(not sequenced)	**HM561705**	(not sequenced)	**HM568399**	(not sequenced)	**HM593085**
Pipistrellus paterculus	M1181	MHNG1926.045	Phôngsaly Province, Lao People's Democratic Republic	**HM561629**	**HM561606**	**HM561706**	**HM561661**	**HM568400**	**HM568360**	**HM593086**
Pipistrellus pipistrellus	M1439	MHNG1956.031	Canton of Genève, Switzerland	**HM561630**	**HM561607**	**HM561707**	**HM561662**	**HM568401**	**HM568361**	**HM593087**
Pipistrellus pygmaeus (analyzed previously as *P. pipistrellus*)	IZEA3403	MHNG1806.032	Barcelona Province, Spain	AF326105	GU328195	GU328273	GU328107	GU328349	**HM568362**	GU328491
Pipistrellus tenuis	FMNH137021	FMNH137021	Sibuyan Island, Philippine Islands	AY495529	**HM561608**	**HM561708**	**HM561663**	**HM568402**	(not sequenced)	**HM593088**
Scotoecus hirundo	FMNH151204	FMNH151204	Kilimanjaro Region, Tanzania	AY495536	**HM561609**	**HM561709**	**HM561664**	**HM568403**	(not sequenced)	**HM593089**
Vespertilio murinus	IZEA3599	MHNG1808.017	Canton of Valais, Switzerland	AY395866	**HM561620**	**HM561720**	**HM561676**	**HM568415**	(not sequenced)	**HM593101**