

VESPERTILIONINAE SYSTEMATICS: USING
MITOCHONDRIAL AND NUCLEAR MARKERS TO
ELUCIDATE PHYLOGENETIC RELATIONSHIPS

By

ZACHARY P. ROEHRS

Bachelor of Science, Fisheries and Wildlife
University of Nebraska – Lincoln
Lincoln, Nebraska, U.S.A.
1999

Master of Science, Museum Studies
University of Nebraska – Lincoln
Lincoln, Nebraska, U.S.A.
2003

Master of Science, Natural Resource Sciences
University of Nebraska – Lincoln
Lincoln, Nebraska, U.S.A.
2004

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July 2009

VESPERTILIONINAE SYSTEMATICS: USING
MITOCHONDRIAL AND NUCLEAR MARKERS TO
ELUCIDATE PHYLOGENETIC RELATIONSHIPS

Dissertation Approved:

Dr. Ronald A. Van Den Bussche

Dissertation Adviser

Dr. Janet K. Braun

Dr. Meredith J. Hamilton

Dr. David M. Leslie, Jr.

Dr. Ronald J. Tyrl

Dr. A. Gordon Emslie

Dean of the Graduate College

ACKNOWLEDGMENTS

It is with gratitude that I thank the funding sources for my education and for making the following research possible. The Department of Zoology has been my academic home for the last 5 years and has provided me with Teaching Associate positions which have not only helped me pay the bills but, more importantly, helped me grow as an educator, academic, and leader. The experiences and knowledge I have gained through this service to the Department have been some of the most important to my future. I hope that I have brought credit to this Department and OSU through my teaching, research, and service. Equally important has been the support of the Oklahoma Cooperative Fish and Wildlife Research Unit at Oklahoma State University. The Unit has employed me over many summers and to which I am also indebted for the experience and knowledge gained during service to the Unit (and for one Dodge 2500 truck). Funding for these summer projects was granted to Dr. Ronald A. Van Den Bussche, Dr. Meredith J. Hamilton, and Dr. David M. Leslie, Jr. from the Oklahoma Department of Wildlife Conservation and administered through the Oklahoma Cooperative Fish and Wildlife Research Unit. Funding for my dissertation research was provided by a grant from the National Science Foundation (DEB-9873657 and DEB-0610844) to Dr. Van Den Bussche.

It is with great respect and admiration that I thank my committee whom has guided my graduate development at OSU, including Dr. Janet K. Braun, Dr. Meredith J. Hamilton, Dr. David M. Leslie, Jr., Dr. Ronald J. Tyrl, and Dr. Ronald A. Van Den Bussche. Dr. Braun is the former Head Curator of the Sam Noble Oklahoma Museum of Natural History and currently Curator of their Department of Mammalogy and Genomic Resources, so I feel she deserves extra thanks for journeying to Stillwater for my committee meetings. As someone who shares similar passions (mammals and natural history collections), she has helped build one of the premier museums in the country and is an expert on mammals in Oklahoma and abroad; it has been an honor to work with her on this and other projects. This project has definitely benefited from having Dr. Braun on my committee and I have gained a great deal from her experience and insight. An expert in cytogenetics, genetics and mammals, Dr. Hamilton is also a caring, compassionate teacher who genuinely wants to help students succeed. Dr. Hamilton has not only been a research mentor insuring that I have a solid broad foundation, but she also has been a motivator through her stories and encouragement, a mentor in teaching through my work under her in the classroom, and in some ways, a parent away from home for our lab, keeping us grounded, providing worldly advice, bandaging our egos, and watching over us. Dr. Tyrl, a Professor Emeritus in Botany and Curator of the OSU Herbarium, should be the example for those who love classification, systematics, and taxonomy of the living world. Dr. Tyrl is an expert on the plants of Oklahoma (among other specialties) and a true naturalist (although an uncouth term today, I consider this title the highest compliment). Amongst these accolades, let me add that he is one of the best teachers I have ever had the privilege of taking a class under and consider myself lucky to have had

the chance to learn about plants under his instruction. Dr. David M. Leslie, Jr. is also an expert on mammals, especially Artiodactyla ecology, a gifted editor, and a champion of the American Society of Mammalogists. Not only has Dr. Leslie supported me and provided me opportunities to work on many projects, he has helped me grow as a researcher through his mentoring on this and other projects. He has helped me become a better writer and has often talked frankly with me about the realities of our field. I truly appreciate his support but also the many discussions we have engaged in about publishing, job hunting, mammals, and synonymies. Finally, I would not be in Stillwater today if not for Dr. Ronald A. Van Den Bussche, who entrusted me with part of his life's work, the systematics of Vespertilionidae. He has given me many opportunities to grow and latitude to develop in my own ways. Dr. Van Den Bussche has taught me what it takes to be a successful researcher through his actions and active mentoring. He is a good judge of character and has helped me play to my strength while helping me work on my many weaknesses. I thank him for everything he has provided for me and given me the opportunity to accomplish.

I especially thank Dr. Karen McBee who, in addition to being a Professor of Zoology, is the Curator of Vertebrates for the Oklahoma State University Collection of Vertebrates. Dr. McBee gave me the opportunity to continue 2 of my passions—teaching and museology. It has always been a dream of mine to be involved in teaching a course in Mammalogy and to continue my work in natural history collections. Not only has Dr. McBee given me these opportunities, but she has immeasurably influenced my knowledge about mammals, being a teacher, and the care of natural history collections. To her I will always be indebted for this and all the support she has given me. There are

many more faculty at OSU that have helped me grow and influenced my thinking in a number of ways but I thanks to Dr. Anthony A. Echelle, Dr. Stanley F. Fox, Dr. Timothy J. O'Connell, and Dr. Michael W. Palmer.

A number of people have been helpful in the course of this research. Justin B. Lack, Alex T. McSpadden, David M. Miller, Craig E. Stanley, Jr., and Lorin W. Stearman all assisted in DNA amplification and sequencing for this project. Justin B. Lack and Brandi S. Coyner were invaluable resources in discussing analysis and statistics and taught me a great deal. They will be well known names (if not already) in the future of mammalian phylogenetic systematics and molecular population ecology. Dr. Geeta N. Eick provided us with a number of primer sequences and helped me in troubleshooting protocols. Dr. Steven R. Hooper, whose excellent work this study is built on and whose shoes I could never fill, was also helpful in discussing protocols and troubleshooting. I would like to thank Janet Rogers and Lisa Whitworth of the Oklahoma State University Recombinant DNA/Protein Core Facility who allowed me access to the equipment in their care, assisted me in troubleshooting, and were always very friendly to me.

Because all writing and no field work truly make Zac a dull boy, I thank Angela Brown, Dr. Hamilton, Dr. McBee, Dr. Leslie, Dr. Van Den Bussche, the Cooperative Fish and Wildlife Research Unit, and the Oklahoma Department of Wildlife Conservation for giving me many opportunities to get outside, learn new techniques, trap new critters, and collect more data. Also I thank the following people for assisting me in field work and other research, putting up with me as a slave driver, and helping me become a mentor: Fred Zac Brown, Brandi S. Coyner, Daniel and Elaine M. Fite, Tasha M. Forman, Ashley A. Foster, Jessica N. Hoops, Sherry J. James, Kim N. King, Justin B.

Lack, Meredith A. Magnuson, Deanna L. Martinez, Bradley A. McNutt, David M. Miller, Christopher L. Roy, Cybil Nikki Smith, Andrew J. Turner, and Thomas B.L. Wilson. I add special thanks to Dr. Daniel J. McGlinn who has helped me numerous times with statistical analysis and ecological theory. Dan is a good friend, a powerful thinker, and an academic inspiration.

One of the reasons I was attracted to OSU was the friendly environment. I have met many wonderful people here and made many friends. I thank the staff of both the Department of Zoology and Oklahoma Cooperative Fish and Wildlife Research Unit: Sandy Cole, Marsha Flasch, Melissa Gentry, Joyce Hufford, Sheryl Lyon, Mylissa Minyard, and Gertie Mulder for putting up with me, all my questions, and requests. You all took care of me and this place would not run without you! I thank my office mates Justin B. Lack, Brandi S. Coyner, Elaine M. Fite, Dustin G. Loftis, and Deanna L. Martinez for their support, encouragement, help, and friendship. There are many friends I could name and recount the many ways you have helped me over the years, please know that I thank you, I hope to see and work with you in the future, and will remember you always!

My graduate schooling has been just as grueling for my parents, Bill and JoAnn Roehrs, who are excited that classes are behind me and I am finally applying for jobs. My parents have always supported, encouraged and loved me without questions despite my provisioning of reasons for them to stop. I love them dearly. My brother Ben has always been my biggest supporter and closest friend and, as he knows, "Much love." Finally, I thank Meredith A. Magnuson. She has assisted me in research, edited this and many other papers, supported and encouraged me, put up with my dark side, and endured

a focus not always on her. For all the challenges, hurdles, and doubts, the ups and downs that graduate school can bring, the most important thing I have gained by coming to OSU is her love. I can say no words to thank her for this!

TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION TO A STUDY OF VESPERTILIONINAE SYSTEMATICS1	
Introduction.....	1
Natural History of Vespertilioninae.....	2
History of Vespertilioninae Systematics.....	3
Unresolved Relationships and the Importance of Phylogeny Resolution.....	6
1) Paucity of phylogenetically useful morphological characters.....	6
2) Parallel or convergent morphological characters.....	7
3) Radiation of incongruent evolutionary hypotheses.....	8
4) Few phylogenetic tests of clade monophyly.....	9
5) Unresolved molecular phylogenetics.....	10
Literature Cited.....	12
II. ELUCIDATING TRIBAL PHYLOGENETIC RELATIONSHIPS WITHIN VESPERTILIONINAE (CHIROPTERA: VESPERTILIONIDAE) BASED ON MITOCHONDRIAL AND NUCLEAR SEQUENCE DATA28	
Introduction.....	29
Materials and Methods.....	32
Taxonomic sampling.....	32
Extraction, amplification, and sequencing.....	33
Phylogenetic analysis.....	34
Results.....	36
Independent gene regions and concordance.....	36
mtDNA sequences.....	36
nDNA sequences.....	37
Combined sequences.....	38
Discussion.....	39
Tribes of Vespertilioninae.....	40
Usefulness of nDNA and combined data.....	46
Acknowledgments.....	47
Literature Cited.....	49
Figure Legends.....	65

Chapter	Page
III. A MOLECULAR PHYLOGENETIC REEVALUATION OF THE TRIBE NYCTICEIINI (CHIROPTERA: VESPERTILIONIDAE).....	71
Introduction.....	72
Materials and Methods.....	75
Extraction, amplification, and sequencing.....	75
Phylogenetic analysis.....	76
Taxonomic sampling.....	77
Results.....	80
mtDNA sequences	80
nDNA sequences.....	81
Combined sequences.....	82
Discussion.....	82
Nycticeiini <i>sensu</i> Tate.....	83
Concordance with baculum morphology and cytogenetics	85
Systematic conclusions	86
Acknowledgments.....	88
Literature Cited	89
Figure Legends.....	95
 IV. MOLECULAR SYSTEMATICS OF THE <i>PIPISTRELLUS</i> -LIKE BATS	 100
Introduction.....	100
Materials and Methods.....	103
Taxonomic sampling.....	103
Extraction, amplification, and sequencing.....	106
Phylogenetic analysis.....	107
Results.....	108
mtDNA sequences	108
nDNA sequences.....	109
Combined sequences.....	110
Discussion.....	111
Systematic conclusions	112
Phylogenetic reevaluation.....	115
Acknowledgments.....	118
Literature Cited	119
Figure Legends.....	131

Chapter	Page
V. CONCLUSIONS ON A PHYLOGENETIC STUDY OF VESPERTILIONINAE	135
Research Synopsis	135
Limitations of These Results	136
Future Research	138
Literature Cited	140
APPENDIX I	146

LIST OF TABLES

Table	Page
1.1 Data from the International Union for Conservation of Nature and Natural Resources (IUCN) Red List from 2 sources: Hutson et al. 2001 and 2008 Red List from http://www.iucnredlist.org/ . Asterisks (*) indicate percentage of 241 recognized species in Vespertilionidae (Simmons 2005 excluding <i>Myotis</i> and <i>Cistugo</i>)	27
2.1 Historic classifications of Vespertilioninae. Superscript "a" denotes that this arrangement can be found in Koopman (1984) and Koopman (1994), but the latter provides the most information and is the basis for depicted classification. Superscript "b" denotes a combination of results taken from Heller and Volleth (1984), Kearney et al. (2002), Volleth and Heller (1994) , Volleth and Tidemann (1991), and Volleth et al. (2001), with most recent papers taking precedence. Taxa marked with asterisks (*) are currently recognized taxa that would have been synonyms in authors' taxonomic system; † denotes these taxa as <i>incertae sedis</i>	61
2.2 Information for the 6 nuclear primers used in this study, with primer sequence, annealing temperatures used, and citations to original primer description. Abbreviations: F refers to forward; R to reverse primers; and Ex refers to external; In to internal primers	63
2.3 Characteristics of individual nDNA 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.	64
4.1 Historic classification of <i>Pipistrellus</i> -like bats	128
4.2 Classification of <i>Pipistrellus</i> -like bats examined in this study	130
5.1 Classification of Vespertilioninae taxa examined in this study	145

LIST OF FIGURES

Figure	Page
<p>2.1 A scatter plot of the percentage of resolved quartets from likelihood-mapping by number of analyzed positions for each individual nDNA gene region and the mtDNA, nDNA, and combined datasets. See Table 2.3 for data and Strimmer and von Haeseler (1997) for likelihood-mapping</p>	67
<p>2.2 Phylogram from Bayesian analysis of the ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA with supported phylogenetic relationships from both maximum parsimony and Bayesian analysis depicted. Asterisks (*) indicate clades supported by both maximum parsimony ($\geq 70\%$ bootstrap values) and Bayesian analysis (≥ 0.95 posterior probability). Taxonomic abbreviations include: A. = <i>Antrozous</i>, Ch. = <i>Chalinolobus</i>, C. = <i>Corynorhinus</i>, E. = <i>Eptesicus</i>, G. = <i>Glauconycteris</i>, H. = <i>Hypsugo</i>, L. = <i>Lasiurus</i>, N. = <i>Neoromicia</i>, Ny. = <i>Nyctalus</i>, P. = <i>Pipistrellus</i>, Pl. = <i>Plecotus</i>, R. = <i>Rhogeessa</i>, S. = <i>Scotophilus</i>, T. = <i>Tylonycteris</i>, and V. = <i>Vespadelus</i>. For species with more than 1 representative, general locality information is provided in parentheses following the species name. Locality abbreviations follow U.S. postal codes or include: Arg. = Argentina, Ca. = Catamarca Province, Ne. = Neuquén Province, and Sa. = Salta Province</p>	68
<p>2.3 Phylogram from Bayesian analysis of the combined nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY with supported phylogenetic relationships from both maximum parsimony ($\geq 70\%$ bootstrap values) and Bayesian analysis (≥ 0.95 posterior probability) depicted. Symbols and abbreviations as in Fig. 2.2.....</p>	69
<p>2.4 Phylogram from Bayesian analysis of the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions with supported phylogenetic relationships from both maximum parsimony ($\geq 70\%$ bootstrap values) and Bayesian analysis (≥ 0.95 posterior probability) depicted. Symbols and abbreviations as in Fig. 2.2</p>	70

- 3.1 Cladogram of supported phylogenetic relationships of the vespertilionid bats included in this study based on ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA. Numbers on clade branches indicate support values for maximum parsimony bootstrap (above) and Bayesian posterior probabilities (below). Bolded numbers indicate those that met clade support qualifications for bootstrap ($\geq 70\%$) and posterior probabilities (≥ 0.95). Taxa highlighted with a gray box indicate taxa historically included in tribe Nycticeiini. Taxonomic genera abbreviations include: C. = *Chalinolobus*, E. = *Eptesicus*, G. = *Glauconycteris*, H. = *Hypsugo*, N. = *Neoromicia*, P. = *Pipistrellus*, S. = *Scotophilus*. Locality abbreviations include: Arg. = Argentina, Eu. = Europe, Tu. = Tunisia96
- 3.2 Cladogram of supported phylogenetic relationships of the vespertilionid bats included in this study based on nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY. Numbers, abbreviations and symbology follow Fig. 3.197
- 3.3 Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Numbers, abbreviations and symbology follow Fig. 3.198
- 3.4 Optimal tree topology from the Bayesian analysis of the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Abbreviations follow Fig. 3.199
- 4.1 Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA. Numbers on clade branches indicate support values for maximum parsimony bootstrap (above) and Bayesian posterior probabilities (below). Bolded numbers indicate those that met clade support qualifications for bootstrap ($\geq 70\%$) and posterior probabilities (≥ 0.95). Circles with numbers and letters are for referencing clades in text. Taxonomic genera abbreviations include: C. = *Chalinolobus*, E. = *Eptesicus*, G. = *Glauconycteris*, H. = *Hypsugo*, N. = *Neoromicia*, Ny. = *Nyctalus*, P. = *Pipistrellus*, S. = *Scotophilus*, T. = *Tylonycteris*, V. = *Vespadelus*132
- 4.2 Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY. Numbers and abbreviations follow Fig. 4.1133

Figure	Page
4.3 Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on the combined ribosomal mtDNA (12S rRNA, tRNA Val, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Numbers and abbreviations follow Fig. 4.1.....	134

CHAPTER I

INTRODUCTION TO A STUDY OF VESPERTILIONINAE SYSTEMATICS

INTRODUCTION

It is clear that bats (Mammalia: Chiroptera) have been of interested humans since recorded history, revered by some societies, hunted by others, and feared by some, but always allocated a place in their cultures (Allen 1967; Freeman 1983; Hill and Smith 1984). Given the nearly worldwide distribution of the family Vespertilionidae (Koopman 1970; Nowak 1994) and the propensity of some of its members to use caves and anthropogenic structures for roosts (Kunz and Reynolds 2003; Nowak 1999), bats of this group may have been some of the most familiar to people. As human society and culture developed, humans interest of the natural world began to evolve into the scientific disciplines of taxonomy and classification (among others; for a historic review see Mayr 1982; Nelson and Platnick 1981; Simpson 1961). The modern systematic study of bats began with Linnaeus (1735) who described the 1st formerly recognized genus of bats, *Vespertilio* (Linnaeus 1758). Etymologically, the name for this group is derived from the Latin word for night, *vesper*, and *vespertilio* is the masculine Latin word for bat (Brown 1956). This group is commonly referred to as the vesper or evening bats. This genus denominates the subfamily of interest (Vespertilioninae) in the following studies. In this opening chapter I provide an introduction to the subfamily Vespertilioninae,

review briefly its changing systematics, and outline the problems that still remain in elucidating Vespertilioninae evolutionary relationships.

NATURAL HISTORY OF VESPERTILIONINAE

Vespertilioninae has a nearly worldwide distribution, being excluded from only the tundra and ice-covered regions of the Northern Hemisphere, Antarctica, alpine zones of mountains ranges, and a few isolated islands (Anderson and Jones 1967; Koopman 1970; Nowak 1994, 1999). This distributional range is greater than any other mammalian subfamilial group (or even familial group if you exclude *Homo sapiens* and a few of its commensals). These bats inhabit environments including tropical and temperate broadleaf and coniferous forests, boreal forests, tropical and temperate grasslands, shrublands and deserts, from mountain slopes to sea level (Koopman 1970; Hill and Smith 1984; Nowak 1994). They spend the day in roosts including caves, rock crevices, tree hollows, under bark, in the foliage, or bird nests (Bogan et al. 2003; Kunz 1982; Kunz and Lumsden 2003; Lewis 1995; Schulz 2002). Many species also commonly use anthropogenic structures such as mines, wells, cellars, bridges, and various parts of buildings depending on season and environmental conditions (Keeley and Tuttle 1999; Kunz and Reynolds 2003; Nowak 1999; Tuttle and Taylor 1998). Vespertilioninae exhibit the gamut of social life styles from solitary to gregarious, forming large roost colonies often segregated by sex and breeding condition (Nowak 1994). Among most Vespertilioninae, mating usually occurs from autumn through spring, but sperm is stored until spring when the egg(s) is fertilized (McCracken and Wilkinson 2000; Nowak 1994; Racey 1982). Most species give birth to 1–2 offspring / year (Barclay and Harder 2003; Hill and Smith 1984; Nowak 1994), but some will have more, with species of *Lasiurus*

producing ≤ 5 pups (Hamilton and Stalling 1972). Bats, including those of Vespertilioninae, are unique in their longevity relative to body size with records of vespertilionins living 15–40 years depending on the species (Brunet-Rossinni and Austad 2004).

Although most are insectivorous, Vespertilioninae show great diversity in foraging ecology, with species that are open and edge space aerial foragers, edge space trawlers, narrow space gleaners (Schnitzler and Kalko 2001; Schnitzler et al. 2003), and terrestrial chasers of prey (Johnston and Fenton 2001). Bats with these foraging styles show predictable trends in wing morphology and echolocation call structure (Neuweiler 1989; Norberg and Rayner 1987). Vespertilionin bats employ frequency-modulated echolocation calls emitted through the mouth (Fenton 1982; Goudy-Trainer and Freeman 2002; Neuweiler 1989, 1990). At dusk, bats leave their day roost to forage, often making use of separate night roosts to rest between foraging bouts before returning to the day roost near dawn. From a utilitarian perspective, the insectivorous nature of Vespertilioninae may be 1 of the most important roles that these bats play in ecological service to humans by feeding on pest insects (Whitaker 1995). They have been shown to reduce both the number of insects on plants and herbivory on plants (Kalka et al. 2008). Bats in more temperate regions often use hibernation, migration, or both to survive insect limited winters.

HISTORY OF VESPERTILIONINAE SYSTEMATICS

Vespertilioninae is a member of the family Vespertilionidae (Simmons 2005) and superfamily Vespertilionoidea (also including Molossidae and Natalidae—Hooper and Van Den Bussche 2003; Miller-Butterworth et al. 2007; Teeling et al. 2005; Van Den

Bussche and Hooper 2001, 2004; Van Den Bussche et al. 2003). The family Vespertilionidae Gray, 1821 is the largest chiropteran family, with >380 species in 46 genera (Simmons 2005; excluding *Miniopterus* which was elevated to its own family by Hooper and Van Den Bussche 2003, and *Cistugo* to its own family by Lack et al. 2009). The phylogenetic diversity of this family is only eclipsed by 2 families of rodents, Cricetidae and Muridae (Wilson and Reeder 2005). The purpose here is to give the reader a historical overview of the scope of the systematic instability in this subfamily that has led to the following research. For complete reviews of the historic literature, see the following chapters herein, Miller (1907) who gives a good review of pre-turn of the century literature and Hill and Harrison (1987) and Hooper and Van Den Bussche (2003) who provide good reviews of literature since then.

The inclusion of 4 subfamilies (Kerivoulinae, Murinae, Myotinae, Vespertilioninae) in this family has been relatively stable since the work of Miller (1907) and Tate (1942) and has been supported by recent molecular analysis (Hooper and Van Den Bussche 2003; Kawai et al. 2003; Lack et al. 2009; Stadelmann et al. 2004; Volleth and Heller 1994b). There have been some incongruencies in the placement of the *Myotis*, whether in its own subfamily (including *Lasionycteris*: Simmons 1998, 2005; or *Myotis* only: Hooper and Van Den Bussche 2003; Kawai et al. 2003; Lack et al. 2009; Stadelmann et al. 2004; Volleth and Heller 1994b) or within Vespertilioninae (tribe Myotini—Tate 1942; Hill and Harrison 1987; Koopman 1994; Koopman and Jones 1970; McKenna and Bell 1997; Miller 1907). However, based on recent phylogenetic analysis of both nuclear and mitochondrial DNA and cladistic analysis of cytological data, it seems clear that Vespertilioninae is paraphyletic with respect to *Myotis*, with *Myotis*

being more closely related to Kerivoulinae and Murinae (Hooper and Van Den Bussche 2003; Kawai et al. 2003; Lack et al. 2009; Stadelmann et al. 2004; Volleth and Heller 1994b). Therefore, the following research only focuses on Vespertilioninae (*sensu* Hooper and Van Den Bussche 2003).

Three other historic incongruencies at the subfamilial rank deserve mention because they fall within the scope of the following studies. First, Miller (1897) and Simmons (2005) argued that *Antrozous* (including *Bauerus*) is morphologically distinct and warrant elevation to its own subfamily Antrozoinae (also see Simmons 1998; Simmons and Geisler 1998). Second, Miller (1907) described the subfamily Nyctophilinae including *Nyctophilus*, *Pharotis*, and *Antrozous*, but this positioning of *Antrozous* has been rejected by more recent studies (Breed and Inns 1985; Hill and Harrison 1987; Hooper and Van Den Bussche 2003; Koopman 1984, 1994; Volleth and Heller 1994b). Finally, Miller (1907) also included the monotypic subfamily Tomopeatinae within Vespertilionidae, which is currently accepted as a subfamily of Molossidae (Simmons 1998, 2005; Sudman et al. 1994). Issues of systematic instability continue at the tribal rank.

Since Gray (1821) 1st described Vespertilioninae (“Race 2” in his words), 9 tribes (excluding Myotini) have been proposed at various times, in various classifications and often with different constituents, to organize the variation 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). Validity of these tribes has been accepted or rejected to varying degrees and their

exact circumscription has been debated. Without a clear supergeneric classification, classification problems also exist at the generic rank with continuing debate as to what constitutes *Eptesicus*-like or *Pipistrellus*-like bats and what species should be included in the genera *Nycticeius*, *Eptesicus*, *Pipistrellus*, and *Vespertilio*, among others (Hill and Harrison 1987; Hofer and Van Den Bussche 2003; Koopman 1994; Koopman and Jones 1970; McKenna and Bell 1997; Simmons 1998, 2005; Simpson 1945; Tate 1942; Volleth and Heller 1994b).

UNRESOLVED RELATIONSHIPS AND THE IMPORTANCE OF PHYLOGENY RESOLUTION

Despite more than a century of work on the evolutionary relationships of Vespertilioninae, there remains no clear, phylogenetically supported, fully resolved hypothesis for the evolution of the subfamily. This situation has been caused by ≥ 5 factors that are not mutually exclusive:

1) *Paucity of phylogenetically useful morphological characters.*—Unlike other vespertilionid subfamilies, Vespertilioninae does not seem to exhibit the same degree of morphological diversity observed in other chiropteran families or vespertilionid taxa. "Of the subfamilies of Vespertilionidae this [Vespertilioninae] is distinctly the most primitive, being perhaps best characterized by the absence of the special modifications that distinguish the other groups" (Miller 1907:197). Tate (1942:221) later commented "...it was found in practice that the remaining Vespertilioninae genera were often too closely integrated one with another to permit satisfactory treatment one by one... In many instances the data upon which certain species or races have been founded are so vague that it has not been possible to reach a conclusion regarding their status." Recent studies of chiropterans also have noted the difficulties of vespertilionid classification due

to a lack of synapomorphic characters for the subfamily Vespertilioninae (Koopman 1993, 1994; Simmons 1998).

2) *Parallel or convergent morphological characters.*—Hill and Harrison (1987:229) stated “...many of the characters used to define taxa and relationships among the Vespertilioninae appear strongly adaptive and of equivocal value in generic and suprageneric systematics.” The historic Vespertilioninae supergeneric and generic classifications of Miller (1907) and Tate (1942) put great emphasis on teeth. Tate (1942) divided many of his Vespertilioninae tribes based on number of premolars and incisors (e.g., P1–3 Myotini [including *Lasionycteris* and most Plecotini], P1–2 Pipistrelloid, only P1 Eptesicoid, only I1 Nycticeiini). Even before Tate’s classic work Ärnäck-Christie-Linde (1909:574) noted: “But however good a generic character the premolars may have proved to afford in most cases, yet there are facts, which make the fitness of the dental formula as a generic character rather doubtful.” Ärnäck-Christie-Linde (1909) cited many examples of individuals from a specific genus not conforming to this premolar classification. Tate himself noted that the use of “...the anterior upper premolar (P²) is unstable” (Tate 1942:232). Since this time there have been a number of other studies that have corroborated the plasticity of the incisors and premolars in both number and cusp pattern and have noted a general convergent trend of tooth row reductions across the lineages of Vespertilioninae (Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill and Harrison 1987; Hill and Topál 1973; Horáček and Zima 1978; Koopman 1975; Rosevear 1962; Volleth and Heller 1994b; Zima and Horáček 1985).

3) *Radiation of incongruent evolutionary hypotheses.*—In the last 2 decades researchers have attempted to use new and potentially useful characters to test hypotheses

of Vespertilioninae evolution. However, these characters have often led to rejection of historic taxonomic groups (e.g., Nycticeiini), the creation of new tribal groups (e.g., Scotophilini, Antrozoini), and changes in circumscription of many tribes (as described in the following chapters). Although bacular morphology has been used since the 1930s, Hill and Harrison (1987) amassed 1 of the largest datasets to specifically address systematic questions in Vespertilionidae. The drawback to bacular morphology is the potential for elevated levels of selection pressures masking true evolutionary relationships (Hill and Harrison 1987; Lanza 1969; Martin 1978).

Cytogenetics also has been used to resolve evolutionary relations within Vespertilioninae (Ao et al. 2006; Baker and Patton 1967; Bickham 1979, 1987; Bogdanowicz et al. 1998; Fedyk and Ruprecht 1983; Hill and Harrison 1987; Kearney et al. 2002; Leniec et al. 1987; Rautenbach et al. 1993; Stock 1983; Volleth 1985, 1987, 1992; Volleth and Heller 1994a, 1994b; Volleth and Tidemann 1989, 1991; Volleth et al. 2001, 2006; Zima 1979, 1982; to name a few examples). These cytogenetic characters are believed to be less prone to parallel or convergent evolution because they represent rare genomic events (Baker 1970). However, the difficulty of producing karyotypes has led to many studies by multiple authors that contain only a few new karyotypes and rely on descriptions and photographs of previous studies to propose a hypothesis of evolutionary relationships. The difficulty of accurately interpreting evolutionary relationships using characters from previous studies is further hampered by differences in quality and potential errors present in some of these studies (Volleth and Heller 1994a).

Mayer and von Helversen (2001) and Mayer et al. (2007) studied relationships among Western Palaearctic vespertilionids using the mitochondrial coding gene ND1.

Kawai et al. (2002) using ND1, the nuclear exon vWF, and short interspersed elements (SINEs) studied relationships among Eastern Palaearctic bats. Hooper and Van Den Bussche (2003) used 2.6 kilobases of mitochondrial ribosomal DNA and modern phylogenetic methods to examine evolutionary relationships among 110 worldwide specimens of Vespertilionidae (cf. Hooper et al. 2003; Hooper and Van Den Bussche 2001; Lack et al. 2009). In molecular studies, we use the actual units of inheritance and therefore remove a level of abstraction caused by plasticity, hereditary factors, and environmental and genetic interactions of gene products. Furthermore, by using molecular datasets, we have access to huge numbers of potentially informative characters that can be used to overcome potential parallel or convergent events in any 1 base or gene. Targeted regions can be chosen based on some background knowledge of the gene region to avoid potential analogy. Finally, based on the simple 4 character code of DNA, relatively simple models of evolution can be predicted for sequence data (Avice 2004; Felsenstein 2004).

4) *Few phylogenetic tests of clade monophyly.*—There have been few tests of monophyly in previously proposed Vespertilioninae supergeneric clades. Jones et al. (2002) in a metadata supertree analysis of all chiropterans recovered little resolution within Vespertilioninae except for the tribes Lasiurini and Plecotini. However, because the datasets they used assumed monophyly of many of these tribes, it was not surprising that recovered tribes were monophyletic. Therefore, the study by Jones et al. (2002) does not constitute a true test of clade monophyly. The large cladistic analysis of cytogenetic data by Volleth and Heller (1994b), although an important and enlightening study of Vespertilioninae relationships, was taxonomically limited in scope (23 genera, 50

species) and included only 1 New World species (*Baeodon alleni*). It was therefore unable to test fully the monophyly of most previously proposed supergeneric clades. To date, the only phylogenetic study able to test monophyly of proposed supergeneric relationships within Vespertilioninae has been Hooper and Van Den Bussche (2003).

5) *Unresolved molecular phylogenetics.*—Hooper and Van Den Bussche (2003), in 1 of the most comprehensive molecular studies of vespertilionid systematics, using 2.6 kilobases of mitochondrial DNA (mtDNA) obtained insufficient resolution to resolve the deep branching patterns within Vespertilioninae. Studies have demonstrated that mtDNA is not as efficient as nuclear DNA (nDNA) at recovering well resolved or supported phylogenies at higher taxonomic levels due to saturation of phylogenetic signal and observed homoplasy (Koepfli and Wayne 2003; Matthee et al. 2001; Springer et al. 2001). Furthermore, results of Hooper and Van Den Bussche (2003) suggested that the potential rapid diversification early in the cladogenesis of Vespertilioninae lineages would prevent accumulation of lineage specific synapomorphic character-states that can be detected in subsequent analysis.

Although composition and evolutionary relationships within Vespertilionidae have been studied using morphological, cytological, and molecular characters, there is no clearly resolved phylogeny for tribal relationships within Vespertilioninae. In the following chapters of this dissertation I used 3 nuclear exon (APOB, DMP1, RAG2) and 3 intron (PRKCI, STAT5A, THY) gene regions in conjunction with ribosomal mtDNA (12S rRNA, tRNA^{Val}, 16S rRNA) to reexamine previous hypotheses about evolutionary relationships of Vespertilioninae bats in a phylogenetic framework. It is only by generating a resolved phylogeny for their evolutionary relationships that research can

begin to address questions about their biogeography and timing of evolutionary events through character mapping and phylogenetic dating. Having a well-resolved phylogeny will be important in insuring an evolution-based classification and helpful in elucidating meaningful morphological characters for use in identification, which is helpful to biologists, managers, and the public.

For over a century our ability to study and understand the 241 bat species in Vespertilioninae has been hampered by our lack of understanding of their evolutionary relationships. Considering their nearly worldwide distribution and great biodiversity, understanding evolutionary relationships of Vespertilioninae is paramount. From the standpoint of human health, bats of Vespertilioninae are implicated as hosts and vectors for disease such as rabies and other lyssaviruses, SARS-like coronaviruses, and Duvenhage virus (Cui et al. 2007; De Serres et al. 2008; Warrell and Warrell 2004; Wong et al. 2007). Therefore, understanding their evolutionary relationships will be important in understanding the evolution of these potentially threatening human diseases. As previously mentioned, vespertilionin bats also are of economic significance by controlling insect pests of crops, and ourselves, and provide guano for fertilizer and explosives. Aside from a utilitarian anthropocentric perspective, Vespertilioninae makes up a significant proportion of chiropteran biodiversity on the planet (22% of bat species). However, >40% of these taxa are listed on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (Table 1.1; Hutson et al. 2001; IUCN 2009) and many species are in need of conservation and management due to habitat loss, logging, cave disturbance, toxicants (especially agricultural and mine related; Clark 1981;

O'Shea et al. 2000, 2001), and diseases like the recent outbreak of white nose syndrome in northeastern North America (Blehert et al. 2009; Cohn 2008).

Modern comparative analysis, behavioral ecology, biogeography, conservation biology, community ecology, evolutionary ecology, functional ecology, life history, physiology, and even medical research all require a foundation based on systematics, the framework for all biological studies. Therefore, a resolved hypothesis for the evolution of taxa within Vespertilioninae is an essential foundation for further research, conservation, and management of this large, economically, and ecologically important group of mammals. The significance of this research includes foremost, a more resolved phylogenetic hypothesis for the evolutionary relationships of Vespertilioninae bats.

LITERATURE CITED

- ALLEN, G. M. 1967. Bats. Dover Publications, Inc., New York.
- ANDERSON, S., AND J. K. JONES, JR. 1967. Recent mammals of the world: a synopsis of families. Ronald Press Company, New York.
- AO, L., ET AL. 2006. Karyotype relationships of six bat species (Chiroptera, Vespertilionidae) from China revealed by chromosome painting and G-banding comparison. *Cytogenetic and Genome Research* 115:145–153.
- ÄRNBÄCK-CHRISTIE-LINDE, A. 1909. On intermediate forms among Chiroptera. *Zoologischer Anzeiger* 34:572–582.
- AVISE, J. C. 2004. Molecular markers, natural history, and evolution. 2nd ed. Sinauer Associates, Inc., Sunderland, Massachusetts.
- BAKER, R. J. 1970. Karyotypic trends in bats. Pp. 65–96 in *Biology of bats* (W. A. Wimsatt, ed.). Academic Press, New York.

- BAKER, R. J., AND J. L. PATTON. 1967. Karyotypes and karyotypic variation of North American vespertilionid bats. *Journal of Mammalogy* 48:270–286.
- BARCLAY, R. M., AND L. D. HARDER. 2003. Life histories of bats: life in the slow lane. Pp. 209–253 in *Bat ecology* (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.
- BICKHAM, J. W. 1979. Chromosomal variation and evolutionary relationships of vespertilionid bats. *Journal of Mammalogy* 60:350–363.
- BICKHAM, J. W. 1987. Chromosomal variation among seven species of lasiurine bats (Chiroptera: Vespertilionidae). *Journal of Mammalogy* 68:837–842.
- BLEHERT, D. S., ET AL. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227.
- BOGAN, M. A., P. M. CRYAN, E. W. VALDEZ, L. E. ELLISON, AND T. J. O’ SHEA. 2003. Western crevice and cavity-roosting bats. Pp. 69–77 in *Monitoring trends in bat populations of the United States and territories: problems and prospects* (T. J. O’Shea and M. A. Bogan, eds.). United States Geological Survey, Biological Resource Discipline, Information and Technology Report, USGS/BRD/ITR–2003–0003.
- BOGDANOWICZ, W., S. KASPER, AND R. D. OWEN. 1998. Phylogeny of plecotine bats: reevaluation of morphological and chromosomal data. *Journal of Mammalogy* 79:78–90.
- BREED, W. G., AND R. W. INNS. 1985. Variation in sperm morphology of Australian Vespertilionidae and its possible phylogenetic significance. *Mammalia* 49:105–108.

- BROWN, R. W. 1956. Composition of scientific words. Smithsonian Books, Washington D.C.
- BRUNET-ROSSINI, A., AND S. N. AUSTAD. 2004. Ageing studies on bats: a review. *Biogerontology* 5:211–222.
- CLARK, D. R., JR. 1981. Bats and environmental contaminants: a review. United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report – Wildlife 235:1–27.
- COHN, J. P. 2008. White-nose syndrome threatens bats. *BioScience* 58:1098.
- CUI, J., ET AL. 2007. Evolutionary relationships between bat coronaviruses and their hosts. *Emerging Infectious Diseases* 13:1526–1532.
- DE SERRES, G., F. DALLAIRE, M. CÔTE, AND D. M. SKOWRONSKI. 2008. Bat rabies in the United States and Canada from 1950 through 2007: human cases with and without bat contact. *Clinical Infectious Diseases* 46:1329–1337.
- ELLERMAN, J. R., AND T. C. S. MORRISON-SCOTT. 1951. Checklist of Palaearctic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London, United Kingdom.
- FEDYK, S., AND A. L. RUPRECHT. 1983. Chromosomes of some species of vespertilionid bats. II. Evolutionary relationships of plecotine bats. *Acta Theriologica* 28:171–182.
- FELSENSTEIN, J. 2004. Inferring phylogenies. Sinauer Associates, Inc., Sunderland, Massachusetts.
- FENTON, M. B. 1982. Echolocation, insect hearing, and feeding ecology of insectivorous bats. Pp. 261–285 in *Ecology of bats* (T. H. Kunz, ed.). Plenum Press, New York.

- FREEMAN, P. W. 1983. Bats: winged maestros of the night. University of Nebraska State Museum, Museum Notes 71:1–4.
- GERVAIS, F. L. P. 1855. Mammifères. Animaux nouveaux, ou rares, recueillis pendant l'expédition dans les parties centrales de l'Amérique du Sud. P. Bertrand, Paris, France.
- GOUDY-TRAINOR, A., AND P. W. FREEMAN. 2002. Call parameters and facial features in bats: a surprising failure of form following function. *Acta Chiropterologica* 4:1–16.
- GRAY, J. E. 1821. On the natural arrangement of vertebrate animals. *London Medical Repository, Monthly Journal, and Review* 15:296–310.
- GRAY, J. E. 1866. Synopsis of the genera of Vespertilionidae and Noctilionidae. *Annals and Magazine of Natural History (ser.3)* 17:89–93.
- HAMILTON, B., AND D. T. STALLING. 1972. *Lasiurus borealis* with five young. *Journal of Mammalogy* 53:190.
- HELLER, K. -G. AND M. VOLLETH. 1984. Taxonomic position of "*Pipistrellus societatis*" Hill, 1972 and the karyological characteristics of the genus *Eptesicus* (Chiroptera: Vespertilionidae). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 22:65–77.
- HILL, J. E., AND D. L. HARRISON. 1987. The baculum in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. *Bulletin of the British Museum of Natural History (Zoology)* 52:225–305.

- HILL, J. E., AND J. D. SMITH. 1984. Bats: a natural history. University of Texas Press, Austin, Texas.
- HILL, J. E., AND G. TOPÁL. 1973. The affinities of *Pipistrellus ridleyi* Thomas, 1898 and *Glischropus rosseti* Oey, 1951 (Chiroptera: Vespertilionidae). Bulletin of the British Museum of Natural History (Zoology) 24:447–454.
- HOOFER, S. R., S. A. REEDER, E. W. HANSEN, AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics and taxonomic review of noctilionoid and vespertilionoid bats (Chiroptera: Yangochiroptera). Journal of Mammalogy 84:809–821.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2001. Phylogenetic relationships of plecotine bats and allies based on mitochondrial ribosomal sequences. Journal of Mammalogy 82:131–137.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropterologica 5(supplement):1–63.
- HORÁČEK, I., AND J. ZIMA. 1978. Specificity of selection pressure as the factor causing parallel evolution in bats. Pp. 115–123 in Natural selection: proceedings of the international symposium, Liblice, Czechoslovakia, June 5–9, 1978 (V. J. A. Novák, V. V. Leonovich, and B. Pacltová, eds.). Czechoslovak Academy of Sciences, Prague, Czechoslovakia.
- HUTSON, A. M., S. P. MICKLEBURGH, AND P. A. RACEY. 2001. Microchiropteran bats: global status and conservation action plan. IUCN/SSC Chiroptera Specialist Group, International Union for Conservation of Nature and Natural Resources, Cambridge, United Kingdom.

- IUCN 2009. IUCN Red List of Threatened Species. Version 2009.1. Online at <http://www.iucnredlist.org>; accessed 13 July 2009.
- JOHNSTON, D. S., AND M. B. FENTON. 2001. Individual and population-level variability in diets of pallid bats (*Antrozous pallidus*). *Journal of Mammalogy* 82:362–373.
- JONES, K. E., A. PURVIS, A. MACLARNON, O. R. P. BININDA-EMONDS, AND N. B. SIMMONS. 2002. A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biological Reviews* 77:223–259.
- KALKA, M. B., A. R. SMITH, AND E. K. V. KALKO. 2008. Bats limit arthropods and herbivory in a tropical forest. *Science* 320:71.
- KAWAI, K., ET AL. 2002. Intra- and Interfamily relationships of Vespertilionidae inferred by various molecular markers including SINE insertion data. *Journal of Molecular Evolution* 55:284–301.
- KEARNEY, T. C., M. VOLLETH, G. CONTRAFATTO, AND P. J. TAYLOR. 2002. Systematic implications of chromosome GTG-band and bacula morphology for southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: Vespertilionidae). *Acta Chiropterologica* 4:55–76.
- KEELEY, B. W., AND M. D. TUTTLE. 1999. Bats in American bridges. Bat Conservation International, Inc. Resource Publication 4:1–41.
- KOEPFLI, K., AND R. K. WAYNE. 2003. Type I STS markers are more informative than cytochrome b in phylogenetic reconstruction of the Mustelidae (Mammalia: Carnivora). *Systematic Biology* 52:571–593.

- KOOPMAN, K. F. 1970. Zoogeography of bats. Pp. 29–50 in About bats: a chiropteran biology symposium (B. H. Slaughter and D. W. Walton, eds.). Southern Methodist University Press, Dallas, Texas.
- KOOPMAN, K. F. 1975. Bats of Sudan. Bulletin of the American Museum of Natural History 154:353–444.
- KOOPMAN, K. F. 1984. A synopsis of the families of bats - part VII. Bat Research News 25:25–27.
- KOOPMAN, K. F. 1993. Order Chiroptera. Pp.137–241 in Mammal species of the world: a taxonomic and geographic reference, 2nd ed. (D. E. Wilson and D. M. Reeder, eds.). Smithsonian Institution Press, Washington D.C.
- KOOPMAN, K. F. 1994. Chiroptera: systematics. Handbook of zoology: a natural history of the phyla of the animal kingdom. Walter de Gruyter, Berlin, Germany. 8(60):1–217.
- KOOPMAN, K. F., and J. K. Jones, Jr. 1970. Classification of bats. Pp. 22–28 in About bats: a chiropteran biology symposium (B. H. Slaughter and D. W. Walton, eds.). Southern Methodist University Press, Dallas, Texas.
- KUNZ, T. H. 1982. Roosting ecology. Pp. 1–55 in Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York.
- KUNZ, T. H., AND L. F. LUMSDEN. 2003. Ecology of cavity and foliage roosting bats. Pp. 209–253 in Bat ecology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.
- KUNZ, T. H., AND D. S. REYNOLDS. 2003. Bat colonies in buildings. Pp. 91–102 in Monitoring trends in bat populations of the United States and territories: problems

and prospects (T. J. O'Shea and M. A. Bogan, eds.). United States Geological Survey, Biological Resource Discipline, Information and Technology Report, USGS/BRD/ITR-2003-0003.

- LACK, J. B., Z. P. ROEHRS, C. E. STANLEY, JR., M. RUEDI, AND R. A. VAN DEN BUSSCHE. 2009. Molecular phylogenetics of *Myotis* suggest familial-level divergence for the genus *Cistugo* (Chiroptera). *Journal of Mammalogy*. *in prep.*
- LANZA, B. 1969. The baculum of *Pteropus* and its significance for the phylogenesis of the genus (Mammalia, Megachiroptera). *Italian Journal of Zoology* 3:37-68.
- LENIEC, H., S. FEDYK, AND A. L. RUPRECHT. 1987. Chromosomes of some species of vespertilionid bats. IV. New data on the plecotine bats. *Acta Theriologica* 32:307-314.
- LEWIS, S. E. 1995. Roost fidelity of bats: a review. *Journal of Mammalogy* 76:481-496.
- LINNAEUS, C. 1735. *Systema naturae, sive regna tria naturae systematice proposita per classes, ordines, genera, and species*. Theodorum Haak, Leiden, Netherlands.
- LINNAEUS, C. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. 10th ed. Laurentii Salvii, Stockholm, Sweden 1:1-824.
- MARTIN, R. L. 1978. The baculum of some bats of Thailand with comments on taxonomic utility and adaptive value. Pp. 199-204 in *Proceedings of the fourth international bat research conference*. Nairobi, Kenya (R. J. Olembo, J. B. Castelino, and F. A. Mutere, eds.). Kenya Academy for Advancement of Arts and Sciences, Kenya Literature Bureau, Nairobi, Kenya.

- MATTHEE, C. A., J. D. BURZLAFF, J. F. TAYLOR, AND S. K. DAVIS. 2001. Mining the mammalian genome for Artiodactyl systematics. *Systematic Biology* 50:367–390.
- MAYER, F., AND O. VON HELVERSEN. 2001. Cryptic diversity in European bats. *Proceedings of the Royal Society B: Biological Sciences* 268:1825–1832.
- MAYER, F., C. DIETZ, AND A. KIEFER. 2007. Molecular species identification boosts bat diversity. *Frontiers in Zoology* 4:4. Online at <http://www.frontiersinzoology.com/content/4/1/4>; accessed 13 July 2009.
- MAYR, E. 1982. *The growth of biological thought*. Harvard University Press, Cambridge, Massachusetts.
- MCCRACKEN, G. F., AND G. S. WILKINSON. 2000. Bat mating systems. Pp. 321–362 in *Reproductive biology in bats* (E. G. Crichton and P. H. Krutzsch, eds.). Academic Press, London, United Kingdom.
- MCKENNA, M. C., AND S. K. BELL. 1997. *Classification of mammals: above the species level*. Columbia University Press, New York.
- MILLER, G. S., JR. 1897. Revision of the North American bats of the family Vespertilionidae. *North American Fauna* 13:1–135.
- MILLER, G. S., JR. 1907. The families and genera of bats. *Bulletin of the United States National Museum* 57:1–282.
- MILLER-BUTTERWORTH, C. M., W. J. MURPHY, S. J. O'BRIEN, D. S. JACOBS, M. S. SPRINGER, AND E. C. TEELING. 2007. A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, *Miniopterus*. *Molecular Biology and Evolution* 24:1553–1561.

- NELSON, G., AND N. PLATNICK. 1981. Systematics and biogeography: cladistics and vicariance. Columbia University Press, New York.
- NEUWEILER, G. 1989. Foraging ecology and audition in echolocating bats. *Trends in Ecology and Evolution* 4:160–166.
- NEUWEILER, G. 1990. Auditory adaptations for prey capture in echolocating bats. *Physiological Reviews* 70:615–641.
- NORBERG, U. M., AND J. M. V. RAYNER. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 316:335–427.
- NOWAK, R. M. 1994. Walker's bats of the world. Johns Hopkins University Press, Baltimore, Maryland.
- NOWAK, R. M. 1999. Walker's mammals of the world, 6th ed. Johns Hopkins University Press, Baltimore, Maryland.
- O'SHEA, T. J., D. R. CLARK, JR., AND T. P. BOYLE. 2000. Impacts of mine-related contaminants on bats. Pp. 205–215 in *Proceedings of bat conservation and mining: a technical interactive forum, November 14–16, 2000, St. Louis, Missouri* (K. C. Voories and D. Throgmorton, eds.). United States Department of Interior, Office of Surface Mining, Alton, Illinois.
- O'SHEA, T. J., A. L. EVERETTE, AND L. E. ELLISON. 2001. Cyclodiene insecticide, DDE, DDT, arsenic and mercury contamination of big brown bats (*Eptesicus fuscus*) foraging at a Colorado Superfund site. *Archives of Environmental Contamination and Toxicology* 40:112–120.

- PETERS, W. C. H. 1865. Über die zu den Vampyri gehörigen Flederthiere und über die natürliche Stellung der Gattung *Antrozous*. Pp. 503–524 in Monatsberichte der Königlichen Preusschen Akademie der Wissenschaften, Berlin, Germany.
- RACEY, P. A. 1982. Ecology of bat reproduction. Pp. 57–104 in Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York.
- RAUTENBACH, I. L., G. N. BRONNER, AND D. A. SCHLITTER. 1993. Karyotypic data and attendant systematic implication of the bats of southern Africa. *Koedoe* 36:87–104.
- ROSEVEAR, D. R. 1962. A review of some African species of *Eptesicus* Rafinesque. *Mammalia* 26:457–477.
- SCHNITZLER, H. -U., AND E. K. V. KALKO. 2001. Echolocation by insect-eating bats. *BioScience* 51:557–569.
- SCHNITZLER, H. -U., C. F. MOSS, AND A. DENZINGER. 2003. From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology and Evolution* 18:386–394.
- SCHULZ, M. 2002. Bats in bird nests in Australia: a review. *Mammal Review* 28:69–76.
- SIMMONS, N. B. 1998. A reappraisal of interfamilial relationships of bats. Pp. 3–26 in *Bat biology and conservation* (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington D.C.
- SIMMONS, N. B. 2005. Order Chiroptera Pp. 312–529 in *Mammal species of the world: a taxonomic and geographic reference*, 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

- SIMMONS, N. B., AND J. H. GEISLER. 1998. Phylogenetic relationships of *Icaronycteris*, *Archaeonycteris*, *Hassianycteris* and *Palaeochiropteryx* to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bulletin of the American Museum of Natural History* 235:1–182.
- SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History* 85:i–xvi,1–350.
- SIMPSON, G. G. 1961. Principles of animal taxonomy. Columbia University Press, New York.
- SPRINGER, M. S., ET AL. 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. *Molecular Biology and Evolution* 18:132–143.
- STADELMANN, B., D. S. JACOBS, C. SCHOEMAN, AND M. RUEDI. 2004. Phylogeny of African *Myotis* bats (Chiroptera, Vespertilionidae) inferred from cytochrome b sequences. *Acta Chiropterologica* 6:177–92.
- STOCK, A. D. 1983. Chromosomal homologies and phylogenetic relationships of the vespertilionid bat genera *Euderma*, *Idionycteris*, and *Plecotus*. *Cytogenetics and Cell Genetics* 35:136–140.
- SUDMAN, P. D., L. J. BARKLEY, AND M. S. HAFNER. 1994. Familial affinity of *Tomopeas ravus* (Chiroptera) based on protein electrophoretic and cytochrome b sequence data. *Journal of Mammalogy* 75:365–377.

- TATE, H. H. 1942. Results of the Archbold expedition. No.47: review of the Vespertilioninae bats, with special attention to genera and species of the Archbold collection. Bulletin of the American Museum of Natural History 80:221–297.
- TEELING, E. C., M. S. SPRINGER, O. MADSEN, P. BATES, S. J. O'BRIEN, AND W. J. MURPHY. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307:580–584.
- TUTTLE, M. D., AND D. A. R. TAYLOR. 1998. Bats and mines. Bat Conservation International, Inc. Resource Publication 3:1–50.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2001. Evaluating monophyly of Nataloidea (Chiroptera) with mitochondrial DNA sequences. Journal of Mammalogy 82:320–327.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2004. Phylogenetic relationships among recent chiropteran families and the importance of choosing appropriate out-group taxa. Journal of Mammalogy 85:321–330.
- VAN DEN BUSSCHE, R. A., S. A. REEDER, E. W. HANSEN, AND S. R. HOOFER. 2003. Utility of the Dentin Matrix Protein 1 (DMP1) gene for resolving mammalian intraordinal phylogenetic relationships. Molecular Phylogenetics and Evolution 26:89–101.
- VOLLETH, M. 1985. Chromosomal homologies of the genera *Vespertilio*, *Plecotus*, and *Barbastella* (Chiroptera: Vespertilionidae). Genetica 66:231–236.
- VOLLETH, M. 1987. Differences in the location of nucleolus organizer regions in European vespertilionid bats. Cytogenetics and Cell Genetics 44:186–197.

- VOLLETH, M. 1992. Comparative analysis of the banded karyotypes of the European *Nyctalus* species (Vespertilionidae: Chiroptera). Pp. 231–236 in Prague studies in mammalogy: collection of papers dedicated to Prof. Dr. Vladimír Hanák on occasion of his 60th birthday (I. Horáček and V. Vohralík, eds.). Charles University Press, Prague, Czech Republic.
- VOLLETH, M., G. BRONNER, M. C. GOPFERT, K. G. HELLER, O. VON HELVERSEN, AND H. S. YOUNG. 2001. Karyotype comparison and phylogenetic relationships of *Pipistrellus*-like bats (Vespertilionidae; Chiroptera; Mammalia). *Chromosome Research* 9:25–46.
- VOLLETH, M., AND K. G. HELLER. 1994a. Karyosystematics of plecotine bats: a reevaluation of chromosomal data. *Journal of Mammalogy* 75:416–419.
- VOLLETH, M., AND K. G. HELLER. 1994b. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 32:11–34.
- VOLLETH, M., K. G. HELLER, AND J. FAHR. 2006. Phylogenetic relationships of three “Nycticeiini” genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. *Zeitschrift für Säugetierkunde* 71:1–12.
- VOLLETH, M., AND C. R. TIDEMANN. 1989. Chromosome studies in three genera of Australian vespertilionid bats. *Zeitschrift für Säugetierkunde* 54:215–222.
- VOLLETH, M., AND C. R. TIDEMANN. 1991. The origin of the Australian Vespertilioninae bats, as indicated by chromosomal studies. *Zeitschrift für Säugetierkunde* 56:321–330.

- WARRELL, M. J., AND D. A. WARRELL. 2004. Rabies and other lyssavirus diseases. *Lancet* 363:959–969.
- WHITAKER, J. O., JR. 1995. Food of the big brown bat *Eptesicus fuscus* from maternity colonies in Indiana and Illinois. *American Midland Naturalist* 134:346–360.
- WILSON, D. E., AND D. M. REEDER. 2005. Mammal species of the world: a taxonomic and geographic reference, 3rd ed. Johns Hopkins University Press, Baltimore, Maryland.
- WONG, S., S. LAU, P. WOO, AND K. -Y. TUEN. 2007. Bats as a continuing source of emerging infections in humans. *Reviews in Medical Virology* 17:67–91.
- ZIMA, J. 1979. Chromosomal homology in the complements of bats of the family Vespertilionidae. I. Karyometric analysis of chromosomal complements of 20 species. *Folia Zoologica* 28:329–336.
- ZIMA, J. 1982. Chromosomal homology in the complements of bats of the family Vespertilionidae. II. G-band karyotypes of some *Myotis*, *Eptesicus* and *Pipistrellus* species. *Folia Zoologica* 31:31–36.
- ZIMA, J., AND I. HORÁČEK. 1985. Synopsis of karyotypes of vespertilionid bats (Mammalia: Chiroptera). *Acta Universitatis Carolinae - Biologica* 1981:311–329.

Table 1.1.—Data from the International Union for Conservation of Nature and Natural Resources (IUCN) Red List from 2 sources: Hutson et al. 2001 and 2008 Red List from <http://www.iucnredlist.org/>. Asterisks (*) indicate percentage of 241 recognized species in Vespertilionidae (Simmons 2005 excluding *Myotis* and *Cistugo*).

Category	2001	2008
Extinct	2	2
Critically Endangered	4	4
Endangered	8	8
Threatened	0	3
Vulnerable	28	11
Near Threatened	45	11
Data Deficient	17	60
Total	104	99
Percent*	43%	41%

CHAPTER II

ELUCIDATING TRIBAL PHYLOGENETIC RELATIONSHIPS WITHIN VESPERTILIONINAE (CHIROPTERA: VESPERTILIONIDAE) BASED ON MITOCHONDRIAL AND NUCLEAR SEQUENCE DATA

ABSTRACT – 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 113 taxa were sequenced and analyzed using maximum parsimony and Bayesian phylogenetic procedures to construct gene trees and test 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 Perimyotinae group and the Hypsuginae group. Tree topologies indicate a Nycticeiini / Eptesicini group, but it is not supported. The heuristically pleasing tribe Plecotini also is unresolved in these gene trees. These results provided further support and greater resolution for previously proposed hypotheses of Vespertilioninae evolution based on mtDNA 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.

INTRODUCTION

Understanding the evolutionary relationships within the subfamily Vespertilioninae (Mammalia: Chiroptera: Vespertilionidae) has been difficult for systematists due to 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) and differences of opinion exist regarding the significance any 1 of these characters should be granted in relation to the divergence of these taxa (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, anterior upper premolar, pelage color) has led to classifications incongruent with evolutionary history within Vespertilioninae (Ärnback-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; e.g., Dobson 1875, 1878; Gill 1885; Gray 1821, 1866; etc.) 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 2.1). This is in stark contrast to Simpson (1945) who rejected a tribal classification rank for Vespertilioninae and synonymized many genera. Most authors since these classic works have followed Tate’s (1942) classification 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). While more recent studies based on bacular morphology and cytogenetics have provided insight into evolutionary relationships of Vespertilioninae, many relationships remain unresolved, many taxa remain unstudied, and some of these findings contradict previous hypotheses about Vespertilioninae evolution (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 (Hofer and Van Den Bussche 2003; Lack et al. 2009; 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), Myotini (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 have been accepted or discredited to varying degrees and their exact rank,

position, circumscription, and composition is of continuing debate (Table 2.1; for a review of this literature see Hill and Harrison 1987; Hooper and Van Den Bussche 2003).

With the development of modern techniques in PCR, DNA sequencing, and molecular data analysis, researchers are reevaluating phylogenetic 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; Hooper and Van Den Bussche 2001; Hooper et al. 2003, 2006; Lack et al. 2009; 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 Hooper and Van Den Bussche (2003) used 2.6 kilobases (kb) 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 (Lack et al. 2009), and subsequent lack of genetic resolution have left our understanding of evolutionary relationships and hence, the taxonomy of 241 bats 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 test the validity of previously proposed tribes (Antrozoini, Eptesicini, Lasiurini, Nycticeiini, Nyctophilini, Pipistrellini, Plecotini, Scotophilini, Vespertilionini) within Vespertilioninae. Production of a resolved and supported phylogeny for Vespertilioninae would enhance our understanding of the evolution of 1 of the most taxonomically diverse, geographically widespread, and ecologically successful groups of mammals and would further our abilities to answer important ecological, evolutionary, and biogeographical questions.

MATERIALS AND METHODS

Taxonomic sampling.—Included in this study are samples from 32 (73%) of the 44 currently recognized genera and 80 (33%) of the 241 species within Vespertilioninae, as well as 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 Murinae 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, Field Museum of Natural History, Durban Natural Science Museum, Indiana State University Vertebrate Collection, Musèum d’Histoire Naturelle de Genève, Museum of Texas Tech University, Museum of Southwestern Biology at the University of New Mexico, 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, University of Lausanne, Institut de Zoologie et d'Ecologie Animale, and the Universidad Nacional Autónoma de Mexico City (Appendix I). Identifications of many specimens were verified by Steven R. Hooper (Hooper and Van Den Bussche 2003) and Manuel Ruedi (pers. comm.); otherwise, I 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 113 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; See Table 2.2 for primer sequence and citations). 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). PCR amplifications were conducted using 200–500 ng of DNA, 1 unit of Taq polymerase, 0.14 mM of each deoxynucleoside triphosphate, 5 μ L of 10X buffer, 3.5 mM MgCl₂, 0.8 mg/mL of bovine serum albumin, and 0.15 μ L of each primer in a 30 μ L total volume reaction. The general PCR 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 (See Table 2.2 for individual primer annealing temperatures). Amplification ended with a final elongation at 72°C for 10 min to ensure all reactions were completed.

PCR 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).

Sequence data were largely generated in the Van Den Bussche laboratory at Oklahoma State University, including previously published mitochondrial ribosomal DNA (mtDNA; comprising 12S rRNA, tRNA^{Val}, and 16S rRNA) for 102 individuals, DMP1 for 3 individuals, and RAG2 for 6 individuals (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003; Lack et al. 2009; Van Den Bussche and Hooper 2000, 2001; Van Den Bussche et al. 2003). Amplifications and sequencing of the mtDNA gene regions were conducted for 20 additional individuals using primers and methods outlined in Van Den Bussche and Hooper (2000). Sequence data for the nuclear DNA (nDNA) also were supplemented with sequences of PRKCI, STAT5A, and THY for 4 individuals published by Eick et al. (2005) and deposited on 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). Lutzoni et al. (2000) procedures for identifying ambiguous sites in sequence data were followed and regions appearing to violate assumptions of positional homology were excluded from phylogenetic analyses. The mtDNA and each of the nDNA gene regions were

independently analyzed using maximum parsimony (MP) in PAUP* v4.0b10 (Swofford 2002) and Bayesian phylogenetic methods in MRBAYES v3.1.2 (Huelsenbeck and Ronquist 2001). An unweighted nucleotide substitution model and a heuristic search with 25 random additions of taxa, a Tree-Bisection-Reconnection branch exchanging algorithm, and 1,000 bootstrap replicates were used as parameters for MP analysis. Bayesian analysis employed a 4 chain (3 hot, 1 cold) parallel Metropolis-coupled Markov chain Monte Carlo which was run for 2×10^6 generations, with sampling every 10 generations, and a 0.02 temperature. 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 generations and locating the region at which model parameters and tree scores stabilized. Nodes in the resulting gene trees were considered supported if they had $\geq 70\%$ MP bootstrap support or ≥ 0.95 Bayesian posterior probabilities.

To examine possible incongruencies between gene regions and evaluate appropriateness of combining gene regions, resulting gene trees were examined using concordance methods of De Queiroz (1993). Previous research using these same sequences documented no significant incongruencies for the 3 mtDNA gene regions and therefore, they were not tested using these procedures (Hofer and Van Den Bussche 2003; Van Den Bussche and Hofer 2000). Based on results of these concordance tests (described in the results section), data were partitioned into mtDNA, nDNA and combined (mtDNA + nDNA) datasets for MP and Bayesian phylogenetic analysis. Finally, the program TREEPUZZLE 5.2 (Strimmer and von Haeseler 1997) was used to conduct likelihood-mapping with a GTR + I + Γ rate model to examine the phylogenetic potential of each independent gene region and combined data partitions.

RESULTS

Independent gene regions and concordance.—The nuclear gene regions analyzed were relatively short (280–1240 bp; Table 2.3) 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 (Fig. 2.1; Strimmer and von Haeseler 1997). The mtDNA, nDNA and combined datasets showed the same trend, but the slope was less positive. Analysis of each of nDNA gene region independently and comparison of these gene trees (not shown) following methods of De Queiroz (1993) provided a high level of 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 PK. The combined dataset was analyzed twice, once excluding APOB and once excluding PK, 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, the independent nDNA gene regions were concatenated for further analysis.

mtDNA sequences.—New ribosomal mtDNA sequence data were generated for 11 taxa: 3 individuals of *Eptesicus macrotus*, and 1 individual of *Arielulus aureocollaris*, *E. magellanicus*, *E. serotinus*, *Lasiurus intermedius*, *Pipistrellus hesperidus*, *P. paterculus*, *P. pipistrellus*, and *Tylonycteris robustula*, which supplemented 102 mtDNA sequences previously generated (Hooper and Van Den Bussche 2003; Lack et al. 2009). These 113 sequences were aligned to provide 2,940 aligned positions, of which 967 were excluded prior to analysis for potential violation of positional homology (Table 2.3). Of the

remaining 1,973 positions, 871 were variable and 697 were phylogenetically informative. MP analysis resulted in 300 parsimonious trees (pre-set max) of 5,905 steps, with 64 supported clades (bootstrap values $\geq 70\%$; Fig. 2.2), a consistency index excluding uninformative characters (CI) of 0.2207, and a retention index (RI) of 0.5679. Bayesian analysis had a burn-in value of 23,340 generations and resulted in 71 supported clades (≥ 0.95 posterior probability; Fig. 2.2).

nDNA sequences.—Sequence data for the concatenated nDNA partition were generated for 113 taxa, of which 18 are missing ≥ 1 gene region (13–23% of nDNA dataset). *Vespadelus vulturinus* was missing the most data without the APOB or DMP1 gene regions, and *Baeodon alleni* was missing the least with approximately the last 470 base pairs of RAG2 missing. In most cases, missing data were from the STAT5A gene region which, proved to be the most difficult to amplify and were not generated for the following 16 taxa: *Eptesicus magellanicus*, *Glauconycteris beatrix*, *G. egeria*, *Hypsugo cadornae*, *H. savii*, *Nyctalus leisleri*, *N. noctula*, *P. coromandra*, *P. hesperidus*, *P. javanicus*, *P. nathusii*, *P. tenuis*, *Scotoecus hirundo*, *T. pachypus*, *T. robustula*, and *Vespertilio murinus*. No changes in clade support or topological resolution were observed when the dataset was analyzed excluding STAT5A (data not shown).

Concatenated alignment of the nDNA gene regions provided 5,570 aligned positions (Table 2.3). With the exclusion of 766 positions for possible violations of positional homology prior to analysis, the remaining 4,804 positions included 2,241 variable positions and 1,665 phylogenetically informative positions. The MP analysis resulted in 12 most parsimonious trees of 8,029 steps, 78 supported clades (bootstrap values $\geq 70\%$), and a CI of 0.4556 and a RI of 0.7161, excluding uninformative characters

(Fig. 2.3). The majority of differences between the 12 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 minus *Nycticeius*). Finally, some variation in topologies was attributed to variability within the genus *Scotophilus*. A burn-in value of 48,320 generations was used for the Bayesian analysis which resulted in a tree with 84 clades supported by posterior probabilities ≥ 0.95 (Fig. 2.3).

Combined sequences.—More than 90% of clades were in concordance between mtDNA and nDNA gene trees and those datasets were concatenated for the combined analysis (De Queiroz 1993). Despite this high level of concordance, there were 3 areas with supported discrepancies between the mtDNA and nDNA gene trees. 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 gene tree (Fig. 2.2) and *P. javanicus* in the nDNA gene tree (Fig. 2.3). The 2nd discrepancy concerned interrelationships of a well-supported clade including *E. dimissus*, *T. pachypus*, and *T. robustula*. In the mtDNA gene tree, the *Tylonycteris* taxa were sister with a basal *E. dimissus* (Fig. 2.2), whereas in the nDNA gene tree *E. dimissus* was sister to *T. robustula* and *T. pachypus* was basal to this clade (Fig. 2.3). The 3rd difference involved relationships within *Lasiurus*, which formed a well-supported clade in both analyses. Concatenation of the mtDNA and nDNA datasets resulted in 8,510 aligned positions. Due to possible violation of positional homology, 1,733 positions were excluded prior to analysis leaving 6,777 positions for phylogenetic

analysis (Table 2.3). Of those remaining positions, 3,112 were variable and 2,362 were phylogenetically informative. The MP analysis resulted in 14 most parsimonious trees, with 14,193 steps and 76 supported clades (bootstrap values $\geq 70\%$; Fig. 2.4). Excluding uninformative characters, the CI was 0.3335 and the RI was 0.6412. Differences among the 14 most parsimonious trees related to relationships among taxa in the New World *Myotis* and relationships among the multiple representatives of *Antrozous*. More in line with the focus of this study are the topological differences in the parsimonious trees related to the variable placement of *Euderma* within Plecotini, the variable position of *Baeodon*, either basal to Antrozoini or Scotophilini, and the variable position of Antrozoini, either sister to Plecotini or basal to *Pipistrellus*-like bats. For the Bayesian analysis, a burn-in of 41,840 generations was used and resulted in a tree with 87 supported clades (posterior probability ≥ 0.95 ; Fig. 2.4).

DISCUSSION

Elucidating evolutionary relationships within Vespertilioninae has historically 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 (Miller 1907; Tate 1942; 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. 2009; 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 elucidate all relationships within Vespertilioninae (Hill

and Harrison 1987; Volleth and Heller 1994b; Volleth et al. 2001, 2006). Recent molecular analyses (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003; Kawai et al. 2002; Mayer and von Helversen 2001; Mayer et al. 2007) have tested previous hypotheses with new informative characters using phylogenetic methods. Using ribosomal mtDNA sequence data, Hooper and Van Den Bussche (2003) completed 1 of the most comprehensive phylogenetic studies 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 base pairs of coding and non-coding gene regions from the nDNA genome were sequenced and combined in subsequent analyses with the previously sequenced mtDNA data to reevaluate previous hypotheses of the evolutionary relationships within Vespertilioninae.

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). I was unable to obtain tissue samples from either *Nyctophilus* or *Pharotis* (Nyctophilini *sensu* Koopman 1994; Simmons 2005) and therefore was unable to address phylogenetic affinities of these taxa. Myotini (Tate 1942) also could not be addressed because it is outside the objectives of this study.

Accumulating evidence of the affinities of *Myotis* to Kerivoulinae and Murinae has required removal of Myotini (excluding *Lasionycteris*) from Vespertilioninae and

elevation of *Myotis* to subfamily rank Myotinae (Hooper and Van Den Bussche 2003; Kawai et al. 2002; Lack et al. 2009; Stadelmann et al. 2004; Volleth and Heller 1994b).

With regard to the remaining 8 traditionally recognized tribes, the combined gene 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. 2.4). *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; Hooper and Van Den Bussche 2003; Koopman 1994; Miller 1907). Results from the combined analysis also support monophyly of Lasiurini (Fig. 2.4). The combined gene 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. 2.4). 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 (Hooper and Van Den Bussche 2003) and was rejected in this study by the combined mtDNA and nDNA gene regions (as well as by each independently; Fig. 2.4). Roehrs (2009: Chapter 3) discussed results of the combined mtDNA and nDNA phylogenetic analysis for tribes Nycticeiini and Scotophilini.

Antrozoini is the 3rd supported clade in the combined gene tree (Fig. 2.4). 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; see also 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 varying 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 2.6 kb of mtDNA (Hooper and Van Den Bussche 2003) or 11 kb of nDNA (Miller-Butterworth et al. 2007). Hooper and Van Den Bussche (2003) redefined Antrozoini by including *Rhogeessa* and *Baeodon* into the tribe. Their arrangement is largely supported by the combined gene tree with a monophyletic *Rhogeessa* sister to a *Antrozous-Bauerus* clade; however, the position of *Baeodon* was unresolved (Fig 2.4). As in Hooper and Van Den Bussche (2003), the mtDNA gene tree supports the inclusion of *Baeodon* in Antrozoini (Fig. 2.2), but topological results of the nDNA gene tree places *Baeodon* basal to the Scotophilini (Fig. 2.3). This relationship is not supported and it is

possible that the incomplete nDNA dataset 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. 2.4) and would constitute a new, yet to be named, tribe referred to here as the Perimyotine group. These results agree with Hooper and Van Den Bussche (2003) in placing these 2 taxa in their own genera, 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 *Pipistrellus*-like bats. Association of *Parastrellus* and *Perimyotis* into their own tribe initially seems difficult 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*, *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. 2.4). However, this tribal level Perimyotine group should be considered tentative until further research corroborates this relationship.

The last 2 supported tribes form a supported sister relationship in the combined gene tree and include most taxa historically considered *Pipistrellus*-like (Fig. 2.4). The 1st of these tribes is composed of *Nyctalus*, *Pipistrellus*, *Scotoecus*, *Tylonycteris*, and *Vespertilio*. Due to inclusion of *Vespertilio* in this tribe and *Vespertilio* having priority, the most appropriate name for this tribe is Vespertilionini. The sample of *E. dimissus* included in this study deserves comment because it was embedded in a supported *Tylonycteris* clade. There are 2 possible explanations for this unexpected position: 1) this specimen represents a misidentified *Tylonycteris* or 2) *E. dimissus* requires a position change to the genus *Tylonycteris*. This specimen was collected from Laos, which is outside of the currently known range of this species (Nepal; peninsular Thailand; Simmons 2005), therefore reevaluation of the identification of the voucher is necessary before systematic conclusions can be made. The other tribe consisted of *Chalinolobus*, *Hypsugo*, *Laephotis*, *Neoromicia*, *Nycticeinops*, and *Vespadelus*. This tribe is currently unnamed, but because *Hypsugo* has priority this, group will be referred to as the Hypsugine group. The intratribal relationships, congruence with historical classifications of these genera, and relationship to other character sets were discussed in detail in Roehrs (2009: Chapter 4).

Two other previously documented tribes, Nycticeiini and Plecotini, deserve mention. The combined gene tree presented here (Fig. 2.4) corroborates recent research (Hill and Harrison 1987; Hofer and Van Den Bussche 2003; Volleth et al. 2006) in rejecting Nycticeiini (*sensu* Tate 1942). These results, as well as a historical review of Nycticeiini systematics, were discussed in detail in Roehrs (2009: Chapter 3). However, with regard to Nycticeiini (*sensu* Hofer and Van Den Bussche 2003), the combined

analysis was in congruence topologically, but the clade was not supported (Fig. 2.4). This lack of support likely stems from a difference between the mtDNA and nDNA gene trees. The mtDNA gene tree from this study is in complete agreement with Hooper and Van Den Bussche (2003) with only the Bayesian analysis supporting Nycticeiini. The nDNA gene tree supports Nycticeiini with the exclusion of *Nycticeius* making this clade more appropriately named Eptesicini. As discussed by Roehrs (2009: Chapter 3), it is apparent that *Arielulus*, *Eptesicus* (including *Histiopus*), *Glauconycteris*, *Lasionycteris*, and *Scotomanes* form a tribal level clade, but more effort will be required to resolve the true 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 described by Gray (1866) as Plecotina (Table 2.1). Handley (1959) is responsible for establishing the core Plecotini genera recognized today: *Barbastella*, *Corynorhinus*, *Euderma*, *Idionycteris*, and *Plecotus*. Other taxa also have been included in Plecotini: *Baeodon*, *Nycticeius*, *Otonycteris*, *Rhogeessa*, *Nyctophilus*, and *Histiopus* (Fig. 2.1; 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 has only recently been explicitly tested (Hooper and Van Den Bussche 2003). Hooper 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 gene tree of this study (as well as the mtDNA and

nDNA gene trees) also was unable to resolve Plecotini leaving this tribe neither supported nor rejected (Fig. 2.4). These taxa may be some of the earliest divergences from Vespertilioninae ancestral stock and appear to have rapidly diverged, not allowing time for these gene regions to accumulate sufficient synapomorphic characters to elucidate their evolutionary histories (Lack et al. 2009). 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 of the evolution of these taxa.

Usefulness of nDNA and combined data.—The nuclear gene regions included in this study were individually relatively short (averaging ~800 bp), had fewer variable positions, and included even fewer potential phylogenetically informative positions (129–415 bp; Table 2.3). Because only 113 taxa were included in this study, for any 1 nDNA gene region, there were relatively few potentially informative positions per taxon, 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. 2.1; Strimmer and von Haeseler 1997). Furthermore, because it is difficult to predict whether a particular gene tree based on a particular gene region reflects true evolutionary relationships, most studies today use a suite of gene regions from multiple genomes to overcome potential problems with nonphylogenetic signals 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 gene regions have been included in a recent study of the phylogenetic relationships of Miniopteridae, *Cistugo*, Myotinae, Kerivoulinae, Murinae, and Vespertilioninae (Lack et al. 2009).

Although results presented here provide a more resolved hypothesis of Vespertilioninae evolutionary relationships 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 exclude taxa, genes, and possibly even codon positions that show rapid rates of evolution compared with the rest of the working dataset to reduce non-phylogenetic signals that suppress evolutionary signals present and decrease resolution, especially in these deep clades that show historic rapid 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: Robert J. Baker of the Museum of Texas Tech University; Nancy B. Simmons of the American Museum of Natural History; Bruce D. Patterson, Lawrence R. Heaney and William T. Stanley of the Field Museum of Natural History; Suzanne B. McLaren of the Carnegie Museum of Natural History; Mark D. Engstrom and Burton Lim of the Royal Ontario Museum; Manuel Ruedi of the Musèum d'Historie Naturelle de Genève and the University of Lausanne, Institut de Zoologie et d'Ecologie Animale; Peter J. Taylor of the Durban Natural Science Museum;

Janet K. Braun and Michael A. Mares of the Sam Noble Oklahoma Museum of Natural History; Terry L. Yates of the Museum of Southwestern Biology at the University of New Mexico; Rodney L. Honeycutt and Duane Schlitter of the Texas Cooperative Wildlife Collection at Texas A&M University; John O. Whitaker, Jr. and Dale S. Sparks of the Indiana State University Vertebrate Collection; Thomas E. Lee, Jr., of Abilene Christian University. I thank Justin B. Lack, Matt Leslie, Craig E. Stanley, Jr., and Sara W. Weyandt for generating a portion of the sequence data included in this project. Geeta Eick was very helpful in providing primers and advice on PCR profiles for the nuclear introns used in this study. Thanks to Steve Hartson, Janet Rogers, and Lisa Whitworth of the Oklahoma State University Recombinant DNA/Protein Core Facility who allowed me to use their equipment and assisted me in trouble-shooting. Janet K. Braun, Meredith J. Hamilton, David M. Leslie, Meredith A. Magnuson, Ronald J. Tylr, and Ronald A. Van Den Bussche reviewed drafts of this document and improved it in the process. 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.

LITERATURE CITED

AMRINE-MADSEN, H., K. -P. KOEPFLI, R. K. WAYNE, AND M. S. SPRINGER. 2003. A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. *Molecular Phylogenetics and Evolution* 28:225–240.

- AO, L., ET AL. 2006. Karyotype relationships of six bat species (Chiroptera, Vespertilionidae) from China revealed by chromosome painting and G-banding comparison. *Cytogenetic and Genome Research* 115:145–153.
- ÄRNBÄCK-CHRISTIE-LINDE, A. 1909. On intermediate forms among Chiroptera. *Zoologischer Anzeiger* 34:572–582.
- BAKER, R. J., S. R. HOOFER, C. A. PORTER, AND R. A. VAN DEN BUSSCHE. 2003. Diversification among New World leaf-nosed bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. *Occasional Papers, Museum of Texas Tech University* 230:1–32.
- BAKER, R. J., AND J. L. PATTON. 1967. Karyotypes and karyotypic variation of North American vespertilionid bats. *Journal of Mammalogy* 48:270–286.
- BAKER, R. J., C. A. PORTER, J. C. PATTON, AND R. A. VAN DEN BUSSCHE. 2000. Systematics of bats of the family Phyllostomidae based on RAG2 DNA sequences. *Occasional Papers, Museum of Texas Tech University* 202:1–16.
- BAURAIN, D., H. BRINKMANN, AND H. PHILIPPE. 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? *Molecular Biology and Evolution* 24:6–9.
- BICKHAM, J. W. 1979. Chromosomal variation and evolutionary relationships of vespertilionid bats. *Journal of Mammalogy* 60:350–363.
- BICKHAM, J. W. 1987. Chromosomal variation among seven species of lasiurine bats (Chiroptera: Vespertilionidae). *Journal of Mammalogy* 68:837–842.
- BICKHAM, J. W., J. C. PATTON, D. A. SCHLITTER, I. L. RAUTENBACH, AND R. L. HONEYCUTT. 2004. Molecular phylogenetics, karyotypic diversity, and partition

- of the genus *Myotis* (Chiroptera: Vespertilionidae). *Molecular Phylogenetics and Evolution* 33:333–338.
- BOGDANOWICZ, W., S. KASPER, AND R. D. OWEN. 1998. Phylogeny of plecotine bats: reevaluation of morphological and chromosomal data. *Journal of Mammalogy* 79:78–90.
- BRINKMANN, H., AND H. PHILIPPE. 2008. Animal phylogeny and large-scale sequencing: progress and pitfalls. *Journal of Systematics and Evolution* 46:274–286.
- DE QUEIROZ, A. 1993. For consensus (sometimes). *Systematic Biology* 42:368–372.
- DOBSON, G. E. 1875. Conspectus of the suborder, families and genera of Chiroptera arranged according to their natural affinities. *Annals and Magazine of Natural History (ser.4)* 16:345–357.
- DOBSON, G. E. 1878. Catalog of the Chiroptera in the collection of the British Museum. British Museum (Natural History), London, United Kingdom.
- EICK, G. N., D. S. JACOBS, AND C. A. MATTHEE. 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Molecular Biology and Evolution* 22:1869–1886.
- ELLERMAN, J. R., AND T. C. S. MORRISON-SCOTT. 1951. Checklist of Palaearctic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London, United Kingdom.
- ENGSTROM, M. D., AND D. E. WILSON. 1981. Systematics of *Antrozous dubiaquercus* (Chiroptera: Vespertilionidae), with comments on the status of *Bauerus* Van Gelder. *Annals of Carnegie Museum* 50:371–383.

- FROST, D. R., AND R. M. TIMM. 1992. Phylogeny of plecotine bats (Chiroptera: "Vespertilionidae"): proposal of a logically consistent taxonomy. *American Museum Novitates* 3034:1–16.
- GERVAIS, F. L. P. 1855. Mammifères. Animaux nouveaux, ou rares, recueillis pendant l'expédition dans les parties centrales de l'Amérique du Sud. P. Bertrand, Paris, France.
- GILL, T. N. 1885. Order IV. Chiroptera. Pp. 159–177 in *The standard natural history*. Vol. 5 (J. S. Kingsley, ed.). S.E. Cassino and Comany, Boston, Massachusetts.
- GRAY, J. E. 1821. On the natural arrangement of vertebrate animals. *London Medical Repository, Monthly Journal, and Review* 15:296–310.
- GRAY, J. E. 1831. Descriptions of some new genera and species of bats. *Zoological Miscellany* 1:37–38.
- GRAY, J. E. 1866. Synopsis of the genera of Vespertilionidae and Noctilionidae. *Annals and Magazine of Natural History (ser.3)* 17:89–93.
- GU, X. -M., S. -Y. HE, AND L. AO. 2008. Molecular phylogenetics among three families of bats (Chiroptera: Rhinolophidae, Hipposideridae, and Vespertilionidae) based on partial sequences of the mitochondrial 12S and 16S rRNA genes. *Zoological Studies* 47:368–378.
- HALL, E. R., AND J. K. JONES, JR. 1961. North American yellow bats, "*Dasypterus*," and a list of the named kinds of the genus *Lasiurus* Gray. University of Kansas Publications, Museum of Natural History 14:73–98.
- HAMILTON, W. J., JR. 1949. The bacula of some North American vespertilionid bats. *Journal of Mammalogy* 30:97–102.

- HANDLEY, C. O., JR. 1959. A revision of American bats of the genera *Euderma* and *Plecotus*. Proceedings of the United States National Museum 110:95–246.
- HANDLEY, C. O., JR. 1960. Descriptions of new bats from Panama. Proceedings of the United States National Museum 112:459–477.
- HELLER, K. -G., AND M. VOLLETH. 1984. Taxonomic position of “*Pipistrellus societatis*” Hill, 1972 and the karyological characteristics of the genus *Eptesicus* (Chiroptera: Vespertilionidae). Zeitschrift für Zoologische Systematik und Evolutionsforschung 22:65–77.
- HILL, J. E. 1966. The status of *Pipistrellus regulus* Thomas (Chiroptera, Vespertilionidae). Mammalia 30:302–307.
- HILL, J. E., AND D. L. HARRISON. 1987. The baculum in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. Bulletin of the British Museum of Natural History (Zoology) 52:225–305.
- HILL, J. E., AND G. TOPÁL. 1973. The affinities of *Pipistrellus ridleyi* Thomas, 1898 and *Glischropus rosseti* Oey, 1951 (Chiroptera: Vespertilionidae). Bulletin of the British Museum of Natural History (Zoology) 24:447–454.
- HOOFER, S. R., S. A. REEDER, E. W. HANSEN, AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics and taxonomic review of noctilionoid and vespertilionoid bats (Chiroptera: Yangochiroptera). Journal of Mammalogy 84:809–821.

- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2001. Phylogenetic relationships of plecotine bats and allies based on mitochondrial ribosomal sequences. *Journal of Mammalogy* 82:131–137.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica* 5(supplement):1–63.
- HOOFER, S. R., R. A. VAN DEN BUSSCHE, AND I. HORÁČEK. 2006. Generic status of the American pipistrelles (Vespertilionidae) with description of a new genus. *Journal of Mammalogy* 87:981–992.
- HORÁČEK, I., AND J. ZIMA. 1978. Specificity of selection pressure as the factor causing parallel evolution in bats. Pp. 115–123 in *Natural selection: proceedings of the international symposium, Liblice, Czechoslovakia, June 5–9, 1978* (V. J. A. Novák, V. V. Leonovich, and B. Pacltová, eds.). Czechoslovak Academy of Sciences, Prague, Czechoslovakia.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- JIANG, Z., C. PRIAT, AND F. GALIBERT. 1998. Traced orthologous amplified sequence tags (TOASTs) and mammalian comparative maps. *Mammalian Genome* 9:577–587.
- KAWAI, K., ET AL. 2002. Intra- and interfamily relationships of Vespertilionidae inferred by various molecular markers including SINE insertion data. *Journal of Molecular Evolution* 55:284–301.
- KOOPMAN, K. F. 1970. Zoogeography of bats. Pp. 29–50 in *About bats: a chiropteran biology symposium* (B. H. Slaughter and D. W. Walton, eds.). Southern Methodist University Press, Dallas, Texas.

- KOOPMAN, K. F. 1975. Bats of Sudan. *Bulletin of the American Museum of Natural History* 154:353–444.
- KOOPMAN, K. F. 1984. A synopsis of the families of bats - part VII. *Bat Research News* 25:25–27.
- KOOPMAN, K. F. 1994. Chiroptera: systematics. *Handbook of zoology: a natural history of the phyla of the animal kingdom*. Walter de Gruyter, Berlin, Germany. 8(60):1–217.
- KOOPMAN, K. F., AND J. K. JONES, JR. 1970. Classification of bats. Pp. 22–28 in *About bats: a chiropteran biology symposium* (B. H. Slaughter, and D. W. Walton, eds.). Southern Methodist University Press, Dallas, Texas.
- LACK, J. B., Z. P. ROEHRS, C. E. STANLEY, JR., M. RUEDI, AND R. A. VAN DEN BUSSCHE. 2009. Molecular phylogenetics of *Myotis* suggest familial-level divergence for the genus *Cistugo* (Chiroptera). *Journal of Mammalogy*. *in prep.*
- LENIEC, H., S. FEDYK, AND A. L. RUPRECHT. 1987. Chromosomes of some species of vespertilionid bats. IV. New data on the plecotine bats. *Acta Theriologica* 32:307–314.
- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers, Museum of Texas Tech University* 163:1–3.
- LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* 49:628–651.

- MADDISON, W. P., AND D. R. MADDISON. 2002. MacClade, v. 4.05. Sinauer Associates, Inc., Sunderland, Massachusetts.
- MATTHEE, C. A., J. D. BURZLAFF, J. F. TAYLOR, AND S. K. DAVIS. 2001. Mining the mammalian genome for artiodactyl systematics. *Systematic Biology* 50:367–390.
- MATTHEE, C. A., AND S. K. DAVIS. 2001. Molecular insight into the evolution of the family Bovidae: a nuclear DNA perspective. *Molecular Biology and Evolution* 18:1220–1230.
- MATTHEE, C. A., G. EICK, S. WILLOWS-MUNRO, C. MONTGELARD, A. T. PARDINI, AND T. J. ROBINSON. 2007. Indel evolution of mammalian introns and the utility of non-coding nuclear markers in eutherian phylogenetics. *Molecular Phylogenetics and Evolution* 42:827–837.
- MATTHEE, C. A., B. J. VAN VUUREN, D. BELL, AND T. J. ROBINSON. 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. *Systematic Biology* 53:433–447.
- MAYER, F., C. DIETZ, AND A. KIEFER. 2007. Molecular species identification boosts bat diversity. *Frontiers in Zoology* 4:DOI:10.1186/1742-9994-4-4.
- MAYER, F., AND O. VON HELVERSEN. 2001. Cryptic diversity in European bats. *Proceedings of the Royal Society B: Biological Sciences* 268:1825–1832.
- MCKENNA, M. C., AND S. K. BELL. 1997. *Classification of mammals: above the species level*. Columbia University Press, New York.
- MILLER, G. S., JR. 1897. Revision of the North American bats of the family Vespertilionidae. *North American Fauna* 13:1–136.

- MILLER, G. S., JR. 1907. The families and genera of bats. Bulletin of the United States National Museum 57:1–282.
- MILLER-BUTTERWORTH, C. M., W. J. MURPHY, S. J. O'BRIEN, D. S. JACOBS, M. S. SPRINGER, AND E. C. TEELING. 2007. A family matter: conclusive resolution of the taxonomic position of the long-fingered bats, *Miniopterus*. Molecular Biology and Evolution 24:1553–1561.
- MURPHY, W. J., E. EIZIRIK, W. E. JOHNSON, Y. P. ZHANG, O. A. RYDER, AND S. J. O'BRIEN. 2001. Molecular phylogenetics and the origins of placental mammals. Nature 409:614–618.
- PETERS, W. C. H. 1865. Über die zu den Vampyri gehörigen Flederthiere und über die natürliche Stellung der Gattung *Antrozous*. Pp. 503–524 in Monatsberichte der Königlichen Preusschen Akademie der Wissenschaften, Berlin, Germany.
- PHILIPPE, H., AND M. J. TELFORD. 2006. Large-scale sequencing and the new animal phylogeny. Trends in Ecology and Evolution 21:614–620.
- PINE, R. H., D. C. CARTER, AND R. K. LAVAL. 1971. Status of *Bauerus* Van Gelder and its relationships to other Nyctophilinae bats. Journal of Mammalogy 52:663–669.
- QUMSIYEH, M. B., AND J. W. BICKHAM. 1993. Chromosomes and relationships of long-eared bats of the genera *Plecotus* and *Otonycteris*. Journal of Mammalogy 74:376–382.
- RODRÍGUEZ-EZPELETA, N., H. BRINKMANN, B. ROURE, N. LARTILLOT, B. F. LANG, AND H. PHILIPPE. 2007. Detecting and overcoming systematic errors in genome-scale phylogenies. Systematic Biology 56:389–399.

- ROEHRS, Z. P. 2009. Vespertilioninae systematics: using mitochondrial and nuclear markers to elucidate phylogenetic relationships. Ph. D. dissertation, Oklahoma State University, Stillwater, Oklahoma.
- ROSEVEAR, D. R. 1962. A review of some African species of *Eptesicus* Rafinesque. *Mammalia* 26:457–477.
- RUEDAS, L. A., J. SALAZAR-BRAVO, J. W. DRAGOO, AND T. L. YATES. 2000. The importance of being earnest: what, if anything, constitutes a “specimen examined?” *Molecular Phylogenetics and Evolution* 17:129–132.
- RUEDI, M., AND F. MAYER. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Molecular Phylogenetics and Evolution* 21:436–448.
- SIMMONS, N. B. 1998. A reappraisal of interfamilial relationships of bats. Pp.3–26 in *Bat biology and conservation* (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington D.C.
- SIMMONS, N. B. 2005. Order Chiroptera Pp. 312–529 in *Mammal species of the world: a taxonomic and geographic reference*. 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.
- SIMMONS, N. B., AND J. H. GEISLER. 1998. Phylogenetic relationships of *Icaronycteris*, *Archaeonycteris*, *Hassianycteris* and *Palaeochiropteryx* to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bulletin of the American Museum of Natural History* 235:1–182.

- SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. Bulletin of the American Museum of Natural History 85:i-xvi,1-350.
- STADELMANN, B., D. S. JACOBS, C. SCHOEMAN, AND M. RUEDI. 2004. Phylogeny of African *Myotis* bats (Chiroptera, Vespertilionidae) inferred from cytochrome b sequences. Acta Chiropterologica 6:177-192.
- STADELMANN, B., L. -K. LIN, T. H. KUNZ, AND M. RUEDI. 2007. Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. Molecular Phylogenetics and Evolution 43:32-48.
- STRIMMER, K., AND A. VON HAESELER. 1997. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proceedings of the National Academy of Sciences 94:6815-6819.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods) 4.0 beta. Sinauer Associates, Inc., Sunderland, Massachusetts.
- TATE, G. H. H. 1941. Results of the Archbold expeditions. No. 40: notes on vespertilionid bats of the subfamilies Miniopterinae, Murinae, Kerivoulinae, and Nyctophilinae. Bulletin of the American Museum of Natural History 78:567-597.
- TATE, G. H. H. 1942. Results of the Archbold expeditions. No. 47: review of the Vespertilioninae bats, with special attention to genera and species of the Archbold collections. Bulletin of the American Museum of Natural History 80:221-296.
- THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence

- weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
- TOYOSAWA, S., C. O'HUIGIN, AND J. KLEIN. 1999. The Dentin Matrix Protein 1 gene of prototherian and metatherian mammals. *Journal of Molecular Evolution* 48:160–167.
- TUMLISON, R., AND M. E. DOUGLAS. 1992. Parsimony analysis and the phylogeny of the plecotine bats (Chiroptera: Vespertilionidae). *Journal of Mammalogy* 73:276–285.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2000. Further evidence for inclusion of the New Zealand short-tailed bat (*Mystacina tuberculata*) within Noctilionoidea. *Journal of Mammalogy* 81:865–874.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2001. Evaluating monophyly of Nataloidea (Chiroptera) with mitochondrial DNA sequences. *Journal of Mammalogy* 82:320–327.
- VAN DEN BUSSCHE, R. A., S. A. REEDER, E. W. HANSEN, AND S. R. HOOFER. 2003. Utility of the Dentin Matrix Protein 1 (DMP1) gene for resolving mammalian intraordinal phylogenetic relationships. *Molecular Phylogenetics and Evolution* 26:89–101.
- VOLLETH, M., AND K. G. HELLER. 1994a. Karyosystematics of plecotine bats: a reevaluation of chromosomal data. *Journal of Mammalogy* 75:416–419.
- VOLLETH, M., AND K. G. HELLER. 1994b. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 32:11–34.

- VOLLETH, M., G. BRONNER, M. C. GOPFERT, K. G. HELLER, O. VON HELVERSEN, AND H. S. YOUNG. 2001. Karyotype comparison and phylogenetic relationships of *Pipistrellus*-like bats (Vespertilionidae; Chiroptera; Mammalia). *Chromosome Research* 9:25–46.
- VOLLETH, M., K. G. HELLER, AND J. FAHR. 2006. Phylogenetic relationships of three “Nycticeiini” genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. *Zeitschrift für Säugetierkunde* 71:1–12.
- WALLIN, L. 1969. The Japanese bat fauna: a comparative study of chorology, species diversity and ecological differentiation. *Zoologiska Bidrag* 37:223–440.
- ZIMA, J., AND I. HORÁČEK. 1985. Synopsis of karyotypes of vespertilionid bats (Mammalia: Chiroptera). *Acta Universitatis Carolinae - Biologica* 1981:311–329.

Table 2.1.—Historic classifications of Vespertilioninae. Superscript "a" denotes that this arrangement can be found in Koopman (1984) and Koopman (1994), but the latter provides the most information and is the basis for depicted classification. Superscript "b" denotes a combination of results taken from Heller and Volleth (1984), Kearney et al. (2002), Volleth and Heller (1994), Volleth and Tidemann (1991), and Volleth et al. (2001), with most recent papers taking precedence. Taxa marked with: asterisks (*) are currently recognized taxa that would have been synonyms in authors taxonomic system; † denotes these taxa as *incertae sedis*.

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

Table 2.1.—Continued.

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

Table 2.2.—Information for the 6 nuclear primers used in this study, with primer sequence, annealing temperatures used, and citations to original primer description. Abbreviations: F refers to forward; R to reverse primers; and Ex refers to external; In to internal primers.

Locus	Primer Name	Primer Sequence (5' to 3')	Annealing Temperature (°C)	Citation	
APOB	APOB (F)	GGCTGGACAGTGAAATATTATGAAC	53 – 58	Jiang et al. 1998	
	APOB (R)	AATCAGAGAGTTGGTCTGAAAAAT		Jiang et al. 1998	
DMP1	Den12 (Ex - F)	GATGAAGACGACAGTGGAGATGACACCTT	51 – 55	Toyosawa et al. 1999	
	Den2 (Ex - R)	ATCTTGGCAATCATTGTCATC		Toyosawa et al. 1999	
	Den2a (Ex - F)	GACACCTTTGGTGATGA		Van Den Bussche et al. 2003	
	Den10 (Ex - R)	GTTGCTCTCTTGATTTGCTGTC		Van Den Bussche et al. 2003	
	DenA (In - F)	TGCARAGYGAYGATCCAGACAC		Van Den Bussche et al. 2003	
	DenB (In - R)	TGATTCTCTTGATTTGACACTGG		Van Den Bussche et al. 2003	
	DenC (In - F)	ACCTCCAGTCACTCAGAAG		Van Den Bussche et al. 2003	
	DenD (In - R)	GGATNTGCTTTCWGAACCTGRAGG		Van Den Bussche et al. 2003	
PRKCI	BatPKa (F)	CTTGTCATGATGATGAGG	40 – 45	Eick et al. 2005	
	BatPKb (R)	CCTATTTTAAAATATGAAAGAAATC		Eick et al. 2005	
	RabbitPKa (F)	AAACAGATCGCATTTATGCAAT		Matthee et al. 2004	
	RabbitPKb (R)	TGTCTGTACCCAGTCAATATC		Matthee et al. 2004	
RAG2	F1 (Ex - F)	GGCYGGCCCAARAGATCCTG	53 – 61	Baker et al. 2000	
	F1Int (In - F)	GRACAGTCGAGGGAARAGCATGG		Baker et al. 2000	
	F2 (In - F)	TTTGTATTGTTGGTGGCTATCAG		Baker et al. 2000	
	F2Int (In - F)	GGAYTCCACTCCCTTTGAAGA		Baker et al. 2000	
	R1 (In - R)	AACYTGYTTATTGTCTCCTGGTATGC		Baker et al. 2000	
	R1Int (In - R)	GGGGCAGGCASTCAGCTAC		Baker et al. 2000	
	R2 (Ex - R)	GRAAGGATTTCTTGGCAGGAGT		Baker et al. 2000	
	R2Int (In - R)	GCAGCAWGTAAATCCAGTAGC		Baker et al. 2000	
	Myotis179F (Ex - F)	CAGTTTTCTCTAAGGAYTCCTGC		52 – 54	Stadelmann et al. 2007
	Myotis428F (In - F)	ATGTGGTATATAGTCGAGGGAAGAGC			Stadelmann et al. 2007
	Myotis968R (In - R)	CCCATGTTGCTTCCAACCATATA	Stadelmann et al. 2007		
	Myotis1458R (Ex - R)	TTGCTATCTTCACATGCTCATGTC	Stadelmann et al. 2007		
	STAT	BatSTATa (F)	CTGCTCATCAACAAGCCCGA	48 – 62	Eick et al. 2005
		BatSTATb (R)	GGCTTCAGGTTCCACAGGTTGC		Eick et al. 2005
ArtiSTATa (F)		GAAGAAACATCACAAAGCCCC	51 – 60	Matthee et al. 2001	
ArtiSTATb (R)		AGACCTCATCCTTGGGCC		Matthee et al. 2001	
THY	BatTHYa (F)	GGGTATGTAGTTCATCTTACTTC	42 – 59	Eick et al. 2005	
	BatTHYb (R)	GGCATCCTGGTATTTCTACAGTCTTG		Eick et al. 2005	
	RabbitTHYa (F)	CATCAACACCACCATCTGTGC	52 – 59	Matthee et al. 2004	
	RabbitTHYb (R)	CACTTGCCACACTTACAGCT		Matthee et al. 2004	

Table 2.3.—Characteristics of individual nDNA 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.

Marker	Number of Taxa	Aligned Positions	Excluded Positions	Analyzed Positions	Variable Positions	Phylogenetically Informative Positions	Percent Resolved ^A	Percent Unresolved ^B
APOB	112	282	0	282	173	129	72	28
DMP1	111	1023	33	990	520	339	89	11
RAG2	112	1239	0	1239	570	415	87	13
PRKCI	113	792	55	737	285	191	64	34
STAT5A	97	1154	667	487	327	283	78	22
THY	113	1080	11	1069	383	308	77	23
mtDNA	113	2940	967	1973	871	697	92	8
nDNA	113	5570	766	4804	2241	1665	94	6
Combined	113	8510	1733	6777	3112	2362	96	4

^A Percent Resolved = $[A_1+A_2+A_3]$ (Strimmer and von Haeseler 1997)

^B Percent Unresolved = $[A_{13}+A_{12}+A_{23}+A^*]$ (Strimmer and von Haeseler 1997)

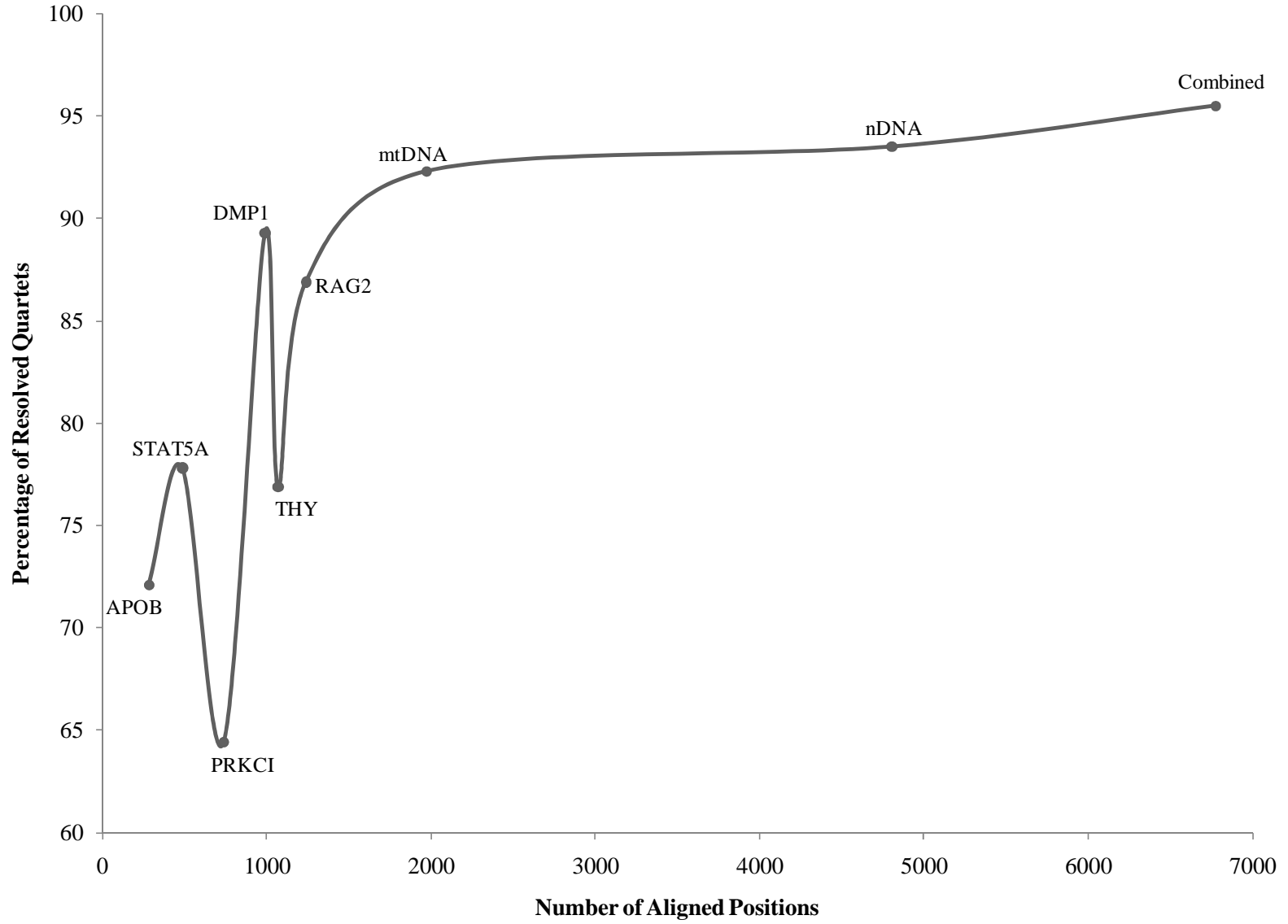
FIGURE LEGENDS

FIG. 2.1.—A scatter plot of the percentage of resolved quartets from likelihood-mapping by number of analyzed positions for each individual nDNA gene region and the mtDNA, nDNA, and combined datasets. See Table 2.3 for data and Strimmer and von Haeseler (1997) for likelihood-mapping.

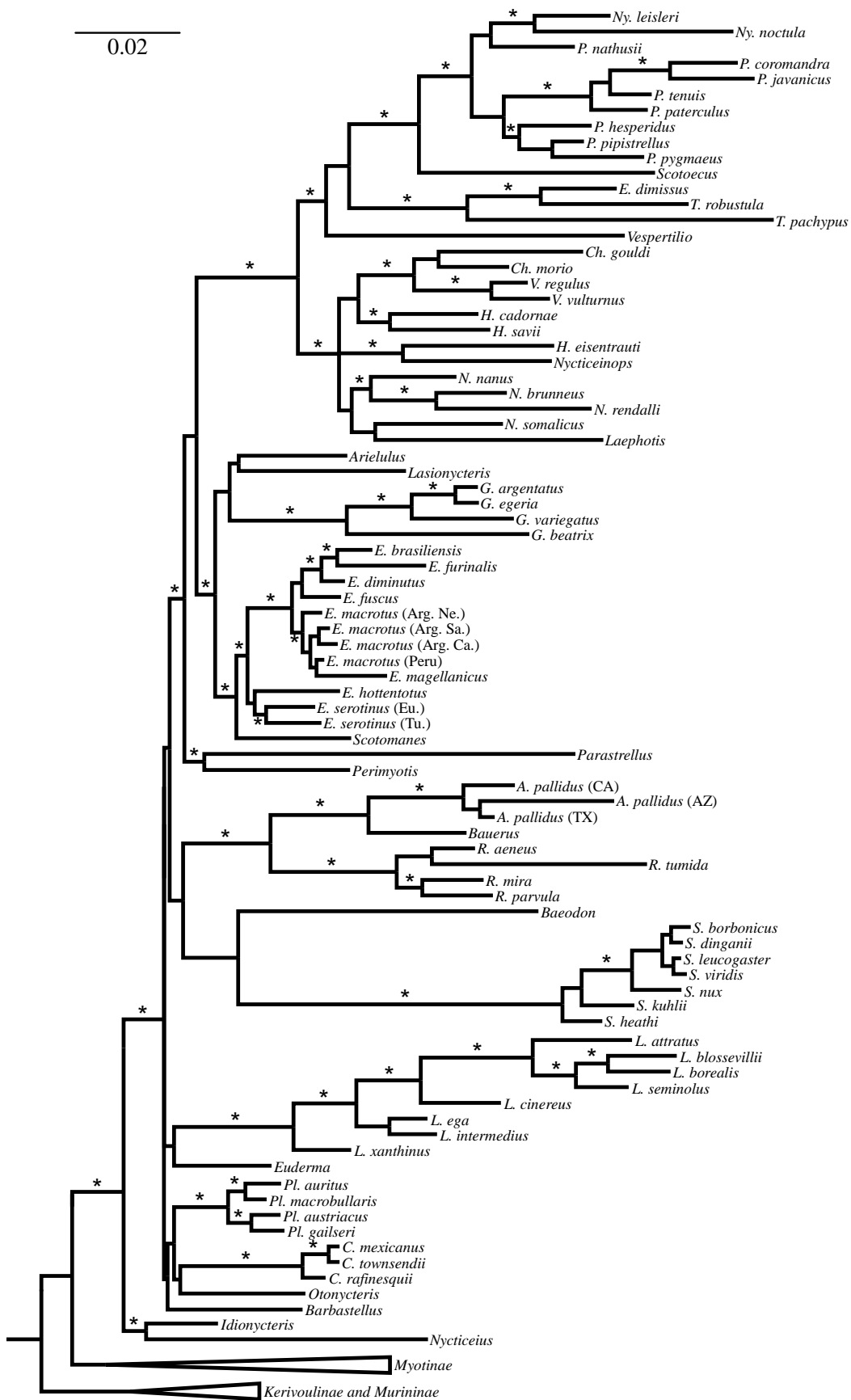
FIG. 2.2.—Phylogram from Bayesian analysis of the ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA with supported phylogenetic relationships from both maximum parsimony and Bayesian analysis depicted. Asterisks (*) indicate clades supported by both maximum parsimony ($\geq 70\%$ bootstrap values) and 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. = *Vespadelus*. For species with more than 1 representative, general locality information is provided in parentheses following the species name. Locality abbreviations follow U.S. postal codes or include: Arg. = Argentina, Ca. = Catamarca Province, Eu. = Europe, Ne. = Neuquén Province, Sa. = Salta Province, and Tu. = Tunisia.

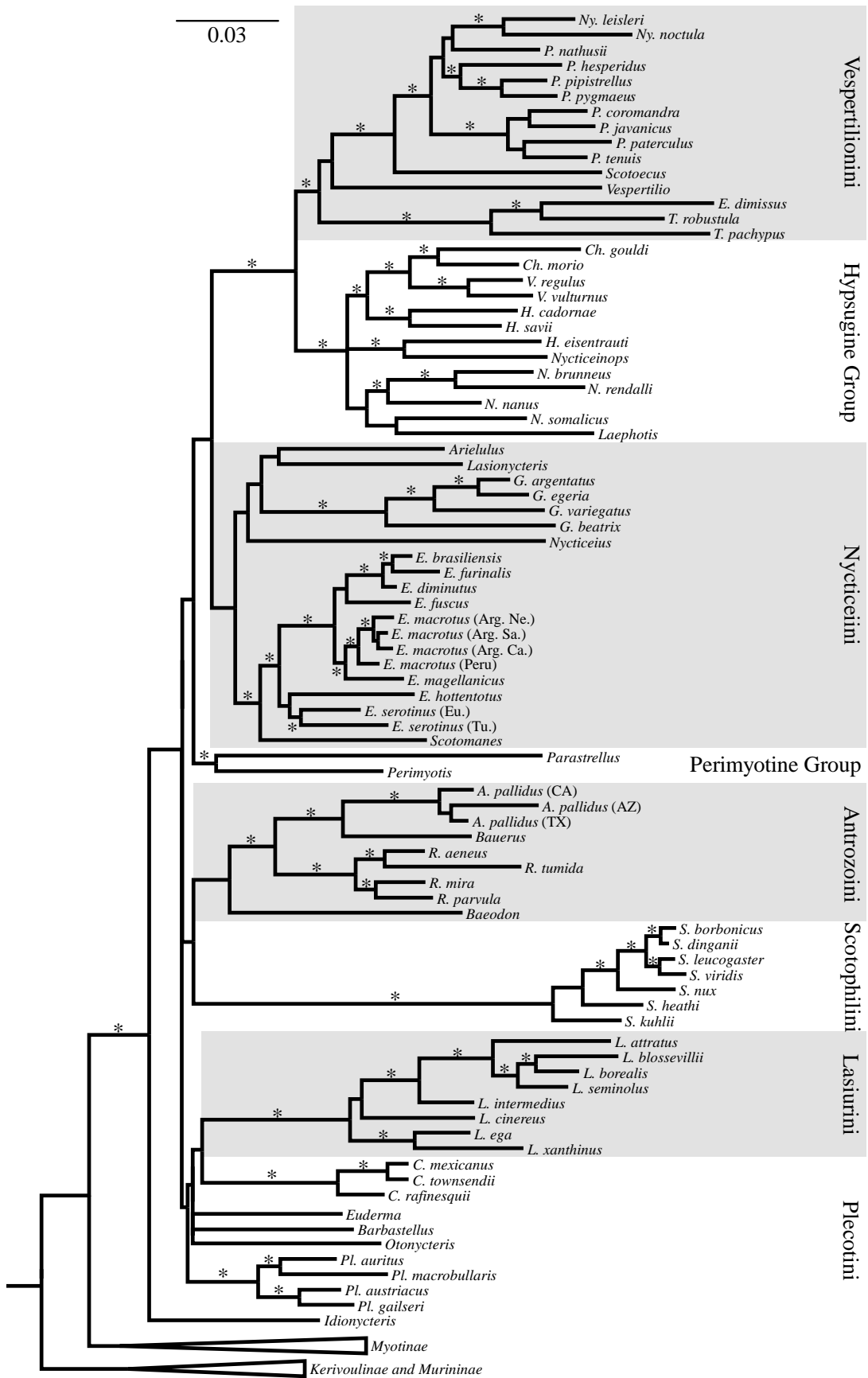
FIG. 2.3.—Phylogram from Bayesian analysis of the combined nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY with supported phylogenetic relationships from both maximum parsimony ($\geq 70\%$ bootstrap values) and Bayesian analysis (≥ 0.95 posterior probability) depicted. Symbols and abbreviations as in Fig. 2.2.

FIG. 2.4.—Phylogram from Bayesian analysis of the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions with supported phylogenetic relationships from both maximum parsimony ($\geq 70\%$ bootstrap values) and Bayesian analysis (≥ 0.95 posterior probability) depicted. Symbols and abbreviations as in Fig. 2.2.









CHAPTER III

A MOLECULAR PHYLOGENETIC REEVALUATION OF THE TRIBE NYCTICEIINI (CHIROPTERA: VESPERTILIONIDAE)

ABSTRACT – The relative taxonomic stability of the tribe Nycticeiini (*Otonycteris*, *Rhogeessa*, *Baeodon*, *Scotomanes*, *Scotophilus*, *Scotoecus*, *Scoteinus* [= *Scoteanax* and *Scotorepens*], *Nycticeius*, and *Nycticeinops*) has been challenged by new datasets over the last 2 decades including baculum morphology, cytogenetics, and mitochondrial ribosomal sequence data. These studies have resulted in new classifications for the *Nycticeius*-like bats, but only 1 study has empirically tested Nycticeiini monophyly. In this study, a suite of nuclear markers including both exon (APOB, DMP1, RAG2) and intron (PRKCI, STAT5A, THY) gene regions were used to test Nycticeiini monophyly and develop new hypotheses for the relationships of the *Nycticeius*-like bats within Vespertilioninae. Although results of these phylogenetic analyses did not fully resolve phylogenetic relationships of all taxa historically included in Nycticeiini, they did reject the validity of Nycticeiini. Taxa historically circumscribed in this tribe were found throughout the gene trees generated, with *Scotoecus* aligning basal to *Pipistrellus-Nyctalus*, *Nycticeinops* with the Hypsugine group, *Scotomanes* with *Eptesicus*, and *Rhogeessa* with *Antrozous*.

INTRODUCTION

The tribe Nycticeiini (Chiroptera: Vespertilionidae) has been 1 of the more taxonomically stable groups throughout the history of systematic study of the subfamily Vespertilioninae. However, over the last 2 decades evaluation of the relationships of bats in this subfamily using new morphological, cytological and molecular datasets has generated doubt as to the validity of this tribe and emphasized need for further studies into evolutionary relationships of these bats. Our modern classification of Nycticeiini is derived from the work of Tate (1942), who included *Otonycteris*, *Rhogeessa*, *Baeodon*, *Scotomanes*, *Scotophilus*, *Scotoecus*, *Scoteinus* [= *Scoteanax* and *Scotorepens*], and *Nycticeius* in his tribe Nycticeiini [= Nycticeiini]. Although Miller (1907) did not formally classify taxa into tribes, he did describe many of the bats that Tate (1942) included in Nycticeiini as being related to *Nycticeius*. Tate (1942) based his classification of Nycticeiini on the absence of P³ and I³ and a reduction of I² to a single cusp (following dentition of Kitchener and Caputi 1985:87). Simpson (1945), in his classic work on mammalian systematics, had a more conservative approach placing many genera in synonymy and employing no supergeneric rank. Many species (*Scotoecus*, *Scoteinus* [= *Scoteanax* and *Scotorepens*], and *Scotomanes*) grouped under Nycticeiini (*sensu stricto* Tate 1942) are synonyms of *Nycticeius* in Simpson's classification, others (*Otonycteris*, *Rhogeessa* [including *Baeodon*], and *Scotophilus*) have been retained at the species rank.

Ellerman and Morrison-Scott (1951:137) felt that Simpson had “gone rather too far” with his reductions in Vespertilioninae genera and they re-elevated many synonymized taxa to species rank including *Scotomanes*. However, they retained

Scoteinus [= *Scoteanax* and *Scotorepens*] and *Scotoecus* as synonyms of *Nycticeius* (including *Nycticeinops schlieffeni*). Nycticeiini (*sensu* Tate 1942 including *Nycticeinops*) has been reaffirmed by many authors based on tooth characteristics noted above (Koopman 1984, 1994; Koopman and Jones 1970; McKenna and Bell 1997). Kitchener and Caputi (1985) supported Nycticeiini, but based on skull and dental measurements they concluded that *Otonycteris* should be excluded from this tribe; *Scotorepens* and *Scoteanax* should be considered distinct taxa with different affinities within Nycticeiini; and *Nycticeius humeralis* and *N. schlieffeni* are not congeneric. This general classification (Nycticeiini excluding *Otonycteris*) was followed by Simmons (2005).

However, more recent work based on baculum morphology (Hill and Harrison 1987), cytogenetics (Volleth and Heller 1994; Volleth et al. 2006), and DNA sequence data (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003) has not supported the traditional classification (Nycticeiini *sensu* Tate 1942). Hill and Harrison (1987) rejected Nycticeiini as an unnatural grouping. To ameliorate this unnatural grouping, Hill and Harrison (1987) assigned *Rhogeessa*, *Baeodon*, *Otonycteris*, and *Nycticeius* to the tribe Plecotini; *Scotophilus* and *Scotomanes* to Scotophilini; and *Scotoecus*, *Scoteanax*, *Scotorepens* and the newly described genus *Nycticeinops* [= *N. schlieffeni*] to Pipistrellini. Studies of Vespertilioninae cytogenetics (Volleth and Heller 1994) initially retained Nycticeiini, including *Scotophilus* and *Rhogeessa alleni* [= *Baeodon alleni*], noting that based on their karyotypes these taxa were similar to *Antrozous pallidus*, *Nycticeius humeralis* and the other *Rhogeessa* (as published by Bickham 1979) and also placed *Scotorepens* in Vespertilionini. However, after further investigation, Volleth et

al. (2006) placed *Scotophilus* in its own tribe Scotophilini, and *Rhogeessa*, *N. humeralis* and *Otonycteris* within Plecotini. Evidence from approximately 2,600 base pairs of ribosomal mitochondrial DNA (mtDNA) also rejected Nycticeiini (*sensu* Tate 1942) and suggested placement of *Rhogeessa* and *Baeodon* into Antrozoini, *Scotophilus* into Scotophilini, *Scotoecus* in Pipistrellini, and *Nycticeinops* in Vespertilionini, with *Otonycteris incertae sedis* (Hooper and Van Den Bussche 2003). Hooper and Van Den Bussche (2003) retained Nycticeiini composed of *Glauconycteris*, *Lasionycteris*, *N. humeralis*, *Scotomanes*, and *Eptesicus* (including *Histiotus*).

Systematic research over the last 20 years has challenged the validity of Nycticeiini (*sensu* Tate 1942) and requires further research with independent datasets to test these hypotheses and provide new data in the scientific process of resolving our understanding of the evolution of Nycticeiini within the subfamily Vespertilioninae. Furthermore, with exception of the work by Hooper and Van Den Bussche (2003), no previous study has explicitly tested Nycticeiini monophyly. Their mtDNA research resulted in a novel composition of taxa for Nycticeiini but was unable to resolve all phylogenetic relationships important to the evolutionary history of taxa traditionally aligned with Nycticeiini (*sensu* Tate 1942). Using both protein-coding nuclear exons and non-coding nuclear introns, this study reevaluated previous hypotheses of systematic relationships among taxa that have been historically assigned to, or aligned with, Nycticeiini. I used a new dataset to test Nycticeiini (*sensu* Tate 1942) monophyly and the divergent classification of Nycticeiini of Hooper and Van Den Bussche (2003), with the goal of providing a working hypothesis of the evolution of *Nycticeius*-like bats.

MATERIALS AND METHODS

Extraction, amplification, and sequencing.—Genomic DNA was isolated from skeletal muscle and organ tissues using the procedures of Longmire et al. (1997) or the DNeasy Tissue Kit (Qiagen, Austin, Texas) for 54 individuals. Included in this study are 50 taxa that have been associated historically with *Eptesicus*, *Nycticeius*, or *Scotophilus*, at some point classified within Nycticeiini (excluding *Scoteanax* and *Scotorepens*), or were included to represent ecological, morphological, or taxonomic diversity of Vespertilioninae. Four species of Myotinae were included as outgroups for character-state polarization. PCR amplification and sequencing reactions focused on 3 nuclear exons Apolipoprotein B (APOB), Dentin Matrix Acidic Phosphoprotein I (DMP1), and Recombination Activating Gene II (RAG2), and 3 nuclear introns Protein Kinase C, Iota (PRKCI), Signal Transducer and Activator of Transcription 5A (STAT5A), and Thyrotropin (THY). Primers, methods, equipment, and protocols used to generate this nuclear dataset (nDNA) can be found in Roehrs (2009: Chapter 2). Sequence data of the PRKCI, STAT5A and THY markers for *Eptesicus hottentotus*, *Nycticeinops schlieffeni*, and *Scotophilus dinganii* were compiled from the previous work of Eick et al. (2005) deposited on GenBank (<http://www.ncbi.nlm.nih.gov/>). The mtDNA dataset used in this study consisting of the 12S rRNA, tRNA^{Val}, and 16S rRNA ribosomal gene regions were primarily generated by previous research in the Van Den Bussche laboratory at Oklahoma State University and deposited on GenBank (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003; Van Den Bussche and Hooper 2000, 2001; Van Den Bussche et al. 2003). Using protocols outlined in Van Den Bussche and Hooper (2000), I generated mtDNA data for 9 additional taxa (*Arielulus aureocollaris*, *E. dimissus*, 2

specimens of *E. macrotus*, *E. magellanicus*, *E. serotinus*, *Hypsugo cadornae*, *Myotis latirostris*, and *Pipistrellus pipistrellus*).

Phylogenetic analyses.—Assembly of forward and reverse sequences for each gene region of each species sample was completed in the program Geneious 4.5.4 (Biomatters Ltd. Auckland, New Zealand) to create contigs that were then aligned in Geneious using ClustalW 1.83.XP (Thompson et al. 1994). Alignments were imported into and manually optimized in the program MacClade 4.05 (Maddison and Maddison 2000). The procedures of Lutzoni et al. (2000) were implemented to identify ambiguously aligned sites in the sequence data caused by insertion of gaps to represent hypothetical indels. Regions identified as possibly violating assumptions of positional homology were excluded from phylogenetic analyses. Three data partitions were used in all phylogenetic analyses, a mtDNA dataset, a nDNA dataset, and a combined mtDNA and nDNA dataset (hereafter referred to as “combined”). Previous studies have demonstrated the congruence of supported topologies of all mitochondrial (Van Den Bussche and Hofer 2000) and nuclear genes (Roehrs 2009: Chapter 2) used in this study and support acceptable concatenation of these gene regions into mtDNA and nDNA datasets, respectively. Therefore, phylogenetic analysis of each separate gene region was not conducted for this study. To examine possible inconsistencies between the mtDNA and nDNA gene trees and the appropriateness of a combined dataset, a concordance test at 90% of supported clades was implemented (De Queiroz 1993).

Each data partition was analyzed using maximum parsimony (MP) in PAUP* v4.0b10 (Swofford 2002) and Bayesian phylogenetic methods in MRBAYES v3.1.2 (Huelsenbeck and Ronquist 2001). Parameters for the MP analysis included unweighted

nucleotide substitutions in a heuristic search with 25 random additions of taxa, a Tree-Bisection-Reconnection branch swapping algorithm, and 1,000 bootstrap replicates to quantify nodal support. The Bayesian analysis was conducted with a 4 chain (3 hot, 1 cold) parallel Metropolis-coupled Markov chain Monte Carlo running for 2×10^6 generations, with sampling every 10 generations, at a 0.02 temperature. Analysis was started with a random unconstrained tree and uniform priors and burn-in values were determined by plotting likelihood scores on generation time and finding the point at which model parameters and tree scores become stationary.

Taxonomic sampling.— Most species included in this study are represented by voucher specimens (Ruedas et al. 2000) 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), Muséum d'Histoire Naturelle, Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB, NK), Museum of Texas Tech University (TTU, TK), Royal Ontario Museum (ROM), Sam Noble Oklahoma Museum of Natural History (OMNH, OCGR), Texas Cooperative Wildlife Collection at Texas A&M University (TCWC), Universidad Nacional Autónoma de México (UNAM), and University of Lausanne, Switzerland, Institut de Zoologie et d'Ecologie Animale (IZEA). Specimen identifications in most cases were verified by Steven R. Hooper and Manuel Ruedi (pers. comm.) otherwise, I relied on the identifications of the above collections. The following specimens were included in this study with voucher specimen

catalog number, tissue catalog number, and a general collecting locality organized alphabetically by family, subfamily, tribe, and species.

Family Vespertilionidae: Subfamily Myotinae – *Myotis bocagii* (FMNH150075, FMNH150075), Tanzania: Tanga Region; *Myotis latirostris* (MHNG, M606), Taiwan: Miao-Li County; *Myotis riparius* (AMNH268591, AMNH268591), French Guiana: Paracou; *Myotis volans* (TTU79545, TK78980), U.S.A.: Texas; Subfamily Vespertilioninae – *Otonycteris hemprichii* (CM, SP7882), Jordan: Maan Government, (SP7908) data not provided, (MBQ1226, SP7933) data not provided; *Parastrellus hesperus* (TTU79269, TK78703), U.S.A.: Texas; *Perimyotis subflavus* (TTU80684, TK90671), U.S.A.: Texas; Tribe Antrozoini – *Antrozous pallidus* (TTU71101, TK49646), U.S.A.: Texas; *Baeodon alleni* (UNAM, TK45023), Mexico: Michoacán; *Rhogeessa parvula* (TTU36633, TK20653), Mexico: Sonora; Tribe Lasiurini – *Lasiurus cinereus* (TTU, TK78926), U.S.A.: Texas; Tribe Nycticeiini – *Arielulus aureocollaris* (ROM106169, F38447), Vietnam: Tuyen Quang; *Eptesicus brasiliensis* (CM76812, TK17809), Suriname: Nickerie; *Eptesicus diminutus* (TTU48154, TK15033), Venezuela: Guárico; *Eptesicus dimissus* (MHNG1926.053, M1187), Laos; *Eptesicus furinalis* (AMNH268583, AMNH268583), French Guiana: Paracou; *Eptesicus fuscus* (CM102826, SP844), U.S.A.: West Virginia; *Eptesicus hottentotus* (type, CM89000, TK33013), Kenya: Rift Valley Province; *Eptesicus macrotus* (CML3230, OCGR2301), Argentina: Neuquén, (FMNH129207, FMNH129207), Peru: Ancash; *Eptesicus magellanicus* (OMNH23500, OCGR2303), Argentina: Neuquén; *Eptesicus serotinus* (MHNG1807.065, M816), Greece, (TTU70947, TK40897), Tunisia: Sidi Bou Zid Governorate; *Glauconycteris argentatus* (FMNH15119, FMNH15119), Tanzania:

Kilimanjaro Region; *Glauconycteris beatrix* (FMNH149417, FMNH149417), Zaire [=Democratic Republic of the Congo]: Haut-Zaïre; *Glauconycteris egeria* (AMNH268381, AMNH268381), Central African Republic, (AMNH109067, AMNH109067), data not provided; *Glauconycteris variegatus* (CM97983, TK33545), Kenya: Western Province; *Lasionycteris noctivagans* (TTU56255, TK24216), U.S.A.: Texas; *Nycticeius humeralis* (TTU49536, TK26380), U.S.A.: Texas, (TTU80664, TK90649), U.S.A.: Texas; *Scotomanes ornatus* (ROM107594, F42568), Vietnam: Tuyen Quang; Tribe Pipistrellini – *Nyctalus leisleri* (FMNH140374, FMNH140374), Pakistan: Malakand Division; *Pipistrellus pipistrellus* (MHNG1956.031, M1439), Switzerland; *Pipistrellus tenuis* (FMNH137021, FMNH137021), Republic of the Philippines: Sibuyan Island; *Scotoecus hirundo* (FMNH151204, FMNH151204), Tanzania: Kilimanjaro Region; Tribe Plecotini – *Barbastella barbastellus* (MHNG1804.094, IZEA3590), Switzerland: Valais Province; *Corynorhinus rafinesquii* (TTU45380, TK5959), U.S.A.: Arkansas; *Idionycteris phyllotis* (ACU736, ACU736), U.S.A.: Arizona, (MSB12091, NK36122), U.S.A.: Utah; *Plecotus auritus* (MHNG1806.047, IZEA2694), Switzerland: Valais Province; Tribe Scotophilini – *Scotophilus dinganii* (FMNH147235, FMNH147235), Tanzania: Tanga Region; *Scotophilus kuhlii* (FMNH145684, FMNH145684), Republic of the Philippines: Sibuyan Island; Tribe Vespertilionini – *Chalinolobus gouldi* (TCWC, RLH27), Australia; *Chalinolobus morio* (TCWC, 05M3), Australia; *Hypsugo cadornae* (MHNG1926.050, M1183), Laos: Phôngsali Province; *Hypsugo eisentrauti* (ROM100532, F34348), Ivory Coast; *Hypsugo nanus* (CM98003, TK33378), Kenya: Eastern Province, (DM7542, DM7542), South Africa: KwaZulu-Natal Province; *Hypsugo savii* (MHNG1804.100, IZEA3586), Switzerland: Valais Province;

Laephotis namibensis (CM93187, SP4160), Namibia: Maltahöhe District; *Neoromicia brunneus* (CM90802, TK21501), Gabon: Estuaire Province; *Neoromicia rendalli* (CM97977, TK33238), Kenya: Coastal Province; *Neoromicia somalicus* (CM97978, TK33214), Kenya: Coastal Province; *Nycticeinops schlieffeni* (CM97998, TK33373), Kenya: Eastern Province; *Tylonycteris pachypus* (ROM106164, F38442), Vietnam: Tuyen Quang; *Vespadelus regulus* (TCWC, RLH30), Australia; *Vespertilio murinus* (MHNG1808.017, IZEA3599), Switzerland: Valais Province.

RESULTS

mtDNA sequences.—A total of 2,891 positions resulted from the alignment of 54 taxa for the 3 ribosomal mtDNA gene regions (12S rRNA, tRNA^{Val}, 16S rRNA). New mtDNA sequence data were generated for 9 taxa: *A. aureocollaris*, *E. dimissus*, 2 specimens of *E. macrotus*, *E. magellanicus*, *E. serotinus*, *H. cadornae*, *M. latirostris*, and *P. pipistrellus*. Of the 2,891 aligned positions, 905 were excluded before analysis for potential violation of positional homology, 794 were variable, and 564 were phylogenetically informative. The MP analysis resulted in 2 most parsimonious trees having 3,776 steps, 21 supported clades (bootstrap values $\geq 70\%$), and excluding uninformative characters, a consistency index (CI) of 0.2496 and a retention index (RI) of 0.4488 (Fig. 3.1). Difference in the topology between the 2 most parsimonious trees related to variable positioning of *Scotoecus* and *P. tenuis* within Pipistrellini. In 1 tree these taxa were sister to each other and in the other, *P. tenuis* was basal to a clade including *Scotoecus*, *P. pipistrellus*, and *Nyctalus*. The Bayesian analysis had a burn-in value of 38,890 generations and resulted in a tree with 27 supported clades (≥ 0.95 posterior probability; Fig. 3.1).

nDNA sequences.—Amplification and sequencing of the STAT5A gene region was at times problematic, and I was unable to generate sequence data for *E. magellanicus*, *G. beatrix*, *G. egeria*, *H. cadornae*, *H. savii*, *N. leisleri*, *P. tenuis*, *S. hirundo*, *T. pachypus*, and *V. murinus*. Furthermore, I was able to generate only 770 positions (approximately first one-half) of RAG2 for *B. alleni*. For the aforementioned taxa, all other nDNA gene regions were sequenced, and despite these problems, I was able to generate full nDNA sequence data for APOB, DMP1, RAG2, PRKCI, STAT5A and THY for 44 taxa with an aligned length of 5,233 positions. Of those positions, 748 were excluded prior to analysis for potential violations of positional homology. The remaining 4,485 positions had 1,848 variable positions, of which 1,109 were phylogenetically informative. The MP analysis resulted in 9 most parsimonious trees of 4,953 steps, 31 supported clades (bootstrap values $\geq 70\%$), and excluding uninformative characters, a CI of 0.4884 and a RI of 0.5883 (Fig. 3.2). Three topological differences occurred in the most parsimonious trees: 1) the interrelationship of Antrozoini (*sensu* Hooper and Van Den Bussche 2003 excluding *Baeodon*), traditional Plecotini and the other Vespertilioninae with Antrozoini and Plecotini sister taxa basal to the remaining Vespertilioninae or serially basal to the remaining Vespertilioninae; 2) the position of a clade consisting of *Arielulus* and *Lasionycteris* within Eptesicini [= Nycticeiini *sensu* Hooper and Van Den Bussche 2003 excluding *Nycticeius*] being either basal to the *Eptesicus* and *Scotomanes* clade or the *Glauconycteris* clade; and 3) the position of *Vespertilio* being basal either to the Pipistrellini (*sensu* Hooper and Van Den Bussche) or the *Tylonycteris* clade. The Bayesian analysis had a burn-in value of 40,660 generations and resulted in a tree with 35 clades supported by posterior probabilities ≥ 0.95 (Fig. 3.2).

Combined sequences.—The placement of *Nyctalus* constituted the only supported discrepancy between the mtDNA and nDNA gene trees. In the mtDNA gene tree (Fig. 3.1) *Nyctalus* formed a supported clade with *P. pipistrellus* causing *Pipistrellus* paraphyly, but *Pipistrellus* was monophyletic with *Nyctalus* supported basally in the nDNA gene tree (Fig. 3.2). Despite this discrepancy, there was 90% concordance between the 2 gene trees, and the datasets were combined for phylogenetic analysis (De Queiroz 1993). This concatenated dataset resulted in 8,124 aligned positions, of which 1,653 positions were excluded prior to analysis for possible violation of positional homology. Of the remaining 6,471 positions, 2,642 were variable and 1,673 were phylogenetically informative. The MP analysis identified 4 most parsimonious trees, with 8,837 steps and 30 supported clades (bootstrap values $\geq 70\%$; Fig. 3.3). Excluding uninformative characters, the MP analysis had a CI of 0.3688 and a RI of 0.5088. Topology differences between the most parsimonious trees included differences in the relationship of the Antrozini clade (*sensu* Hofer and Van Den Bussche 2003), a clade made up of Plecotini, *Lasiurus*, and New World pipistrelles, and the other Vespertilioninae, which was similar to the nDNA parsimony results. The position of *P. auritus* also varied, being positioned sometimes basal to Plecotini and other times basal to New World pipistrelles. Burn-in value for the Bayesian analysis was 41,830 generations and resulted in a tree with 35 supported clades (posterior probability ≥ 0.95 ; Fig. 3.3).

DISCUSSION

In the time since Miller (1907) 1st recognized morphological similarities between these taxa and Tate (1942) officially attributed to circumscribe *Otonycteris*, *Rhogeessa*, *Baeodon*, *Scotomanes*, *Scotophilus*, *Scotoecus*, *Scoteinus* [= *Scoteanax* and *Scotorepens*],

Nycticeinops, and *Nycticeius*, the tribe Nycticeiini has been a relatively stable taxon throughout the history of Vespertilioninae systematics. However, studies over the last 20 years using new datasets and methods have not supported this traditional classification, which was largely based on reductions in dentition of members of this tribe (Koopman 1984, 1994; Koopman and Jones 1970; McKenna and Bell 1997; Tate 1942). Many authors have questioned the primary and sole reliance on dental formulae and cusp patterns and feel that these characters may be too plastic, leading to systematic conclusions incongruent with the actual evolution in these taxa (Ärnäck-Christie-Linde 1909; Heller and Volleth 1984; Hill and Harrison 1987; Koopman 1975; Zima and Horáček 1985). Tate (1942:228) even noted "...profound specializations of various sorts have obscured the basic pattern first indicated [by tooth characters of the tribe Nycticeiini]," implying that he was having difficulty in fitting these characters with his evolutionary hypothesis.

Nycticeiini sensu Tate.—In this reevaluation of Nycticeiini (*sensu* Tate 1942), results from > 8,100 base pairs of data including separate mtDNA, nDNA, and combined analyses do not support this tribe. While these results leave evolutionary relationships of some taxa included in Nycticeiini unresolved (Antrozoini [including *Rhogeessa*], *Baeodon*, *Nycticeius*, *Otonycteris*, Scotophilini), it clearly demonstrates polyphyly of this taxon as defined by Tate (1942). Results from each analysis of all 3 datasets demonstrated that *Scotoecus* is most closely related to *Pipistrellus* and *Nyctalus*, while *Nycticeinops* groups with *H. eisentrauti*. Furthermore, these taxa (*Scotoecus*, *Nycticeinops*) are embedded in a larger clade that incorporates taxa historically included in the tribes Pipistrellini and Vespertilionini. Both nDNA and combined analyses

supported the basal position of *Scotomanes* to a clade including both Old and New World *Eptesicus* and *Rhogeessa* has been supported as sister to *Antrozous* in all gene trees. The position of *Baeodon* in these results is problematic because it is largely unsupported; sometimes it grouped with or basal to a clade made up of *Antrozous* and *Rhogeessa* (mtDNA and combined gene trees), forming a supported clade with *Scotophilus* (nDNA MP analysis) or *Lasiurus* (nDNA Bayesian analysis). It is possible that this lack of resolution was caused by data missing from the last part of the RAG2 gene region, but given the relatively small amount of missing data (~470 bp) compared with the total aligned (>8100 bp), this is an unlikely explanation. The species of *Scotophilus* included in this study formed a well-supported clade that appears to have a long independent evolutionary history (Fig. 3.4), but as with *Otonycteris*, their relationship to other Vespertilioninae taxa is unresolved. Finally, the relationship of the namesake taxon for this tribe (*Nycticeius*) to other taxa also is unresolved. The nDNA gene tree places *N. humeralis* basal to all members of the Vespertilioninae except possibly *I. phyllotis* (Fig. 3.2). This position and relationship of *N. humeralis* to *I. phyllotis* in the gene trees could be due to mutational saturation of non-phylogenetically informative characters that tend to accumulate in taxa with long branches. Given the general lack of resolution for deep branches in these gene trees, it is not surprising that a monotypic genus such as *Nycticeius* or *Idionycteris* would appear in an unsupported basal position. Alternatively, *Nycticeius* has been linked to Plecotini based on bacular morphology (Hill and Harrison 1987) and Antrozoini (Bickham 1979) through cytogenetics and although the relationship between these clades is unsupported in this analysis, the position of *Nycticeius* may reflect its relationship to these basal lineages.

Concordance with baculum morphology and cytogenetics.—Based on baculum morphology Hill and Harrison (1987) rejected Nycticeiini placing *Rhogeessa*, *Baeodon*, *Nycticeius*, and *Otonycteris* into Plecotini, *Scotomanes* with *Scotophilus* in Scotophilini, and *Scotoecus*, *Scoteanax* and *Scotorepens* in Pipistrellini. Although the results of this study concur with their rejection of Nycticeiini (*sensu* Tate 1942), they generally do not support reclassification of *Nycticeius*-like bats made by Hill and Harrison (1987). Hill and Harrison (1987:257) also addressed potential limitations of bacular characters in intergeneric systematic studies noting: “The structure of the baculum in the Vespertilioninae suggests some modifications to tribal classification within the subfamily, although clearly other morphological characters need to be given equivalent or greater weight.” Although this study cannot support or reject Plecotini (*sensu* Hill and Harrison 1987), results from each gene tree support a relationship between *Rhogeessa* and *Antrozous*, which are in separate tribes in the classification of Hill and Harrison (1987; Plecotini and Antrozoini, respectively). Hill and Harrison (1987:258) noted that *Antrozous* and *Bauerus* (as well as *Lasiurus*) have saddle shaped, derived bacula similar to taxa included in Plecotini, but state that they “are quite distinctive on other morphological grounds.” Whether these undisclosed morphological characters warrant separate tribal status for *Antrozous* and *Bauerus* seems less likely based on the results of this study. Molecular results are in agreement with cytogenetic data (Volleth et al. 2006) in rejecting the sister relationship between *Scotomanes* and *Scotophilus* proposed by Hill and Harrison (1987). These results support the basal position of *Scotomanes* to a monophyletic group composed of members of the genus *Eptesicus* (including *Histiotus*) in the nDNA and combined gene trees. In a recent reevaluation of *Scotophilus*, Horáček

et al. (2006) supported this result based on tooth morphology. However, these molecular results support Hill and Harrison (1987) in aligning *Scotoecus* with *Pipistrellus* and *Nycticeinops* within the *Pipistrellus*-like bats. Baculum morphology seems to be useful in separating *Pipistrellus*-like bats (Vespertilionini and Pipistrellini) with their long slender bifurcating tipped baculum from the more shield-like Vespertilioninae (Fig. 3.4), a character possibly ancestral for the subfamily.

Analysis of cytological data also rejected Nycticeiini (*sensu* Tate 1942), initially retaining the tribe (Volleth and Heller 1994) and later, based on additional data, excluding the tribe altogether (Volleth et al. 2006). Volleth and Heller (1994) initially retained Nycticeiini including a potential close relationship between *Scotophilus* and *Baeodon alleni*. The grouping of Scotophilini and Antrozoini was often found in phylogram topologies in this study (Fig. 3.4), but was not supported in any analyses except by the MP bootstrap analysis in the nDNA gene tree (Fig. 3.2). Volleth et al. (2006) proposed that the karyotype of *Scotoecus hirundo* was intermediate between Pipistrellini and Vespertilionini. Results of this study demonstrated that *Scotoecus* was a pipistrelloid bat, but firmly placed it basal to the clade including *Pipistrellus* and *Nyctalus* in Pipistrellini (*sensu* Hooper and Van Den Bussche 2003). Finally, these results cannot refute inclusion of *Rhogeessa* in Plecotini (*sensu* Volleth et al. 2006); however, *Antrozous* also would have to be included in this tribe to be valid based on molecular data.

Systematic conclusions.—Based on results of this study, in corroboration with baculum and cytogenetic data, it is apparent that Nycticeiini (*sensu* Tate 1942) is an unnatural grouping and molecular data fail to support, but cannot refute, other previously

proposed compositional arrangements for tribe Nycticeiini (Hooper and Van Den Bussche 2003; Volleth and Heller 1994). Results from the nDNA gene region and combined dataset were most in line with the systematic conclusion based on molecular ribosomal data (Hooper and Van Den Bussche 2003), but provided more resolution of deeper divergent clades. Results from the mtDNA gene region (and Hooper and Van Den Bussche 2003) placed *Nycticeius* in a clade with *Arielulus*, *Eptesicus* (including *Histiotus*), *Glauconycteris*, *Lasionycteris*, and *Scotomanes* (their Nycticeiini). Hooper and Van Den Bussche (2003:31) denominate this clade Nycticeiini because *Nycticeius* has priority. With the exception of *Nycticeius*, the nDNA gene region was in concordance with the results from the mtDNA gene region. However, with removal of *Nycticeius* from this clade, tribal nomenclatural priority is transferred to *Eptesicus*, and the most appropriate name for this clade is Eptesicini based on nDNA. It is apparent from both the mtDNA and nDNA that *Arielulus*, *Eptesicus*, *Glauconycteris*, *Lasionycteris*, and *Scotomanes* comprise a supported clade. The only confounding factor is the variable position of *Nycticeius* between these 2 gene trees. Bacular morphology, cytogenetics, and nDNA put the position of *Nycticeius* closer to the tribe Antrozoini and Plecotini, but until its position is resolved, the full circumscription of this tribe (Eptesicini / Nycticeiini) and its nomenclature remain equivocal. Furthermore, these results clearly support *Eptesicus* paraphyly with relation to *Histiotus*, removal of *Neoromicia* and *Vespadelus* from *Eptesicus*, and the basal position of *Scotomanes* to *Eptesicus* (including *Histiotus*).

The sequence data clearly support the tribe Pipistrellini (*sensu* Hooper and Van Den Bussche 2003) including *Scotoecus* as a basal lineage of the tribe. *Nycticeinops*,

once a synonym of *Nycticeius*, is most related to *H. eisentrauti*. Hooper and Van Den Bussche (2003) transferred *H. eisentrauti* to *Nycticeinops* and included it in their tribe Vespertilionini. Based on results from the combined mtDNA and nDNA data, this change in position is supported, but this *Nycticeinops* clade is a member of the Hypsugine group not Vespertilionini. *Scotophilus* forms a supported clade in all gene trees and appears to have a long independent evolutionary history (at least for the genes included in this study; Fig. 3.4). This would lend support to the tribe Scotophilini, but without full resolution of their position in Vespertilioninae, this taxonomic arrangement is only tentative. *Rhogeessa* forms a supported clade with *Antrozous* in all gene trees and these results would lend support to Antrozoini (*sensu* Hooper and Van Den Bussche 2003), but refutes Antrozoidae (Simmons 1998; Simmons and Geisler 1998) and Antrozinae (Miller 1897; Simmons 2005).

ACKNOWLEDGMENTS

I thank Sara W. Weyandt and Matt Leslie who generated RAG2 sequence data in early phases of this project. Justin B. Lack and Craig E. Stanley, Jr. who have generated portions of the sequence data included in this project. Geeta Eick was helpful in providing primers and advice on PCR profiles for some of the nuclear genes used in this project. Janet K. Braun, Meredith J. Hamilton, David M. Leslie, Meredith A. Magnuson, Ronald J. Tyrl, and Ronald A. Van Den Bussche reviewed this manuscript and are deserving of thanks. I thank Steve Hartson, Janet Rogers, and Lisa Whitworth of the Oklahoma State University Recombinant DNA/Protein Core Facility for use of equipment and trouble shooting. National Science Foundation grants DEB-9873657 and DEB-0610844 to RAVDB funded this research. Any opinion, findings, and conclusions

or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. This research could not have possible without the support and loan of tissues by the following individuals and institutions: Robert J. Baker of the Natural Sciences Research Laboratory of the Museum of Texas Tech University; Nancy B. Simmons of the American Museum of Natural History; Bruce D. Patterson, Lawrence R. Heaney and William T. Stanley of the Field Museum of Natural History; Suzanne B. McLaren of the Carnegie Museum of Natural History; Mark D. Engstrom and Burton Lim of the Royal Ontario Museum; Manuel Ruedi of the Muséum d'Histoire Naturelle, Genève, and the University of Lausanne, Institut de Zoologie et d'Ecologie Animale; Peter J. Taylor of the Durban Natural Science Museum; Janet K. Braun and Michael A. Mares of the Sam Noble Oklahoma Museum of Natural History; Terry L. Yates and Joseph Cook of the Museum of Southwestern Biology at the University of New Mexico; Thomas E. Lee, Jr., of Abilene Christian University; and Rodney L. Honeycutt and Duane Schlitter of the Texas Cooperative Wildlife Collection at Texas A&M University.

LITERATURE CITED

- ÄRNBÄCK-CHRISTIE-LINDE, A. 1909. On intermediate forms among Chiroptera. *Zoologischer Anzeiger* 34:572–582.
- BICKHAM, J. W. 1979. Chromosomal variation and evolutionary relationships of vespertilionid bats. *Journal of Mammalogy* 60:350–363.
- DE QUEIROZ, A. 1993. For consensus (sometimes). *Systematic Biology* 42:368–372.

- EICK, G. N., D. S. JACOBS, AND C. A. MATTHEE. 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Molecular Biology and Evolution* 22:1869–1886.
- ELLERMAN, J. R., AND T. C. S. MORRISON-SCOTT. 1951. Checklist of Palaearctic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History). London, United Kingdom.
- HILL, J. E., AND D. L. HARRISON. 1987. The baculum in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. *Bulletin of the British Museum (Natural History), Zoology* 52:225–305.
- HELLER, K. -G., AND M. VOLLETH. 1984. Taxonomic position of “*Pipistrellus societatis*” Hill, 1972 and the karyological characteristics of the genus *Eptesicus* (Chiroptera: Vespertilionidae). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 22:65–77.
- HOOFER, S. R., S. A. REEDER, E. W. HANSEN, AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics and taxonomic review of noctilionoid and vespertilionoid bats (Chiroptera: Yangochiroptera) *Journal of Mammalogy* 84:809–821.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2001. Phylogenetic relationships of plecotine bats and allies based on mitochondrial ribosomal sequences. *Journal of Mammalogy* 82:131–137.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica* 5(supplement):1–63.

- HORÁČEK, I., O. FEJFAR, AND P. HULVA. 2006. A new genus of vespertilionid bat from Early Miocene of Jebel Zelten, Libya, with comments on *Scotophilus* and early history of vespertilionid bats (Chiroptera). *Lynx* 37:131–150.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- KITCHENER, D. J., AND N. CAPUTI. 1985. Systematic revision of Australian *Scoteanax* and *Scotorepens* (Chiroptera: Vespertilionidae), with remarks on relationships to other Nycticeiini. *Records of the Western Australia Museum* 12:85–146.
- KOOPMAN, K. F. 1975. Bats of Sudan. *Bulletin of the American Museum of Natural History* 154:353–444.
- KOOPMAN, K. F. 1984. A Synopsis of the families of bats - part VII. *Bat Research News* 25:25–27.
- KOOPMAN, K. F. 1994. Chiroptera: systematics. *Handbook of zoology: a natural history of the phyla of the animal kingdom*. Walter de Gruyter, Berlin, Germany. 8(60):1–217.
- KOOPMAN, K. F., and J. K. Jones, Jr. 1970. Classification of bats. Pp. 22–28 in *About bats: a chiropteran biology symposium* (B. H. Slaughter and D. W. Walton, eds.). Southern Methodist University Press, Dallas, Texas.
- LONGMIRE, J. L., M. MALTBIÉ, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers, Museum of Texas Tech University* 163:1–3.

- LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* 49:628–651.
- MADDISON, W. P., AND D. R. MADDISON. 2002. *MacClade*, v. 4.05. Sinauer Associates, Inc., Sunderland, Massachusetts.
- MCKENNA, M. C., AND S. K. BELL. 1997. *Classification of mammals: above the species level*. Columbia University Press, New York.
- MILLER, G. S., JR. 1897. Revision of the North American bats of the family *Vespertilionidae*. *North American Fauna* 13:1–136.
- MILLER, G. S., JR. 1907. The families and genera of bats. *Bulletin of the United States National Museum* 57:1–282.
- ROEHRS, Z. P. 2009. *Vespertilioninae systematics: using mitochondrial and nuclear markers to elucidate phylogenetics relationships*. Ph.D. dissertation, Oklahoma State University, Stillwater, Oklahoma.
- RUEDAS, L. A., J. SALAZAR-BRAVO, J. W. DRAGOO, AND T. L. YATES. 2000. The importance of being earnest: what, if anything, constitutes a “specimen examined?” *Molecular Phylogenetics and Evolution* 17:129–132.
- SIMMONS, N. B. 1998. A reappraisal of interfamilial relationships of bats. Pp. 3–26 in *Bat biology and conservation* (T. H. Kunz and P. A. Racey, eds.). Smithsonian Institution Press, Washington D.C.
- SIMMONS, N. B. 2005. Order Chiroptera Pp. 312–529 in *Mammal species of the world: a taxonomic and geographic reference*. 3rd ed. (D. E. Wilson, and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.

- SIMMONS, N. B., AND J. H. GEISLER. 1998. Phylogenetic relationships of *Icaronycteris*, *Archaeonycteris*, *Hassianycteris* and *Palaeochiropteryx* to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bulletin of the American Museum of Natural History* 235:1–182.
- SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History* 85:i–xvi,1–350.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods) 4.0 beta. Sinauer Associates, Inc., Sunderland, Massachusetts.
- TATE, G. H. H. 1942. Results of the Archbold expeditions. No. 47: review of the Vespertilioninae bats, with special attention to genera and species of the Archbold collections. *Bulletin of the American Museum of Natural History* 80:221–296.
- THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL_W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2000. Further evidence for inclusion of the New Zealand short-tailed bat (*Mystacina tuberculata*) within Noctilionoidea. *Journal of Mammalogy* 81:865–874.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2001. Evaluating monophyly of Nataloidea (Chiroptera) with mitochondrial DNA sequences. *Journal of Mammalogy* 82:320–327.

- VAN DEN BUSSCHE, R. A., S. A. REEDER, E. W. HANSEN, AND S. R. HOOFER. 2003. Utility of the Dentin Matrix Protein 1 (DMP1) gene for resolving mammalian intraordinal phylogenetic relationships. *Molecular Phylogenetics and Evolution* 26:89–101.
- VOLLETH, M., AND K. G. HELLER. 1994. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 32:11–34.
- VOLLETH, M., K. G. HELLER, AND J. FAHR. 2006. Phylogenetic relationships of three “Nycticeiini” genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. *Zeitschrift für Säugetierkunde* 71:1–12.
- ZIMA, J., AND I. HORÁČEK. 1985. Synopsis of karyotypes of vespertilionid bats (Mammalia: Chiroptera). *Acta Universitatis Carolinae - Biologica* 1981:311–329.

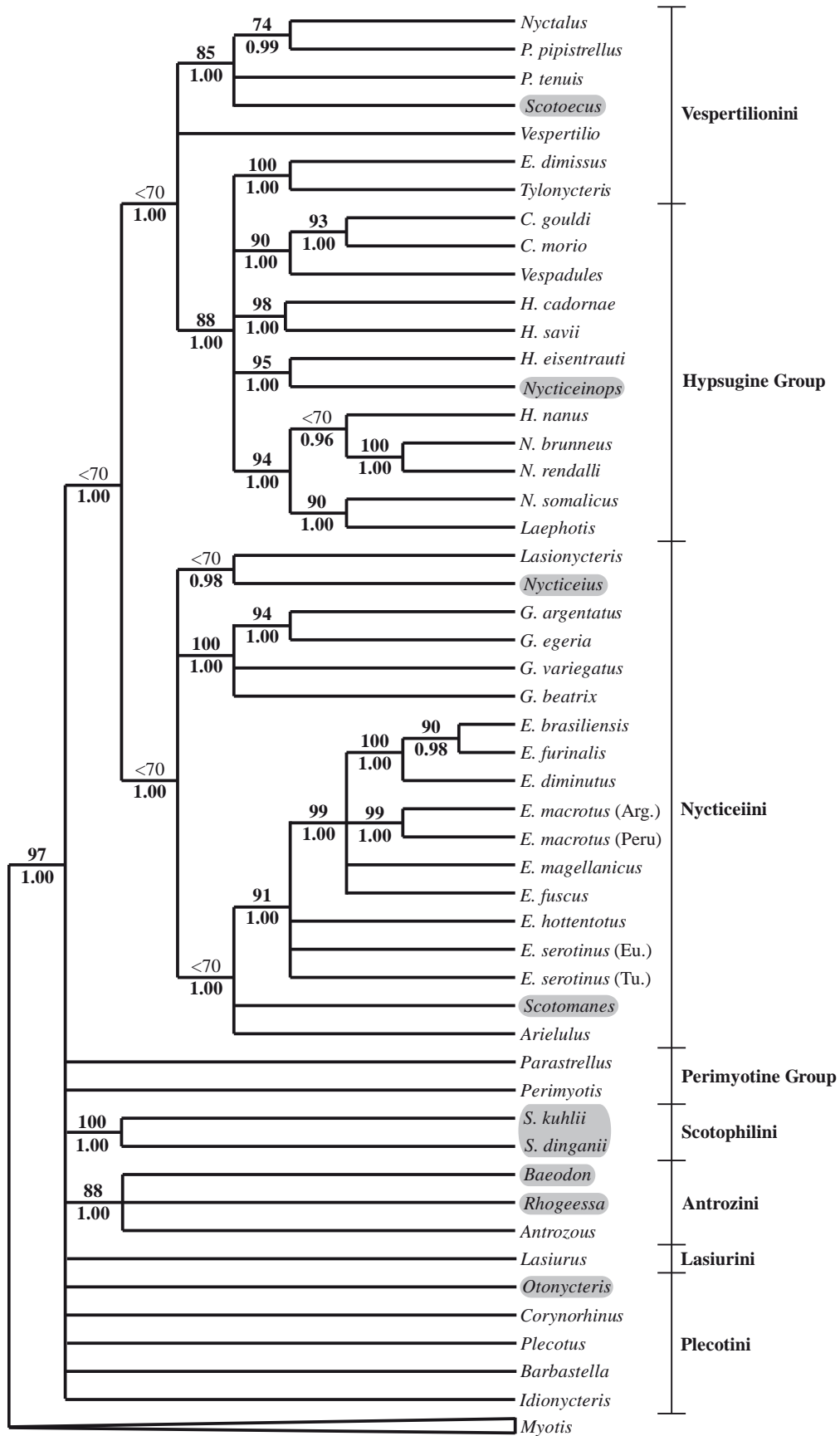
FIGURE LEGENDS

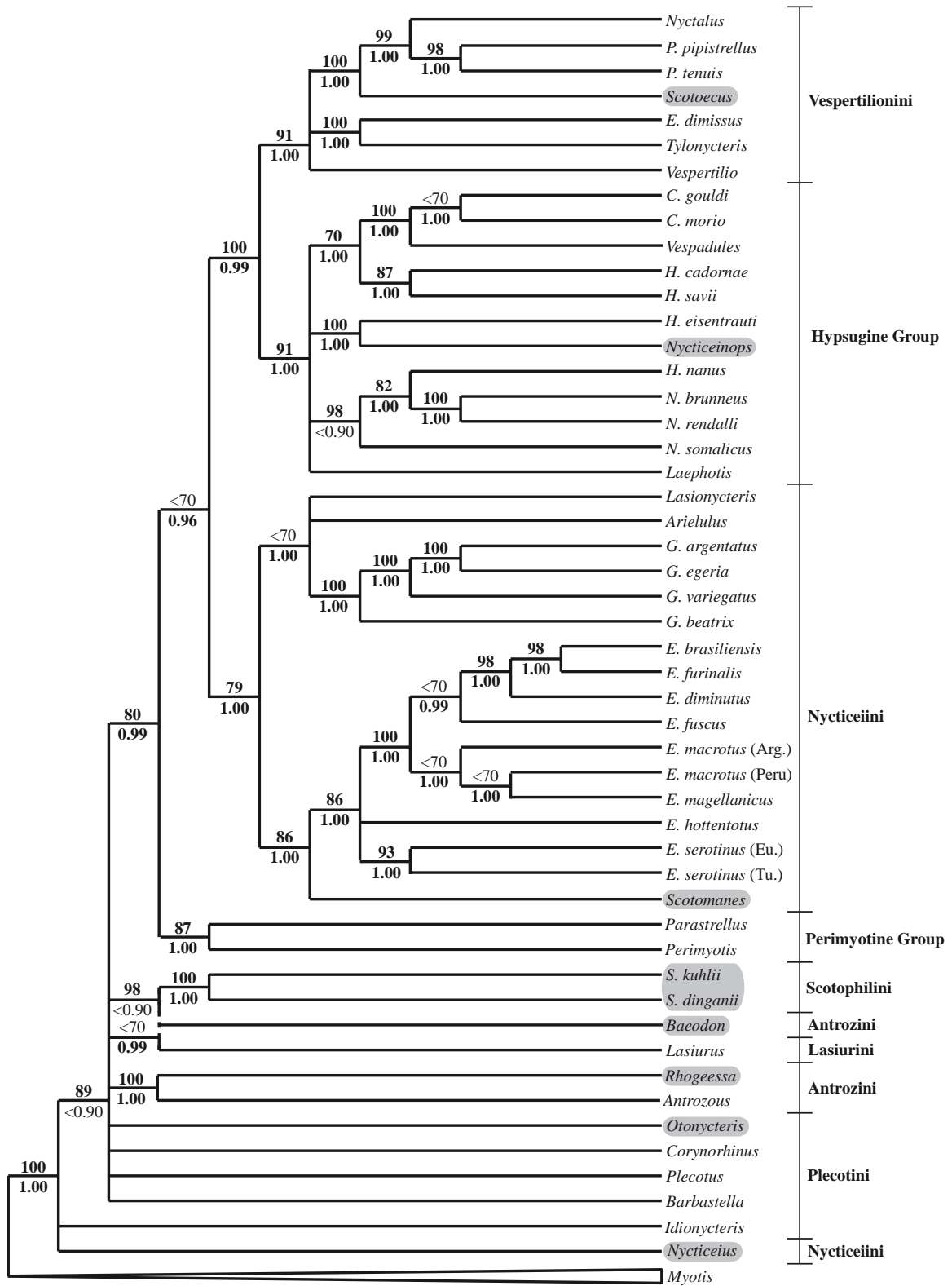
FIG. 3.1.—Cladogram of supported phylogenetic relationships of the vespertilionid bats included in this study based on ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA. Numbers on clade branches indicate support values for maximum parsimony bootstrap (above) and Bayesian posterior probabilities (below). Bolded numbers indicate those that met clade support qualifications for bootstrap ($\geq 70\%$) and posterior probabilities (≥ 0.95). Taxa highlighted with a gray box indicate taxa historically included in tribe Nycticeiini. Taxonomic genera abbreviations include: C. = *Chalinolobus*, E. = *Eptesicus*, G. = *Glauconycteris*, H. = *Hypsugo*, N. = *Neoromicia*, P. = *Pipistrellus*, S. = *Scotophilus*. Locality abbreviations include: Arg. = Argentina, Eu. = Europe, Tu. = Tunisia.

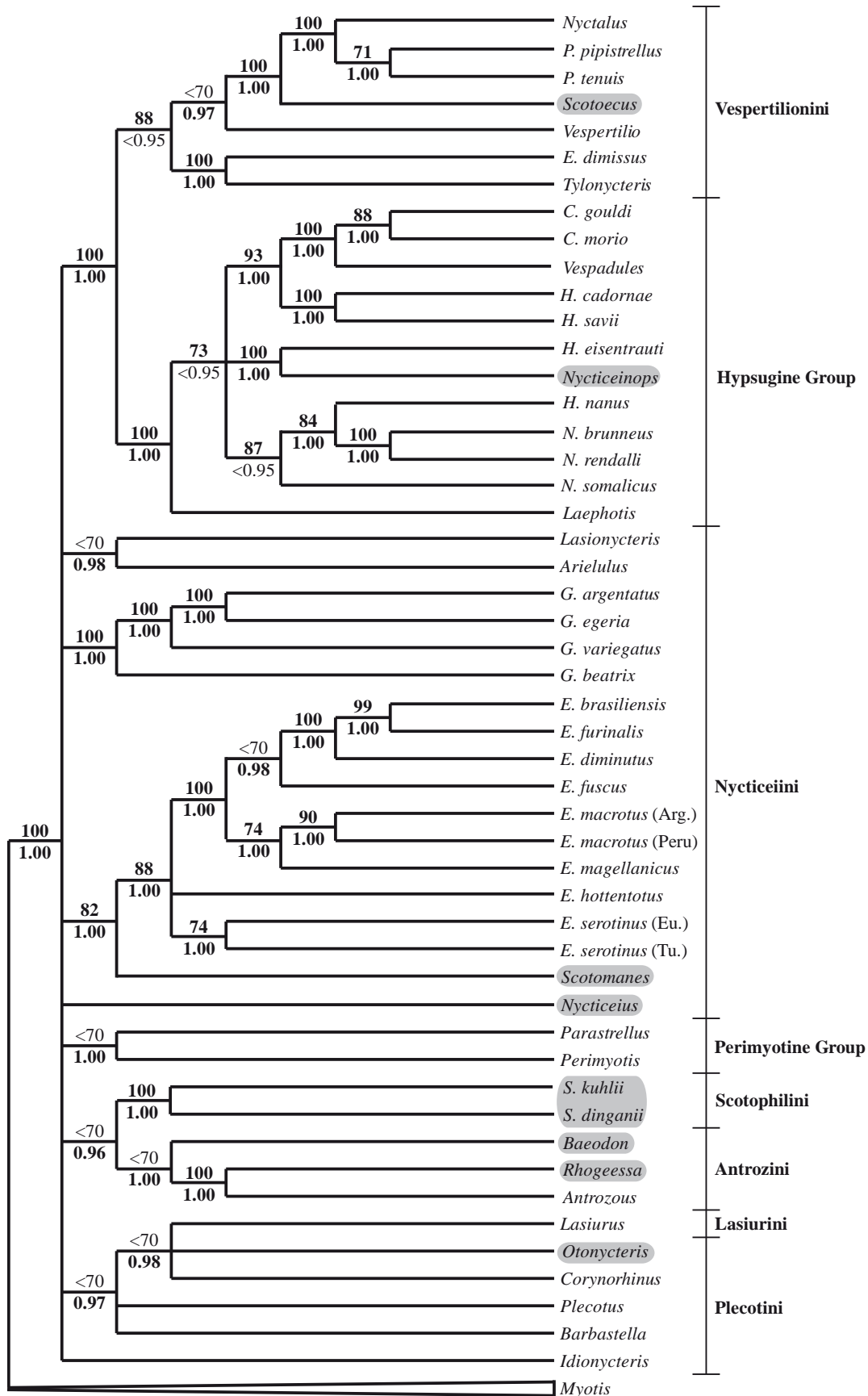
FIG. 3.2.—Cladogram of supported phylogenetic relationships of the vespertilionid bats included in this study based on nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY. Numbers, abbreviations and symbology follow Fig. 3.1.

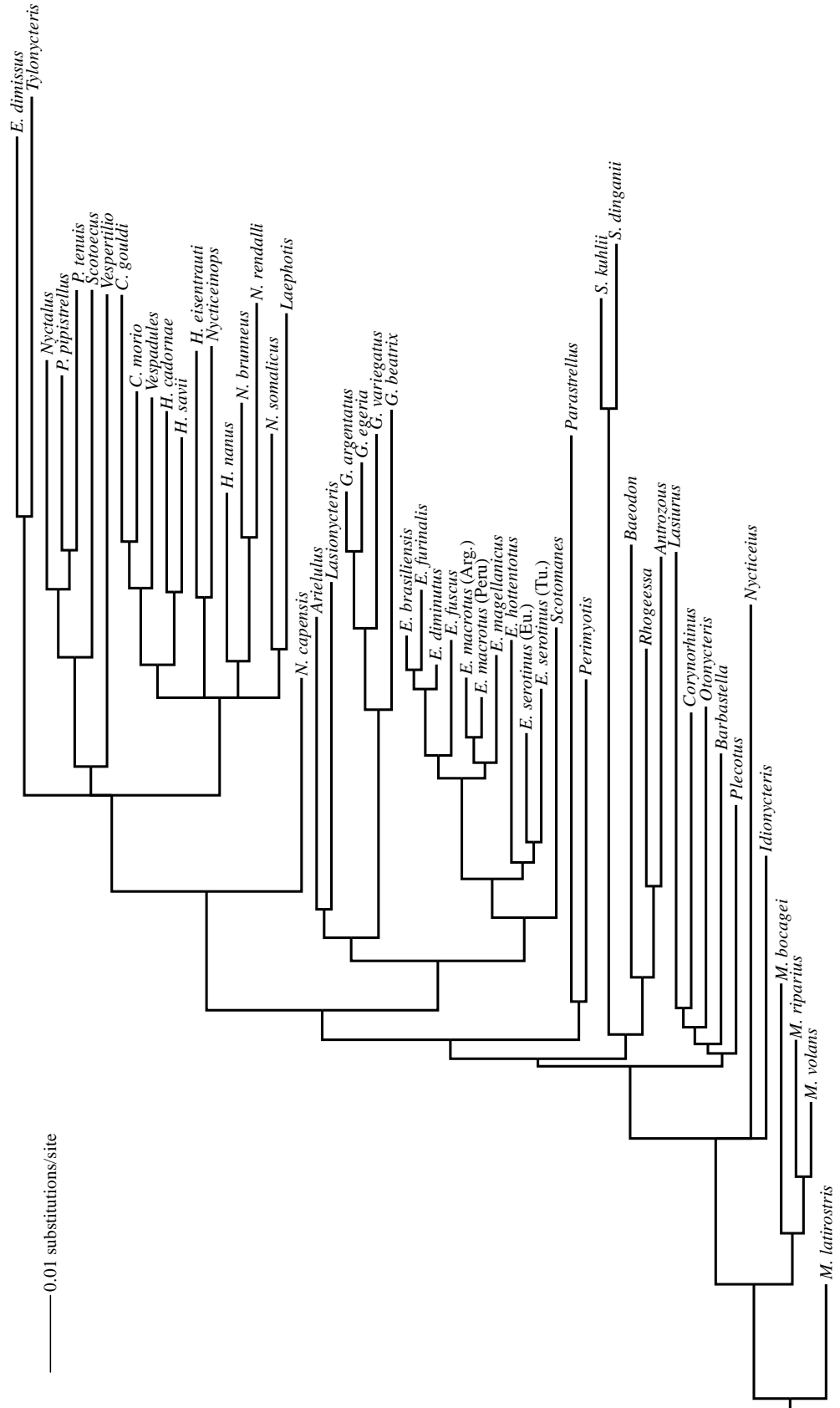
FIG. 3.3.—Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Numbers, abbreviations and symbology follow Fig. 3.1.

FIG. 3.4.—Optimal tree topology from the Bayesian analysis of the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Abbreviations follow Fig. 3.1.









CHAPTER IV

MOLECULAR SYSTEMATICS OF THE *PIPISTRELLUS*-LIKE BATS

ABSTRACT – Reconstructing evolutionary relationships of *Pipistrellus*-like bats has been historically challenging due to evolutionary success of these taxa, a paucity of useful morphological characters, and potential convergent evolution. Three nuclear exons (APOB, DMP1, RAG2) and 3 introns (PRKCI, STAT5A, THY) were sequenced and phylogenetically analyzed in combination with available ribosomal mitochondrial DNA to reexamine previously proposed hypotheses for the evolutionary relationships of *Pipistrellus*-like bats. Phylogenetic analysis of 8,395 aligned positions supported recognition of 4 tribal level clades of *Pipistrellus*-like bats (Eptesicini-Nycticeiini, Hypsugine group, Perimyotine group, and Vespertilionini). Results of this study are largely in agreement with previous research based on mitochondrial DNA and cytogenetics. The only exceptions related to inclusion of *Tylonycteris* and *Vespertilio* in a clade with *Pipistrellus*, *Nyctalus*, and *Scotozous* and a deeply divergent sister relationship between the New World pipistrelles.

INTRODUCTION

Of all the difficulties in reconstructing evolutionary relationships of bats in the subfamily Vespertilioninae, the *Pipistrellus*-like bats (*Arielulus*, *Chalinolobus*, *Eptesicus*,

Eudiscopus, *Falsistrellus*, *Glauconycteris*, *Glischropus*, *Hesperoptenus*, *Histiotus*, *Hypsugo*, *Ia*, *Laephotis*, *Mimetillus*, *Neoromicia*, *Parastrellus*, *Perimyotis*, *Philetor*, *Pipistrellus*, *Nyctalus*, *Scotozous*, *Tylonycteris*, *Vespadelus*, and *Vespertilio*) have drawn the most attention and have had the greatest instability. Early systematists split most of these taxa into *Pipistrellus*-like and *Eptesicus*-like supergeneric groups with differing compositions. Miller (1907), in his seminal work on bats, did not assign formal taxonomic names to a supergeneric rank, but instead described bats as either *Pipistrellus*-like or *Eptesicus*-like based largely on dentition and cranial morphology (Table 4.1). In his foundational work on Vespertilioninae, Tate (1942) grouped all these bats into the tribe Pipistrellini based on the absence of the P³ (shared with his “Nycticeini”) and presence of I² (distinct from his “Nycticeini”). This group was further subdivided into 2 subgroups, Eptesicoid genera with P² absent and Pipistrelloid genera with P² retained (Table 4.1). However, Miller (1907) and Tate (1942) had different constituent taxa in their *Eptesicus*-like and *Pipistrellus*-like bats.

Simpson (1945), on the other hand, synonymized many of these taxa under *Eptesicus* and *Pipistrellus* preserving the relationships of Miller (1907) but demoting their taxonomic rank (Table 4.1). In their studies of Palaearctic and Southern Africa bats, Ellerman and Morrison-Scott (1951) and Ellerman et al. (1953) retained *Pipistrellus* but noted “... *Pipistrellus* is not more than a subgenus of *Eptesicus*, which itself might well be referred to *Vespertilio*” (Ellerman and Morrison-Scott 1951:162). They also felt that *Glauconycteris* was a subgenus of *Chalinolobus*, despite retaining its generic rank. Although Ellerman and Morrison-Scott (1951:137) felt that Simpson had “gone rather too far” in his synonymical taxonomic revision, their systematic conclusions were different

but equally gestalt when it came to *Pipistrellus*-like bats, believing most to belong to the genus *Vespertilio* (Table 4.1). The principle of this latter idea was followed by Koopman (1994) and McKenna and Bell (1997) who placed all *Pipistrellus*-like bats into 1 tribe, Vespertilionini (since *Vespertilio* not *Pipistrellus* had priority), but retained *Eptesicus*, *Pipistrellus*, and *Vespertilio* as distinct genera. Koopman (1994) and McKenna and Bell (1997) also considered *Glauconycteris* as a synonym of *Chalinolobus* and many currently recognized genera (*Arielulus*, *Falsistrellus*, *Hypsugo*, *Neoromicia*, *Perimyotis*, *Parastrellus*, *Scotozous*, and *Vespadelus*) as synonyms of *Pipistrellus*.

Morphological similarity of *Pipistrellus*-like bats has supported the inclusion of all these bats into the subfamily Vespertilioninae, but has made understanding their evolutionary relationships below this rank difficult, as the studies above attest (Ellerman and Morrison-Scott 1951; Miller 1907; Tate 1942). Of particular contention is the usefulness of dentition and tooth morphology to distinguish phylogenetically informative groups within Vespertilioninae (Ärnback-Christie-Linde 1909; Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill and Harrison 1987; Koopman 1975; Rosevear 1962; Tate 1942; Volleth and Heller 1994; Zima and Horáček 1985). The contentious phylogenetic utility of dentition and tooth morphology has led to the search for other characters useful for systematic study of these bats. Over the last 2 decades, 3 additional character sets have been used in systematic studies of *Pipistrellus*-like bats including baculum morphology (Hill and Harrison 1987), cytogenetics (Volleth and Heller 1994; Volleth et al. 2001), and mitochondrial DNA (mtDNA) sequence data (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003). These studies have resulted in relatively unique taxonomic arrangements with results from the cytogenetic and mtDNA sequence

data being largely congruent (Table 4.1). To date, only mtDNA studies have phylogenetically tested previously proposed relationships (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003; Van Den Bussche and Hooper 2000, 2001; Van Den Bussche et al. 2003).

The purpose of this study was to reevaluate evolutionary relationships of *Pipistrellus*-like bats using new sequence data from the nuclear genome combined with previously generated mtDNA sequence data to provide a digenomic reassessment of phylogenetic relationships. These data will provide unique characters to test previously proposed systematic hypotheses (Table 4.1) in a phylogenetic framework. The focus of this study was on higher-level relationships (= ranks: infrafamily, tribe, subtribe) of *Pipistrellus*-like bats. The goal is to provide a resolved and supported phylogenetic hypothesis of evolutionary relationships of these historically problematic taxa and to serve as a starting architecture for elucidating evolutionary relationships of taxa at the genus and species rank. Understanding these evolutionary relationships also provides the foundation for understanding the biogeography and evolution of these taxa.

MATERIALS AND METHODS

Taxonomic sampling.—Tissues from 51 taxa were assembled with the intent of including representatives of most genera historically associated with *Pipistrellus* and *Vespertilio* or included in Pipistrellini or Vespertilionini. Four representatives of *Myotis* were included as outgroups. These 55 taxa are listed below, organized alphabetically by family, subfamily, tribe, and species, with voucher specimen catalog number, tissue catalog number, and a general collecting locality. Taxa included in this study are represented by voucher specimens in the following institutions (Ruedas et al. 2000):

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), Muséum d'Histoire Naturelle, Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB, NK), Museum of Texas Tech University (TTU, TK), Royal Ontario Museum (ROM, F), Texas Cooperative Wildlife Collection at Texas A&M University (TCWC), Universidad Nacional Autónoma de México (UNAM), and University of Lausanne, Switzerland, Institut de Zoologie et d'Ecologie Animale (IZEA).

Family Vespertilionidae: Subfamily Myotinae – *Myotis bocagii* (FMNH150075, FMNH150075), Tanzania: Tanga Region; *Myotis latirostris* (MHNG, M606), Taiwan: Miao-Li County; *Myotis riparius* (AMNH268591, AMNH268591), French Guiana: Paracou; *Myotis volans* (TTU79545, TK78980), U.S.A.: Texas; Subfamily Vespertilioninae – *Otonycteris hemprichii* (CM, SP7882), Jordan: Maan Government, (SP7908) data not provided, (MBQ1226, SP7933) data not provided; *Parastrellus hesperus* (TTU79269, TK78703), U.S.A.: Texas; *Perimyotis subflavus* (TTU80684, TK90671), U.S.A.: Texas; Tribe Antrozoini – *Antrozous pallidus* (TTU71101, TK49646), U.S.A.: Texas; *Baeodon alleni* (UNAM, TK45023), Mexico: Michoacán; *Rhogeessa parvula* (TTU36633, TK20653), Mexico: Sonora; Tribe Lasiurini – *Lasiurus cinereus* (TTU, TK78926), U.S.A.: Texas; Tribe Nycticeiini – *Arielulus aureocollaris* (ROM106169, F38447), Vietnam: Tuyen Quang; *Eptesicus dimissus* (MHNG1926.053, M1187), Laos; *Eptesicus fuscus* (CM102826, SP844), U.S.A.: West Virginia; *Eptesicus hottentotus* (type, CM89000, TK33013), Kenya: Rift Valley Province; *Eptesicus*

macrotus (FMNH129207, FMNH129207), Peru: Ancash; *Glauconycteris argentatus* (FMNH15119, FMNH15119), Tanzania: Kilimanjaro Region; *Glauconycteris beatrix* (FMNH149417, FMNH149417), Zaire [=Democratic Republic of the Congo]: Haut-Zaire; *Glauconycteris egeria* (AMNH268381, AMNH268381), Central African Republic, (AMNH109067, AMNH109067), data not provided; *Glauconycteris variegatus* (CM97983, TK33545), Kenya: Western Province; *Lasionycteris noctivagans* (TTU56255, TK24216), U.S.A.: Texas; *Nycticeius humeralis* (TTU49536, TK26380), U.S.A.: Texas, (TTU80664, TK90649), U.S.A.: Texas; *Scotomanes ornatus* (ROM107594, F42568), Vietnam: Tuyen Quang; Tribe Pipistrellini – *Nyctalus leisleri* (FMNH140374, FMNH140374), Pakistan: Malakand Division; *Nyctalus noctula* (IZEA, Nno1), Switzerland: Canton of Berne; *Pipistrellus coromandra* (FMNH140377, FMNH140377), Pakistan: Malakand Division; *Pipistrellus javanicus* (FMNH147069, FMNH147069), Republic of the Philippines: Mindanao Island; *Pipistrellus hesperidus*, (DM8013, DM8013), South Africa: KwaZulu-Natal Province; *Pipistrellus nathusii* (MHNG1806.003, IZEA2830), Switzerland: Vaud, (MHNG1806.001, IZEA3406), Switzerland: Vaud, (TTU, TK81167), data not provided; *Pipistrellus paterculus* (MHNG1926.045, M1181), Laos: Phôngsali Province; *Pipistrellus pipistrellus* (MHNG1956.031, M1439), Switzerland; *Pipistrellus pygmaeus* (MHNG1806.032, IZEA3403), Spain: Barcelona Province; *Pipistrellus tenuis* (FMNH137021, FMNH137021), Republic of the Philippines: Sibuyan Island; *Scotoecus hirundo* (FMNH151204, FMNH151204), Tanzania: Kilimanjaro Region; Tribe Plecotini – *Barbastella barbastellus* (MHNG1804.094, IZEA3590), Switzerland: Valais; *Corynorhinus rafinesquii* (TTU45380, TK5959), U.S.A.: Arkansas; *Idionycteris phyllotis*

(ACU736, ACU736), U.S.A.: Arizona, (MSB12091, NK36122), U.S.A.: Utah; *Plecotus auritus* (MHNG1806.047, IZEA2694), Switzerland: Valais; Tribe Scotophilini – *Scotophilus kuhlii* (FMNH145684, FMNH145684), Republic of the Philippines: Sibuyan Island; Tribe Vespertilionini – *Chalinolobus gouldi* (TCWC, RLH27), Australia; *Chalinolobus morio* (TCWC, 05M3), Australia; *Hypsugo cadornae* (MHNG1926.050, M1183), Laos: Phôngsali Province; *Hypsugo eisentrauti* (ROM100532, F34348), Ivory Coast; *Hypsugo savii* (MHNG1804.100, IZEA3586), Switzerland: Valais; *Laephotis namibensis* (CM93187, SP4160), Namibia: Maltahöhe District; *Neoromicia brunneus* (CM90802, TK21501), Gabon: Estuaire Province; *Neoromicia nanus* (CM98003, TK33378), Kenya: Eastern Province, (DM7542, DM7542), South Africa: KwaZulu-Natal Province; *Neoromicia rendalli* (CM97977, TK33238), Kenya: Coastal Province; *Neoromicia somalicus* (CM97978, TK33214), Kenya: Coastal Province; *Nycticeinops schlieffeni* (CM97998, TK33373), Kenya: Eastern Province; *Tylonycteris pachypus* (ROM106164, F38442), Vietnam: Tuyen Quang; *Tylonycteris robustula* (MHNG1926.059, M1203), Laos: Phôngsali Province; *Vespadelus regulus* (TCWC, RLH30), Australia; *Vespadelus vulturinus* (TCWC, RLH16), Australia; *Vespertilio murinus* (MHNG1808.017, IZEA3599), Switzerland: Valais.

Extraction, amplification, and sequencing.—The procedures of Longmire et al. (1997) or the DNeasy Tissue Kit (Qiagen, Austin, Texas) were used to extract genomic DNA from tissue samples for each taxon included in this study. PCR amplification and sequencing reactions for 3 nuclear exons Apolipoprotein B (APOB), Dentin Matrix Acidic Phosphoprotein I (DMP1), and Recombination Activating Gene II (RAG2), and 3 nuclear intron regions from Protein Kinase C, Iota (PRKCI), Signal Transducer and

Activator of Transcription 5A (STAT5A), and Thyrotropin (THY) follow procedures outlined in Roehrs (2009: Chapter 2). Sequence data for the 12S rRNA, tRNA^{Val}, and 16S rRNA ribosomal genes were largely obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) from previous research in the Van Den Bussche laboratory at Oklahoma State University (Hooper and Van Den Bussche 2001, 2003; Hooper et al. 2003; Van Den Bussche and Hooper 2000, 2001; Van Den Bussche et al. 2003). To supplement these data 7 additional taxa (*A. aureocollaris*, *E. dimissus*, *H. cadornae*, *P. hesperidus*, *P. paterculus*, *P. pipistrellus*, and *T. robustula*) were sequenced for these same ribosomal mtDNA genes by Roehrs (2009: Chapter 2) and included here. Also obtained from GenBank were sequence data for the PRKCI, STAT5A and THY markers of *E. hottentotus* and *N. schlieffeni* as published by Eick et al. (2005).

Phylogenetic analysis.—The program Geneious 4.5.4 (Biomatters Ltd. Auckland, New Zealand) was used to assemble forward and reverse sequences for each gene region and then align them in Geneious using the ClustalW 1.83.XP algorithm (Thompson et al. 1994). These alignments were then manually optimized in the program MacClade 4.05 (Maddison and Maddison 2002). During alignment optimization, ambiguously aligned sites were identified using procedures of Lutzoni et al. (2000) and subsequently excluded from phylogenetic analysis because they could possibly violate assumptions of positional homology. Concatenation of gene regions for phylogenetic analysis was employed to create 3 data partitions: 1) mtDNA = concatenation of 12S rRNA, tRNA^{Val}, and 16S rRNA; 2) nDNA = concatenation of APOB, DMP1, RAG2, PRKCI, STAT5A, and THY; 3) combined = concatenation of mtDNA and nDNA. Since gene regions can have differing compositional biases and substitution rates, concatenation of these gene regions

was only conducted after separate analysis of each gene region and comparison of resulting gene trees. Congruence of supported topologies has been previously documented for the mtDNA (Van Den Bussche and Hooper 2000) and nDNA (Lack et al. 2009; Roehrs 2009: Chapter 2) gene regions. Therefore, phylogenetic analysis for each independent gene region was not conducted in this study. However, possible inconsistencies between mtDNA and nDNA gene trees and the appropriateness of concatenation of these datasets was examined using a concordance test requiring 90% agreement of supported clades (De Queiroz 1993). Clades were considered supported if they had a maximum parsimony (MP) bootstrap value $\geq 70\%$ and a Bayesian posterior probability of ≥ 0.95 .

Each data partition was analyzed using MP in PAUP* v4.0b10 (Swofford 2002) and Bayesian phylogenetic methods in MRBAYES v3.1.2 (Huelsenbeck and Ronquist 2001). Parameters for the MP analysis included unweighted nucleotide substitutions in a heuristic search with 25 random additions of taxa, Tree-Bisection-Reconnection branch swapping, and 1,000 bootstrap replicates to quantify nodal support. The Bayesian analysis was conducted with a 4 chain (3 hot, 1 cold) parallel Metropolis-coupled Markov chain Monte Carlo running for 2×10^6 generations with sampling every 10 generations at a 0.02 temperature. Analysis began with a random unconstrained tree, uniform priors, and burn-in values were determined by plotting likelihood scores on generation time and identifying the point at which model parameters and tree scores become stationary.

RESULTS

mtDNA sequences.—Fifty five ribosomal mtDNA sequences were previously generated (Hooper and Van Den Bussche 2003; Roehrs 2009: Chapter 2) and provided

2,889 aligned positions, of which 903 were excluded prior to analysis for potential violation of positional homology. Of the remaining 1,986 positions, 784 were variable and 561 were phylogenetically informative. Fifteen trees of 3,824 steps were retained in the MP analysis, with 22 supported clades (bootstrap values $\geq 70\%$; Fig. 4.1), a consistency index excluding uninformative characters (CI) of 0.2418, and a retention index (RI) of 0.4305. The majority of differences between these 15 trees was due to relationships between out-group taxa; taxa traditionally aligned with Antrozoini, Plecotini, and *Lasiurus*; or the position of *Nycticeius* and *Idionycteris* within Nycticeiini (*sensu* Hooper and Van Den Bussche 2003) and are not the focus of this study. However, 2 issues of variable topology are of direct interest in this study. The 1st relates to the relationship of the 4 clades comprising the Hypsugine group with the topology ((1,2),(3,4)) in some trees and (2,(3,(1,4))) in others (Fig. 4.1). The 2nd issue in topology variation dealt with interrelations of the *Neoromicia-Laephotis* taxa, where (C,(A,B)) was reflected in some topologies and (B,(A,C)) in others (Fig. 4.1). The Bayesian analysis supported a topology most similar to ((1,2),(3,3)); however, relationships between (1,2), 3 and 4 were not resolved. This analysis also supported a (C,(A,B)) cladel arrangement. A burn-in value of 25,740 generations was used for the Bayesian analysis and resulted in 32 supported clades (≥ 0.95 posterior probability; Fig. 4.1).

nDNA sequences.—Of the 55 taxa included in this study, 38 had complete sequence data for the 6 nDNA gene regions; the remaining 17 taxa are missing some sequence data (20–25% of nDNA dataset). The STAT5A gene region was the most difficult to amplify and was not generated for 15 taxa: *G. beatrix*, *G. egeria*, *N. leisleri*, *N. noctula*, *P. coromandra*, *P. hesperidus*, *P. javanicus*, *P. nathusii*, *P. tenuis*, *S. hirundo*,

H. cadornae, *H. savii*, *T. pachypus*, *T. robustula*, and *V. murinus*. Reanalyzing data by removing STAT5A from the dataset does not result in changes in clade support or topological resolution. APOB and DMP1 sequence data also were missing for *V. vulturinus*, and only the 1st 770 positions of RAG2 were available for *B. alleni* (Roehrs 2009: Chapter 2). All other gene regions were sequenced completely and included for these taxa. Despite missing data, there were 5,506 aligned positions in the nDNA dataset, of which 783 were excluded prior to analysis for potential violations of positional homology. The remaining 4,723 positions had 1,869 variable positions of which 1,118 were phylogenetically informative. The MP analysis resulted in 6 most parsimonious trees of 5,111 steps, 31 supported clades (bootstrap values $\geq 70\%$), and a CI of 0.4752 and a RI of 0.6117 excluding uninformative characters (Fig. 4.2). Differences between the 6 most parsimonious trees were due to differences in topological relationships between taxa historically associated with Antrozoini, Plecotini, *Lasiurus* and *Scotophilus* and variation in the positions of *Arielulus* and *Lasionycteris* within Nycticeiini (*sensu* Hooper and Van Den Bussche 2003 minus *Nycticeius*). In some tree topologies *Arielulus* and *Lasionycteris* were sister to a clade consisting of *Eptesicus* and *Scotomanes* and in others a clade of *Glauconycteris*. The Bayesian analysis had a burn-in value of 28,580 generations and resulted in a tree with 38 clades supported by posterior probabilities ≥ 0.95 (Fig. 4.2).

Combined sequences.—Despite 2 supported discrepancies between the mtDNA and nDNA gene trees, these data met the 90% concordance rule (De Queiroz 1993) and were concatenated for the combined analysis. Both discrepancies were at tip branches and beyond the primary focus of this study. The 1st of these discrepancies related to the

sister taxon of *P. coromandra*, which was *P. tenuis* in the mtDNA gene tree (Fig. 4.1) and *P. javanicus* in the nDNA gene tree (Fig. 4.2). The 2nd discrepancy was in the relationships of *E. dimissus*, *T. pachypus*, and *T. robustula* that together formed a supported clade in both gene trees. In the mtDNA gene tree, the 2 *Tylonycteris* taxa were sister and *E. dimissus* was basal to them (Fig. 4.1), whereas in the nDNA gene tree, *E. dimissus* was sister to *T. robustula* and *T. pachypus* was basal to this clade (Fig. 4.2). The concatenated dataset resulted in 8,395 aligned positions, of which 1,687 positions were excluded prior to analysis for possible violation of positional homology. Of the remaining 6,708 positions, 2,653 were variable and 1,679 were phylogenetically informative. The MP analysis resulted in 3 most parsimonious trees, with 9,055 steps and 28 supported clades (bootstrap values $\geq 70\%$; Fig. 4.3). The MP analysis had a CI of 0.3612 and a RI of 0.5166, excluding uninformative characters. Differences in tree topology of the 3 most parsimonious trees were related to the variable relationships between unresolved *Pipistrellus* clades (Fig. 4.3). The Bayesian analysis had a burn-in of 26,950 generations and resulted in a tree with 30 supported clades (posterior probability ≥ 0.95 ; Fig. 4.3).

DISCUSSION

Elucidating evolutionary relationships of *Pipistrellus*-like bats has been historically challenging, primarily because they constitute a large number of taxa in the evolutionarily successful Vespertilioninae, these taxa have a paucity of useful morphological characters for systematic study, and there is evidence of convergent evolution in different subclades (Ärnäck-Christie-Linde 1909; Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill and Harrison 1987; Horáček and Zima 1978;

Koopman 1975; Rosevear 1962; Tate 1942; Volleth and Heller 1994; Zima and Horáček 1985). The goal of this study was to use available mtDNA and newly generated nDNA data to provide a resolved phylogeny allowing for reexamination of previous hypotheses of evolutionary relationships of these taxa. This goal was largely achieved by generating a combined gene tree that is resolved at many nodes relevant to the supergeneric focus of this study. Although there is agreement (90% of supported nodes) between the nDNA and mtDNA dataset used in this study and the results of Hooper and Van Den Bussche (2003) based on mtDNA sequence data, the results presented here provide a slightly different picture of the evolutionary relationships between *Pipistrellus*-like taxa.

Systematic conclusions.—The combined gene tree supported 3 clades of the *Pipistrellus*-like bats included in this study that are here assigned tribal taxonomic rank (Fig. 4.3; Table 4.2). This is in agreement with a concomitant study of phylogenetic relationships within Vespertilioninae (Roehrs 2009: Chapter 2). The 1st clade is made up of the genera *Nyctalus*, *Pipistrellus* (*sensu* Simmons 2005), *Scotoecus*, *Tylonycteris*, and *Vespertilio*. The inclusion of *Vespertilio* in this clade (which has priority) would require this clade be named Vespertilionini (Roehrs 2009: Chapter 2). The close relationship of *Nyctalus* and *Pipistrellus* has been recognized and generally supported since Tate (1942), but *Scotoecus* has been associated historically with *Nycticeius* (see Roehrs 2009: Chapter 3 for detailed discussion). Furthermore, the relationship of *Tylonycteris* and *Vespertilio* to these other pipistrelloid taxa is a rather unique phylogenetic hypothesis. Although inclusion of *Tylonycteris* and *Vespertilio* in a tribe with *Nyctalus* and *Pipistrellus* is supported in the combined and nDNA gene trees, this position is unresolved in the mtDNA gene tree (unsupported by MP analysis) and in disagreement with the mtDNA

results of Hooper and Van Den Bussche (2003) with respect to *Tylonycteris*. As reported previously (Roehrs 2009: Chapter 2), inclusion of *E. dimissus* in the *Tylonycteris* clade is in need of further examination including verification of the voucher specimen identification before drawing any taxonomic conclusions. The genus *Pipistrellus* is paraphyletic with respect to the supported position of *Nyctalus* in the mtDNA gene tree (as found by Hooper and Van Den Bussche 2003), but the combined (and nDNA) analysis can neither support nor refute this conclusion. These results do support a close relationship of *Nyctalus* and *Pipistrellus*, but the combined gene tree does not resolve the relationship of *Nyctalus*, *P. nathusii*, and 2 *Pipistrellus* clades. The 1st of these *Pipistrellus* clades includes the Southeast Asian centered species *P. coromandra*, *P. javanicus*, *P. paterculus*, and *P. tenuis*, and the 2nd clade consists of a sister relationship of western Eurasian *P. pipistrellus* and *P. pygmaeus* with a basal African *P. hesperidus*. *Scotoecus* is supported basal to the *Pipistrellus-Nyctalus* clade. It is obvious based on these results and recent discovery of a number of cryptic species (Benda et al. 2004; García-Mudarra et al. 2009; Hulva et al. 2004, 2007; Ibáñez et al. 2006; Racey et al. 2007) that more research will be necessary to elucidate phylogenetic relationships within *Pipistrellus*.

The 2nd tribal clade supported in the combined gene tree consists of the genera *Chalinolobus*, *Vespadelus*, *Hypsugo*, *Nycticeinops*, *Neoromicia*, and *Laephotis*. The most appropriate tribe name for this clade would be ‘Hypsugini’ because *Hypsugo* Kolenati, 1856 has priority. However, because this tribe name is currently a *nomen nudum*, it will be referred to as the Hypsugine group through the remainder of this paper. This designation is only tentative and warrants further study before being formally

adopted. Relationships of taxa within the Hypsugine group largely concur with results of Hooper and Van Den Bussche (2003), except aforementioned *Tylonycteris* and *Vespertilio*. The Hypsugine group is divided into 3 clades whose relationship to each other is unresolved. One Hypsugine clade consists of a sister relationship between Australasian taxa in the genera *Chalinolobus* and *Vespadelus*, with a clade consisting of *H. cadornae* and *H. savii* basal to that clade. Another clade in the Hypsugine group consists of the African *Nycticeinops* and *H. eisentrauti*. To avoid *Hypsugo* paraphyly, *H. eisentrauti* is transferred to the genus *Nycticeinops* as recommended previously by Hooper and Van Den Bussche (2003). It is apparent from these results that *Hypsugo*, as currently defined, may be paraphyletic and will require further investigation of species not included here. The remaining clade within the Hypsugine group contains the African genera *Laephotis* and *Neoromicia*. It also includes the only topological difference between the nDNA and mtDNA gene trees. This topological difference relates to the position of *Laephotis*, who forms a sister relation to *N. somalicus* in the mtDNA gene tree and is unresolved within the Hypsugine group in the nDNA gene tree. It has been suggested previously that *Neoromicia* is paraphyletic with respect to *Laephotis* based on bacular morphology (Kearney et al. 2002) and mtDNA (Hooper and Van Den Bussche 2003). Based on biogeography, a close relationship between *Laephotis* and *Neoromicia* is not surprising, but a systematic review of all taxa in these genera will be necessary before taxonomic revision can be made. I recommend tentative retention of these genera until this issue is more fully examined.

The 3rd *Pipistrellus*-like tribe contains the deeply split sister relationship between the 2 New World pipistrelles, *Parastrellus* and *Perimyotis* as discussed in Roehrs (2009:

Chapter 2). Although this relationship is supported in the combined analysis, it is unresolved in all mtDNA gene trees (Fig. 4.1; Hooper and Van Den Bussche 2003). If this relationship is found to be supported by further research, the most appropriate name for a tribe including these extant taxa would be 'Perimyotini' because the genus *Perimyotis* Menu, 1984 has priority. Finally, evolutionary relationships of the remaining *Pipistrellus*-like bats (*Arielulus*, *Eptesicus*, *Glauconycteris*, and *Lasionycteris*) remained unresolved in the combined gene tree with the exception of support for independent *Eptesicus* (including *Histiotus*) and *Glauconycteris* clades (Fig. 4.3). Hooper and Van Den Bussche (2003) grouped these taxa along with *Nycticeius* into the tribe Nycticeiini. In their analysis and our mtDNA gene tree, only the Bayesian posterior probabilities supported this relationship (Fig. 2.1); the nDNA gene tree also supported that relationship, except for the exclusion of *Nycticeius* (Fig. 2.2). As discussed previously (Roehrs 2009: Chapter 2 and 3), until the position of *Nycticeius* is resolved what appears to be the 4th tribe of *Pipistrellus*-like bats (Eptesicini or Nycticeiini) remains ambiguous.

Phylogenetic reevaluation.—Historically, systematic study of *Pipistrellus*-like bats has resulted in a myriad of phylogenetic hypotheses, with some taxa changing rank, position, or circumscription in each new reexamination (Table 4.1). Of all previous work in this area, results presented here are most in line with those based on mitochondrial ribosomal DNA (Hooper and Van Den Bussche 2003) and cytogenetics (Volleth and Heller 1994; Volleth et al. 2001). The only major difference between their phylogenetic hypotheses and those presented here is the placement of *Tylonycteris* and *Vespertilio* in a clade with *Pipistrellus* and the necessary changes in tribal nomenclature that result. This result at 1st glance would be rejected by the relationship of bacular morphology

suggested by Hill and Harrison (1987), but considering the triangular saddle-like bacula to be ancestral in Vespertilioninae while elongation and alternate tip shapes to be derived, then it can be hypothesized that *Tylonycteris* and *Vespertilio* are members of Vespertilionini (possibly basal) that retain the ancestral character. Explanations for the chromosomal patterns observed by Volleth and Heller (1994) would be less parsimonious based on the nDNA gene tree. The tentative nature of this result must be stressed because a number of taxa are absent from these data including *Falsistrellus*, *Glischropus*, *Nyctophilus*, *Philetor*, *Scotorepens*, *Scotozous*, and many *Pipistrellus* species, which may be important in gaining confident resolution of this issue.

With regards to the tentatively proposed Perimyotine group, the mtDNA gene tree presented in this study and results of Hooper and Van Den Bussche (2003) can neither support nor reject this hypothesis. Bacular, cytogenetic, and molecular sequence data indicate a distant evolutionary relationship between *Perimyotis* and *Parastrellus*, but only the nDNA data support a sister relationship between these taxa. In some of the earliest work on Vespertilionidae bacula, Hamilton (1949) suggested these taxa were so dissimilar that they warranted, at minimum, subgeneric distinction. In 1 of the 1st comparisons of chromosomes, Baker and Patton (1967) felt that these genera could only be distantly related due to *Parastrellus* lacking a pair of chromosomes present in *Perimyotis*. Menu (1984), who described the genus *Perimyotis*, separated it from other *Pipistrellus* based on its dentition, skeletal, and bacular morphology and felt the genus was more closely related to *Myotis* than Old World *Pipistrellus* and *Parastrellus*. *Parastrellus* was 1st suggested by Horáček and Hanák (1985; 1985-1986) and formally described by Hooper et al. (2006) based on dental, bacular, and karyotypic characters.

Heller and Volleth (1984) felt the relationship of these taxa to *Pipistrellus* was unclear based on karyology, but Hill and Harrison (1987) included them within *Pipistrellus* based on bacular morphology. Hill and Harrison (1987) hypothesized that *Parastrellus* was more aligned to the subgenus *Hypsugo* and *Perimyotis* to the subgenus *Arielulus* based on bacular morphology yet *Perimyotis* was related to the subgenus *Pipistrellus* based on rostral and dental features (Hill and Harrison 1987). There is substantial evidence to reject inclusion of these taxa in a monophyletic *Pipistrellus*, but their relationship to *Pipistrellus*-like bats has been unclear until this study. The 2 most likely hypotheses for the relationship between *Parastrellus* and *Perimyotis* observed in the combined gene tree is that they are sister taxa with very distant relationships and therefore warrant tribal recognition or, alternatively, this relation is an artifact of mutational saturation causing long-branch attraction within *Pipistrellus*-like bats.

Finally, these results clearly reject a close association between *Chalinolobus* and *Glauconycteris* (Dobson 1878; Ellerman and Morrison-Scott 1951; Koopman 1994; McKenna and Bell 1997; Miller 1907; Simpson 1945); the paraphyletic inclusion of *Arielulus*, *Hypsugo*, *Neoromicia*, and *Vespadelus* in *Pipistrellus* (Ellerman and Morrison-Scott 1951; Hill and Harrison 1987; Koopman 1994; McKenna and Bell 1997; Simpson 1945; Tate 1942); or alternatively the alignment of *Neoromicia* and *Vespadelus* with *Eptesicus* (Adams et al. 1987; Hall and Woodside 1989; Hayman and Hill 1971; Kingdon 1974; Kitchener et al 1987; McKean et al. 1978; Rosevear 1965; Tate 1942). These results lend support to the contention that convergent evolutionary forces during diversification of Vespertilioninae have led to the development of similar forms in different biogeographic regions and raises interesting questions about ecological and

biogeographic constraints that have led to this pattern. Further study into Vespertilioninae is needed to resolve remaining evolutionary relationships and phylogenetic discrepancies. Despite implementation of a large multigenetic dataset comprised of gene regions successfully used to resolve similar evolutionary relationships in other groups, these results demonstrate that future studies will require more taxa and more DNA data to resolve this historically difficult group. Recent studies have underscored the importance of proper prior selection of these taxa and post alignment evaluation of gene regions and codon positions for the removal of data that show elevated rates of evolution (Baurain et al. 2007; Brinkmann and Philippe 2008; Rodríguez-Ezpeleta et al. 2007).

ACKNOWLEDGMENTS

Justin B. Lack, Matt Leslie, Craig E. Stanley, Jr., and Sara W. Weyandt deserve thanks for generating various portions of sequence data included in this project. I thank Geeta Eick for providing primers and advice on PCR profiles for nuclear introns used in this study. Steve Hartson, Janet Rogers, and Lisa Whitworth of the Oklahoma State University Recombinant DNA/Protein Core Facility I also thank for allowing me to use their equipment and for assistance in trouble shooting. I thank Janet K. Braun, Meredith J. Hamilton, David M. Leslie, Meredith A. Magnuson, Ronald J. Tyrl, and Ronald A. Van Den Bussche for reviewing 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. Any project like this would not be possible without the support and loan of tissues from

individuals and institutions including: Robert J. Baker of the Natural Sciences Research Laboratory of the Museum of Texas Tech University; Nancy B. Simmons of the American Museum of Natural History; Bruce D. Patterson, Lawrence R. Heaney and William T. Stanley of the Field Museum of Natural History; Suzanne B. McLaren of the Carnegie Museum of Natural History; Mark D. Engstrom and Burton Lim of the Royal Ontario Museum; Manuel Ruedi of the Muséum d'Histoire Naturelle, Genève; Peter J. Taylor of the Durban Natural Science Museum; Terry L. Yates and Joseph Cook of the Museum of Southwestern Biology at the University of New Mexico; Thomas E. Lee, Jr., of Abilene Christian University; and Rodney L. Honeycutt and Duane Schlitter of the Texas Cooperative Wildlife Collection at Texas A&M University.

LITERATURE CITED

- ADAMS, M., P. R. BAVERSTOCK, C. H. S. WATTS, AND T. REARDON. 1987. Electrophoretic resolution of species boundaries in Australian Microchiroptera. I. *Eptesicus* (Chiroptera: Vesperilionidae). Australian Journal of Biological Sciences 40:143–162.
- ÄRNBÄCK-CHRISTIE-LINDE, A. 1909. On intermediate forms among Chiroptera. Zoologischer Anzeiger 34:572–582.
- BAKER, R. J., AND J. L. PATTON. 1967. Karyotypes and karyotypic variation of North American vespertilionid bats. Journal of Mammalogy 48:270–286.
- BAURIAN, D., H. BRINKMANN, AND H. PHILIPPE. 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? Molecular Biology and Evolution 24:6–9.

- BENDA, P., P. HULVA, AND J. GAISLER. 2004. Systematic status of African populations of *Pipistrellus pipistrellus* complex (Chiroptera: Vespertilionidae), with a description of a new species from Cyrenaica, Libya. *Acta Chiropterologica* 6:193–217.
- BRINKMANN, H., AND H. PHILIPPE. 2008. Animal phylogeny and large-scale sequencing: progress and pitfalls. *Journal of Systematics and Evolution* 46:274–286.
- DE QUEIROZ, A. 1993. For consensus (sometimes). *Systematic Biology* 42:368–372.
- DOBSON, G. E. 1878. Catalog of the Chiroptera in the collection of the British Museum. British Museum (Natural History), London, United Kingdom.
- EICK, G. N., D. S. JACOBS, AND C. A. MATTHEE. 2005. A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Molecular Biology and Evolution* 22:1869–1886.
- ELLERMAN, J. R., AND T. C. S. MORRISON-SCOTT. 1951. Checklist of Palaearctic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London, United Kingdom.
- ELLERMAN, J. R., T. C. S. MORRISON-SCOTT, AND R. W. HAYMAN. 1953. Southern African mammals 1758 to 1951: a reclassification. Trustees of the British Museum (Natural History), London, United Kingdom.
- GARCÍA-MUDARRA, J. L., C. IBÁÑEZ, J. JUSTE. 2009. The Straits of Gibraltar: barrier or bridge to Ibero-Moroccan bat diversity? *Biological Journal of the Linnean Society* 96:434–450.
- HALL, L. S., AND D. P. WOODSIDE. 1989. Vespertilionidae. Pp. 1–41 in *Fauna of Australia, Volume 1B Mammalia* (D. W. Walton and B. J. Richardson, eds.).

Australian Government Publishing Service, Canberra, Australian Capital Territory, Australia.

HAMILTON, W. J., JR. 1949. The bacula of some North American vespertilionid bats.

Journal of Mammalogy 30:97–102.

HAYMAN, R. W., AND J. E. HILL. 1971. Part 2. Order Chiroptera. Pp. 1–73 in The

mammals of Africa: an identification manual (J. A. J. Meester and H. W. Seteer, eds.). Smithsonian Institution Press, Washington D.C.

HELLER, K. G., AND M. VOLLETH. 1984. Taxonomic position of “*Pipistrellus societatis*”

Hill, 1972 and the karyological characteristics of the genus *Eptesicus* (Chiroptera: Vespertilionidae). Zeitschrift für Zoologische Systematik und Evolutionsforschung 22:65–77.

HILL, J. E., AND D. L. HARRISON. 1987. The baculum in the Vespertilioninae (Chiroptera:

Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. Bulletin of the British Museum (Natural History), Zoology 52:225–305.

HOOFER, S. R., S. A. REEDER, E. W. HANSEN, AND R. A. VAN DEN BUSSCHE. 2003.

Molecular phylogenetics and taxonomic review of noctilionoid and vespertilionoid bats (Chiroptera: Yangochiroptera) Journal of Mammalogy 84:809–821.

HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2001. Phylogenetic relationships of

plecotine bats and allies based on mitochondrial ribosomal sequences. Journal of Mammalogy 82:131–137.

- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica* 5(supplement):1–63.
- HOOFER, S. R., R. A. VAN DEN BUSSCHE, AND I. HORÁČEK. 2006. Generic status of the American pipistrelles (Vespertilionidae) with description of a new genus. *Journal of Mammalogy* 87:981–992.
- HORÁČEK, I., AND V. HANÁK. 1985. Genetic status of *Pipistrellus savii* (Bonaparte, 1837) and remarks on systematics of the genus *Pipistrellus*. *Bat Research News* 26:62.
- HORÁČEK, I., AND V. HANÁK. 1985–1986. Genetic status of *Pipistrellus savii* and comments on classification of the genus *Pipistrellus* (Chiroptera, Vespertilionidae). *Myotis* 23/24:9–16.
- HORÁČEK, I., AND J. ZIMA. 1978. Specificity of selection pressure as the factor causing parallel evolution in bats. Pp. 115–123 in *Natural selection: proceedings of the international symposium, Liblice, Czechoslovakia, June 5–9, 1978* (V. J. A. Novák, V. V. Leonovich, and B. Pacltová, eds.). Czechoslovak Academy of Sciences, Prague, Czechoslovakia.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- HULVA, P., P. BENDA, V. HANÁK, A. EVIN, AND I. HORÁČEK. 2007. New mitochondrial lineages within the *Pipistrellus pipistrellus* complex from Mediterranean Europe. *Folia Zoologica* 56:378–388.
- HULVA, P., I. HORÁČEK, P. P. STRELKOV, AND P. BENDA. 2004. Molecular architecture of *Pipistrellus pipistrellus* / *Pipistrellus pygmaeus* complex (Chiroptera:

- Vespertilionidae): further cryptic species and Mediterranean origin of the divergence. *Molecular Phylogenetics and Evolution* 32:1023–1035.
- IBÁÑEZ, C., J. L. G. GARCÍA-MUDARRA, M. RUEDI, B. STADELMANN, AND J. JUSTE. 2006. The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterologica* 8:277–297.
- KEARNEY, T. C., M. VOLLETH, G. CONTRAFATTO, AND P. J. TAYLOR. 2002. Systematic implications of chromosome GTG-band and bacula morphology for southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: Vespertilionidae). *Acta Chiropterologica* 4:55–76.
- KINGDON, J. 1974. East African mammals: an atlas of evolution in Africa. Academic Press, London, United Kingdom.
- KITCHENER, D. J., B. JONES, AND N. CAPUTI. 1987. Revision of Australian *Eptesicus* (Microchiroptera: Vespertilionidae). *Records of the Western Australia Museum* 13:427–500.
- KOLENATI, F. A. 1856. Europa's Chiroptern. I. Synopsis der Europäischen Chiroptern. *Allgemeine deutsche Naturhistorische Zeitung* 2: 121–133.
- KOOPMAN, K. F. 1975. Bats of Sudan. *Bulletin of the American Museum of Natural History* 154:353–444.
- KOOPMAN, K. F. 1994. Chiroptera: systematics. *Handbook of zoology: a natural history of the phyla of the animal kingdom*. Walter de Gruyter, Berlin, Germany. 8(60):1–217.

- LACK, J. B., Z. P. ROEHRS, C. E. STANLEY, JR., M. RUEDI, AND R. A. VAN DEN BUSSCHE. 2009. Molecular phylogenetics of *Myotis* suggest familial-level divergence for the genus *Cistugo* (Chiroptera). *Journal of Mammalogy*. *in prep.*
- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers, Museum of Texas Tech University* 163:1–3.
- LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* 49:628–651.
- MADDISON, W. P., AND D. R. MADDISON. 2002. *MacClade*, v. 4.05. Sinauer Associates, Inc., Sunderland, Massachusetts.
- MCKEAN, J. L., G. C. RICHARDS, AND W. J. PRICE. 1978. A taxonomic appraisal of *Eptesicus* (Chiroptera: Mammalia) in Australia. *Australian Journal of Zoology* 26:529–537.
- MCKENNA, M. C., AND S. K. BELL. 1997. *Classification of mammals: above the species level*. Columbia University Press, New York.
- MENU, H. 1984. Revision du statut de *Pipistrellus subflavus* (F. Cuvier, 1832). Proposition d’un taxon generique nouveau: *Perimyotis* nov. gen.. *Mammalia* 48:408–416.
- MILLER, G. S., JR. 1907. The families and genera of bats. *Bulletin of the United States National Museum* 57:1–282.

- RACEY, P. A., ET AL. 2007. Microsatellite DNA polymorphism confirms reproductive isolation and reveals differences in population genetic structure of cryptic pipistrelle bat species. *Biological Journal of the Linnean Society* 90:539–500.
- RODRÍGUEZ-EZPELETA, N., H. BRINKMANN, B. ROURE, N. LARTILLOT, B. F. LANG, AND H. PHILIPPE. 2007. Detecting and overcoming systematic errors in genome-scale phylogenies. *Systematic Biology* 56:389–399.
- ROEHRS, Z. P. 2009. Vespertilioninae systematics: using mitochondrial and nuclear markers to elucidate phylogenetics relationships. Ph.D. dissertation, Oklahoma State University, Stillwater, Oklahoma.
- ROSEVEAR, D. R. 1962. A review of some African species of *Eptesicus* Rafinesque. *Mammalia* 26:457–477.
- ROSEVEAR, D. R. 1965. The bats of west Africa. Trustees of the British Museum (Natural History), London, United Kingdom.
- RUEDAS, L. A., J. SALAZAR-BRAVO, J. W. DRAGOO, AND T. L. YATES. 2000. The importance of being earnest: what, if anything, constitutes a “specimen examined?” *Molecular Phylogenetics and Evolution* 17:129–132.
- SIMMONS, N. B. 2005. Order Chiroptera Pp. 312–529 in *Mammal species of the world: a taxonomic and geographic reference*. 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.
- SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History* 85:i–xvi, 1–350.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods) 4.0 beta. Sinauer Associates, Inc., Sunderland, Massachusetts.

- TATE, G. H. H. 1942. Results of the Archbold expeditions. No. 47: review of the Vespertilioninae bats, with special attention to genera and species of the Archbold collections. *Bulletin of the American Museum of Natural History* 80:221–296.
- THOMPSON, J. D., D. G. HIGGINS, AND T. J. GIBSON. 1994. CLUSTAL_W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673–4680.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2000. Further evidence for inclusion of the New Zealand short-tailed bat (*Mystacina tuberculata*) within Noctilionoidea. *Journal of Mammalogy* 81:865–874.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2001. Evaluating monophyly of Nataloidea (Chiroptera) with mitochondrial DNA sequences. *Journal of Mammalogy* 82:320–327.
- VAN DEN BUSSCHE, R. A., S. A. REEDER, E. W. HANSEN, AND S. R. HOOFER. 2003. Utility of the Dentin Matrix Protein 1 (DMP1) gene for resolving mammalian intraordinal phylogenetic relationships. *Molecular Phylogenetics and Evolution* 26:89–101.
- VOLLETH, M., G. BRONNER, M. C. GOPFERT, K. G. HELLER, O. VON HELVERSEN, AND H. S. YOUNG. 2001. Karyotype comparison and phylogenetic relationships of *Pipistrellus*-like bats (Vespertilionidae; Chiroptera; Mammalia). *Chromosome Research* 9:25–46.

- VOLLETH, M., AND K. G. HELLER. 1994. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für zoologische Systematik und Evolutionsforschung* 32:11–34.
- VOLLETH, M., AND C. R. TIDEMANN. 1991. The origin of the Australian Vespertilioninae bats, as indicated by chromosomal studies. *Zeitschrift für Säugetierkunde* 56:321–330.
- ZIMA, J., AND I. HORÁČEK. 1985. Synopsis of karyotypes of vespertilionid bats (Mammalia: Chiroptera). *Acta Universitatis Carolinae - Biologica* 1981:311–329.

Table 4.1.—Historic classification of *Pipistrellus* -like bats.

Miller 1907	Tate 1942	Simpson 1945	Ellerman ^a	Hill and Harrison 1987
<i>Chalinolobus</i> -like	Pipistrellini	<i>Chalinolobus</i>	? <i>Chalinolobus</i>	Pipistrellini
<i>Chalinolobus</i>	<i>Discopus</i> [= <i>Eudiscopus</i>]	* <i>Glauconycteris</i>	<i>Discopus</i> [= <i>Eudiscopus</i>]	<i>Chalinolobus</i>
<i>Glauconycteris</i>	Eptesicoid	<i>Discopus</i> [= <i>Eudiscopus</i>]	§ <i>Glauconycteris</i>	<i>Eudiscopus</i>
<i>Eptesicus</i> -like	<i>Eptesicus</i>	<i>Eptesicus</i>	? <i>Laephotis</i>	<i>Glischropus</i>
<i>Eptesicus</i>	* <i>Hypsugo</i>	* <i>Hesperoptenus</i>	? <i>Mimetillus</i>	<i>Hesperoptenus</i>
* <i>Neoromicia</i>	* <i>Vespadelus</i>	* <i>Histiotus</i>	<i>Vespertilio</i> -like	<i>Laephotis</i>
<i>Hesperoptenus</i>	<i>Histiotus</i>	* <i>Laephotis</i>	<i>Hesperoptenus</i>	<i>Nyctalus</i>
<i>Histiotus</i>	<i>Laephotis</i>	* <i>Mimetillus</i>	<i>Tylonycteris</i>	<i>Nycticeinops</i>
<i>Laephotis</i>	<i>Vespertilio</i>	* <i>Philetor</i>	<i>Vespertilio</i>	<i>Philetor</i>
<i>Mimetillus</i>	Pipistrelloid	* <i>Tylonycteris</i>	<i>Eptesicus</i> -like	<i>Pipistrellus</i>
<i>Philetor</i>	<i>Barbastella</i>	<i>Pipistrellus</i>	† <i>Eptesicus</i>	* <i>Arielulus</i>
<i>Tylonycteris</i>	<i>Chalinolobus</i>	* <i>Glischropus</i>	* <i>Neoromicia</i>	* <i>Falsistrellus</i>
<i>Vespertilio</i>	<i>Glauconycteris</i>	* <i>Ia</i>	<i>Pipistrellus</i> -like	* <i>Hypsugo</i>
<i>Pipistrellus</i> -like	<i>Glischropus</i>	* <i>Nyctalus</i>	<i>Barbastella</i>	* <i>Neoromicia</i>
<i>Glischropus</i>	<i>Hesperoptenus</i>	* <i>Scotozous</i>	<i>Glischropus</i>	* <i>Perimyotis</i>
<i>Ia</i>	<i>Ia</i>	<i>Vespertilio</i>	<i>Nyctalus</i>	* <i>Parastrellus</i>
<i>Pipistrellus</i>	<i>Mimetillus</i>		‡ <i>Pipistrellus</i>	* <i>Vespadelus</i>
* <i>Hypsugo</i>	<i>Nyctalus</i>		* <i>Arielulus</i>	<i>Scoteanax</i>
* <i>Parastrellus</i>	<i>Philetor</i>		* <i>Falsistrellus</i>	<i>Scotoecus</i>
* <i>Perimyotis</i>	<i>Pipistrellus</i>		* <i>Hypsugo</i>	<i>Scotorepens</i>
<i>Pterygistes</i> [= <i>Nyctalus</i>]	* <i>Arielulus</i>		* <i>Ia</i>	<i>Scotozous</i>
<i>Scotozous</i>	* <i>Falsistrellus</i>		* <i>Scotozous</i>	Vespertilionini
	* <i>Hypsugo</i>			<i>Eptesicus</i>
	* <i>Parastrellus</i>			<i>Glauconycteris</i>
	* <i>Perimyotis</i>			<i>Histiotus</i>
	* <i>Vespadelus</i>			<i>Ia</i>
	<i>Scotozous</i>			<i>Mimetillus</i>
	<i>Tylonycteris</i>			<i>Tylonycteris</i>
				<i>Vespertilio</i>

Table 4.1.—Continued.

Koopman 1994	Volleth ^b	McKenna and Bell 1997	Hooper and Van Den Bussche 2003	Simmons 2005
Vespertilionini	Eptesicini	Vespertilionini	<i>Perimyotis</i>	Eptesicini
<i>Chalinolobus</i>	<i>Eptesicus</i>	<i>Chalinolobus</i>	<i>Parastrellus</i>	<i>Arielulus</i>
* <i>Glauconycteris</i>	* <i>Arielulus</i>	* <i>Glauconycteris</i>	Nycticeiini	<i>Eptesicus</i>
<i>Eptesicus</i>	<i>Hesperoptenus</i>	<i>Eptesicus</i>	<i>Eptesicus</i>	<i>Hesperoptenus</i>
<i>Eudiscopus</i>	<i>Histiotus</i>	<i>Eudiscopus</i>	* <i>Histiotus</i>	Pipistrellini
<i>Glischropus</i>	<i>Ia</i>	<i>Glischropus</i>	<i>Glauconycteris</i>	<i>Glischropus</i>
<i>Hesperoptenus</i>	Pipistrellini	<i>Hesperoptenus</i>	<i>Lasionycteris</i>	<i>Nyctalus</i>
<i>Histiotus</i>	<i>Glischropus</i>	<i>Histiotus</i>	<i>Nycticeius</i>	<i>Pipistrellus</i>
<i>Ia</i>	<i>Nyctalus</i>	<i>Ia</i>	<i>Scotomanes</i>	* <i>Perimyotis</i>
<i>Laephotis</i>	<i>Pipistrellus</i>	<i>Laephotis</i>	Pipistrellini	* <i>Parastrellus</i>
<i>Mimetillus</i>	* <i>Parastrellus</i>	<i>Mimetillus</i>	<i>Pipistrellus</i>	<i>Scotozous</i>
<i>Nyctalus</i>	* <i>Perimyotis</i>	<i>Nyctalus</i>	* <i>Nyctalus</i>	Vespertilionini
<i>Philetor</i>	<i>Scotozous</i>	<i>Nycticeinops</i>	<i>Scotoecus</i>	<i>Chalinolobus</i>
<i>Pipistrellus</i>	Vespertilionini	<i>Philetor</i>	Vespertilionini	<i>Eudiscopus</i>
* <i>Arielulus</i>	<i>Chalinolobus</i>	<i>Pipistrellus</i>	<i>Chalinolobus</i>	<i>Falsistrellus</i>
* <i>Falsistrellus</i>	<i>Falsistrellus</i>	* <i>Arielulus</i>	<i>Hypsugo</i>	<i>Glauconycteris</i>
* <i>Hypsugo</i>	<i>Hypsugo</i>	* <i>Falsistrellus</i>	<i>Laephotis</i>	<i>Histiotus</i>
* <i>Neoromicia</i>	<i>Laephotis</i>	* <i>Hypsugo</i>	<i>Neoromicia</i>	<i>Hypsugo</i>
* <i>Perimyotis</i>	<i>Neoromicia</i>	* <i>Neoromicia</i>	<i>Nycticeinops</i>	<i>Ia</i>
* <i>Parastrellus</i>	<i>Nyctophilus</i>	* <i>Perimyotis</i>	<i>Nyctophilus</i>	<i>Laephotis</i>
* <i>Scotozous</i>	<i>Philetor</i>	* <i>Parastrellus</i>	<i>Tylonycteris</i>	<i>Mimetillus</i>
* <i>Vespadelus</i>	<i>Scotorepens</i>	* <i>Scotozous</i>	Unnamed Genus	<i>Neoromicia</i>
<i>Tylonycteris</i>	<i>Tylonycteris</i>	* <i>Vespadelus</i>	<i>Vespadelus</i>	<i>Philetor</i>
<i>Vespertilio</i>	<i>Vespadelus</i>	<i>Tylonycteris</i>	<i>Vespertilio</i>	<i>Tylonycteris</i>
	<i>Vespertilio</i>	<i>Vespertilio</i>		<i>Vespadelus</i>
				<i>Vespertilio</i>

^a denotes a combination of results taken from both Ellerman and Morrison-Scott 1951 and Ellerman et al. 1953.

^b denotes a combination of results taken from Heller and Volleth 1984, Kearney et al. 2002, Volleth et al. 2001, Volleth and Heller 1994, and Volleth and Tidemann 1991, with most recent papers taking precedence.

* denotes currently recognized taxa that would have been synonyms in authors taxonomic system.

? denotes recognized genera, but relationship to other taxa are unclear.

§ *Glauconycteris* was retained at full generic rank as a matter of convenience, but authors felt taxon was no more than a subgenus of *Chalinolobus*.

† *Eptesicus* was retained at full generic rank as a matter of convenience, but authors felt taxon should be referred to *Vespertilio*.

‡ *Pipistrellus* was retained at full generic rank as a matter of convenience, but authors felt taxon was a subgenus of *Eptesicus*.

Table 4.2.—Classification of *Pipistrellus* -like bats examined in this study.

Subfamily Vespertilioninae
Tribe Unnamed Tribe ^a
Genus <i>Chalinolobus</i>
Genus <i>Vespadelus</i>
Genus <i>Hypsugo</i> ^b
Genus <i>Nycticeinops</i> ^c
Genus <i>Neoromicia</i> ^d
Genus <i>Laephotis</i> ^e
Tribe Vespertilionini
Genus <i>Pipistrellus</i> ^f
Genus <i>Scotoecus</i>
Genus <i>Tylonycteris</i> ^g
Genus <i>Vespertilio</i>
Tribe Eptesicini ^h
Genus <i>Arielulus</i>
Genus <i>Lasionycteris</i>
Genus <i>Glauconycteris</i>
Genus <i>Eptesicus</i>
Genus <i>Scotomanes</i>
Tribe Unnamed Tribe ⁱ
Genus <i>Parastrellus</i>
Genus <i>Perimyotis</i>

^a 'Hypsugini' would be suggested name since *Hypsugo* Kolenati, 1856 has priority.

^b *Hypsugo* includes *H. cadornae* and *H. savii*.

^c *Nycticeinops* includes *H. eisentrauti* and *N. schlieffeni*.

^d *Neoromicia* includes *N. nanus* formerly a member of the genus *Hypsugo*.

^e *Laephotis* is tentatively retained.

^f *Pipistrellus* includes *Nyctalus*.

^g *Tylonycteris* includes specimen of *E. dimissus* used in this study.

^h Tentatively supported in nDNA gene tree and mtDNA Bayesian analysis, but unresolved in combined gene tree. If *Nycticeius* is found to be included in this clade most appropriate name would be Nycticeini.

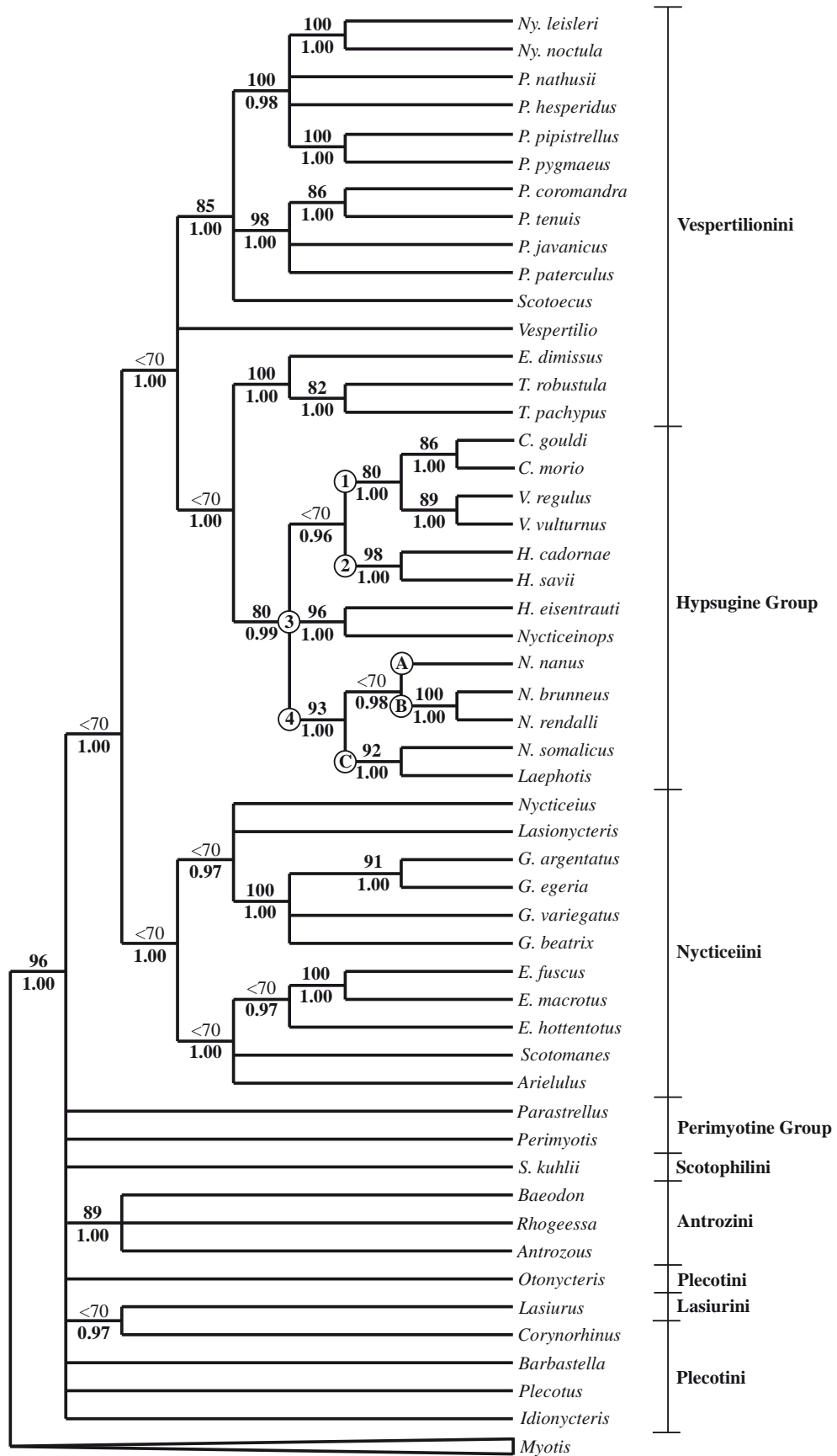
ⁱ 'Perimyotini' would be suggested name since *Perimyotis* Menu, 1984 has priority.

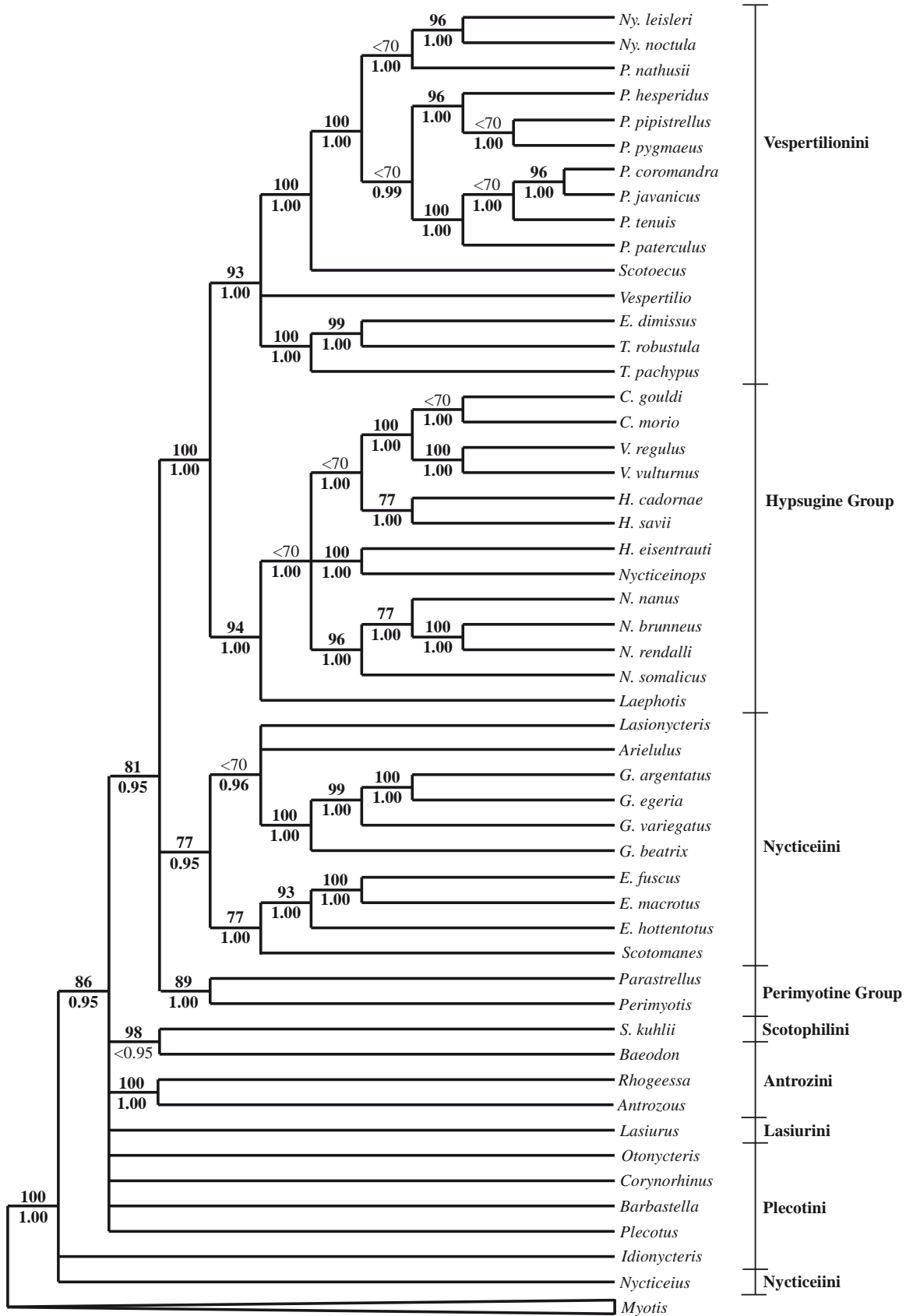
FIGURE LEGENDS

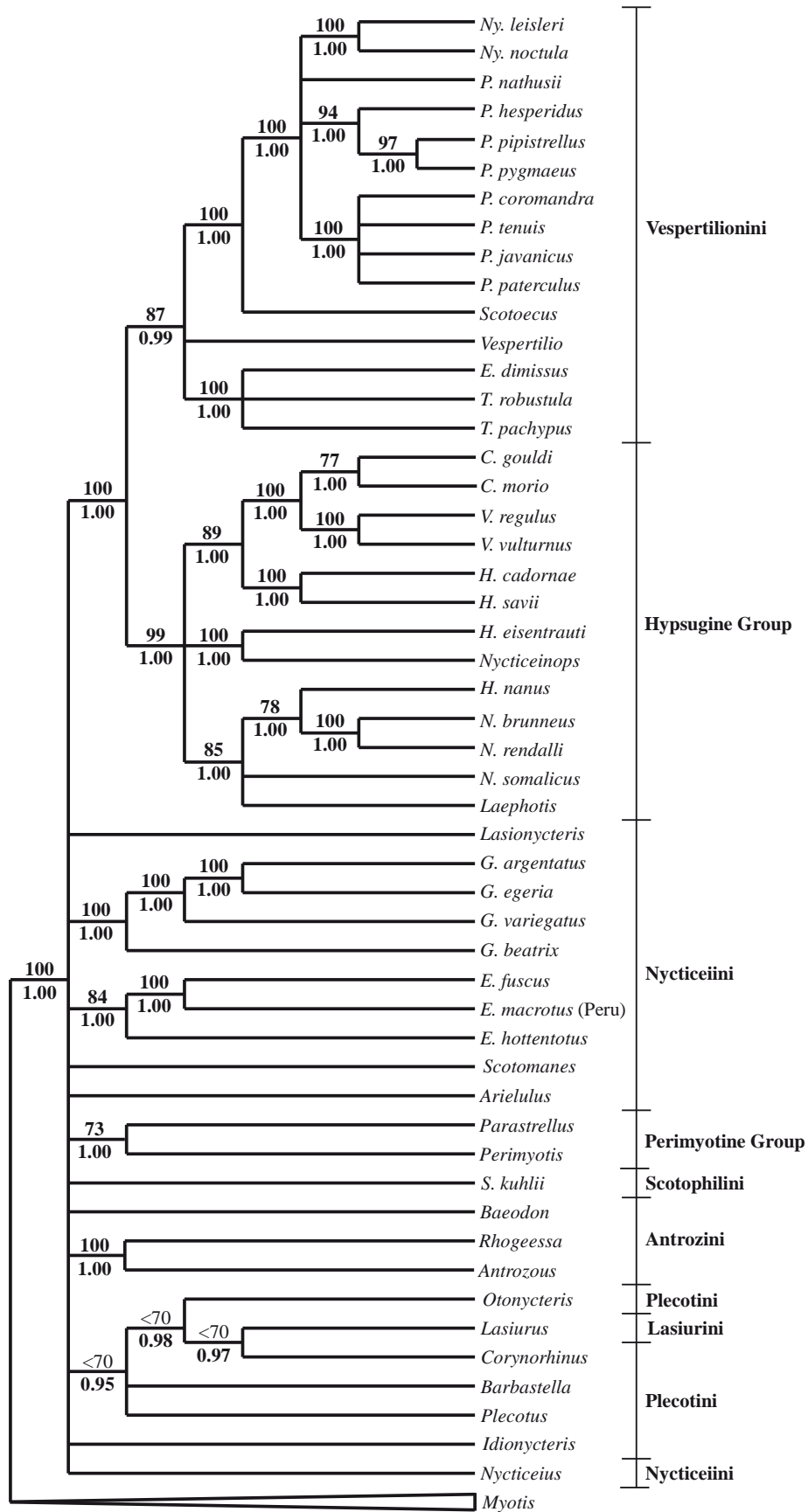
FIG. 4.1.—Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on ribosomal mtDNA genes 12S rRNA, tRNA^{Val}, and 16S rRNA. Numbers on clade branches indicate support values for maximum parsimony bootstrap (above) and Bayesian posterior probabilities (below). Bolded numbers indicate those that met clade support qualifications for bootstrap ($\geq 70\%$) and posterior probabilities (≥ 0.95). Circles with numbers and letters are for referencing clades in text. Taxonomic genera abbreviations include: C. = *Chalinolobus*, E. = *Eptesicus*, G. = *Glauconycteris*, H. = *Hypsugo*, N. = *Neoromicia*, Ny. = *Nyctalus*, P. = *Pipistrellus*, S. = *Scotophilus*, T. = *Tylonycteris*, V. = *Vespadelus*.

FIG. 4.2.—Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on nDNA genes regions APOB, DMP1, RAG2, PRKCI, STAT5A, and THY. Numbers and abbreviations follow Fig. 4.1.

FIG. 4.3.—Cladogram of supported phylogenetic relationships of vespertilionid bats included in this study based on the combined ribosomal mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) and nDNA (APOB, DMP1, RAG2, PRKCI, STAT5A, and THY) genes regions. Numbers and abbreviations follow Fig. 4.1.







CHAPTER V

CONCLUSIONS OF A PHLYOGENETIC STUDY OF VESPERTILIONINAE

RESEARCH SYNOPSIS

The primary goal of this dissertation was to elucidate evolutionary relationships of bats in Vespertilioninae using previously published ribosomal mitochondrial DNA and newly generated mtDNA and nuclear DNA sequence data. Results presented in preceding chapters (Roehrs 2009: Chapters 2, 3 and 4) have demonstrated that > 8 kilobases of digenomic DNA from these gene regions provided increased resolution and more supported clades than previous studies of mtDNA alone (Hooper and Van Den Bussche 2003). Furthermore, within a phylogenetic structure, they provide a working hypothesis for the evolution of Vespertilioninae. Specifically, these results have supported the existence of at least 7 tribes within Vespertilioninae (Table 5.1).

Antrozoini (*sensu* Hooper and Van Den Bussche 2003) is generally supported by these analyses despite the unresolved position of *Baeodon* ostensibly caused by missing data and sequencing problems with this sample. These phylogenetic analyses support the long recognized Lasiurini and also support the more recently proposed tribe Scotophilini (Hill and Harrison 1987 excluding *Scotomanes*; Hooper and Van Den Bussche 2003; Volleth et al. 2006). Nycticeiini (*sensu* Tate 1942) is clearly rejected by these data which confirms earlier suppositions based on bacular morphology and cytogenetics (Hill and Harrison 1987; Volleth and Heller 1994). However, Nycticeiini (*sensu* Hooper and Van

Den Bussche 2003) is neither supported nor rejected by these results. The position of *Nycticeius* in these gene trees is *incertae sedis* within Vespertilioninae, but because the position of this taxon affects the nomenclature of this tribe (due to issues of priority) it remains equivocal (Nycticeiini / Eptesicini).

The last 3 tribes supported by the phylogenetic analyses conducted in this research are unique, previously unproposed tribal arrangements. The Perimyotine group consists of the New World pipistrelles and, based on nomenclatural principles, 'Perimyotini' is suggested as *nomen nudum* pending formal description (Ride et al. 1999). The remaining tribes (Hypsugine group and Vespertilionini) form a supported sister relationship. The Hypsugine group includes the genera *Chalinolobus*, *Vespadelus*, *Hypsugo*, *Nycticeinops*, *Neoromicia*, and *Laephotis* and, pending description, should be assigned the *nomen nudum* 'Hypsugini' (Ride et al. 1999). Vespertilionini is phylogenetically supported in these gene trees as consisting of the genera *Nyctalus*, *Pipistrellus*, *Scotoecus*, *Tylonycteris*, and *Vespertilio*. The only historically recognized tribe that these data could not support or reject was Plecotini. This heuristically pleasing tribe has been 1 of the more stable taxa aside from Lasiurini within Vespertilioninae. However, with the exception of Hooper and Van Den Bussche (2003) and this study, the monophyly of Plecotini (as well as most of these other tribes) has not been phylogenetically examined, and although this tribe has generally been accepted based on morphological and cytogenetic grounds, it is interesting that it remains unresolved in these analyses.

LIMITATIONS OF THESE RESULTS

Results of these phylogenetic studies have indicated relationships and new tribal arrangements not previously documented. Of the 7 supported tribes, 3 have unique arrangements of constituent taxa and are essentially new tribes (Perimotine group, Hypsugine group, and Vespertilionini). Although these systematic hypotheses are based on robust phylogenetic analyses of a relatively large digenomic dataset, they must be perceived as hypotheses requiring further study. Only when cladistic analyses of multiple datasets converge in support of these proposed hypotheses will there be confidence that these hypotheses reflect true evolutionary relationships. Therefore, the hypotheses presented in this dissertation will require further testing using independent datasets.

Furthermore, despite the important phylogenetic information gained through analyses of this relatively large dataset, I was still unable to fully explicate the deep phylogenetic relationships within Vespertilioninae. Relationships between many of the aforementioned tribes remain ambiguous. Previous molecular studies on a number of mammalian groups, across many taxonomic levels (e.g., higher level relationships within Eutheria; families within Chiroptera; interrelationships with Artiodactyla, Bovidae, Leporidae, and Phyllostomidae, independently), using less base pairs (bp; $\bar{X} = 5,448$ bp; range: 2,958–7,806 bp), and including some of the same gene regions used in this dissertation have been able to resolve most nodes in their resulting gene trees (e.g., Baker et al. 2003; Matthee and Davis 2001; Matthee et al. 2001, 2004, 2007; Teeling et al. 2002; Van Den Bussche and Hofer 2004). This may reflect a difference in the tempo of evolution in the history of Vespertilioninae as a whole, and effects of systematic error in

phylogenetic analysis. Despite many previous studies of the evolutionary relationships within this subfamily, full resolution of the phylogenetic relationships of these taxa remains elusive (Hill and Harrison 1987; Hooper and Van Den Bussche 2003; Koopman 1994; McKenna and Bell 1997; Miller 1907; Simmons 2005; Simpson 1945; Tate 1942; Volleth and Heller 1994).

FUTURE RESEARCH

Resolving deep evolutionary relationships within Vespertilioninae is important because it provides the structure in which accurate examination of inter- and intrageneric relationships can be conducted and is necessary for other facets of research, conservation, and management of Vespertilioninae taxa (Roehrs 2009: Chapter 1). Explicating these deep branching patterns within Vespertilioninae will require the addition of more DNA sequence data for 2 reasons. First, short branch lengths of unresolved nodes may indicate a rapid diversification event occurring early in the evolutionary history of Vespertilioninae. An increase in the tempo of diversification would limit development of synapomorphic characters available in the genome and useful for elucidating evolutionary relationships. Results from likelihood-mapping indicated a logistic relationship between resolving power and the number of analyzed positions indicating that it will require an increasing number of base pairs of data to resolve these remaining nodes (Roehrs 2009: Chapter 2, Fig. 2.1, Table 2.3). If this trend was to remain consistent, providing a fully resolved tree would require an estimated minimum of 6 Kbp of additional sequence data. Further research into factors that are confounding explication of Vespertilioninae evolutionary relationships is currently being conducted in the Van Den Bussche laboratory.

Second, the historic response in systematic research to a lack of resolution has been to add more sequence data leading eventually to studies of whole genomes, which does not necessarily resolve all nodes (Baurain et al. 2007; Philippe and Telford 2006). Recently it has been demonstrated in genomic studies that the addition of more sequence data can overcome stochastic error but increases the likelihood that systematic error is influencing phylogenetic results. These systematic errors are largely caused by violations in the model of sequence evolution used in analysis and leads to nonphylogenetic signals influencing resulting gene trees. Mutational saturation at any position or extent within the dataset increases the effect of nonphylogenetic signals (Baurain et al. 2007; Brinkmann and Philippe 2008; Rodríguez-Ezpeleta et al. 2007). A number of approaches have been proposed to deal with overcoming systematic error including character-recoding (Rodríguez-Ezpeleta et al. 2007), express sequencing tags (EST; Philippe and Telford 2006), and a site heterogeneous mixture model (Lartillot and Philippe 2004). However, these methods have only been applied to genomic datasets, or require the generation of cDNA libraries. Another approach is to sequence more data (often entire genomes) and then remove any taxa, gene regions, or codon positions that demonstrate elevated rates of evolution before phylogenetic analysis (Baurain et al. 2007; Brinkmann and Philippe 2008; Rodríguez-Ezpeleta et al. 2007). These techniques have mainly been used on genomic datasets but have been used recently in phylogenetic analysis on rodents having a similarly sized dataset to the one in my research with improved resolution (Montgelard et al. 2008).

Of course improving taxonomic sampling is also helpful in breaking long branches and may be of some use in certain regions of the gene trees presented in this

study (e.g. Vespertilionini and the Hypsugine group), especially evolutionary relationships within tribes, but this will likely not significantly improve resolution of intertribal relationships within Vespertilioninae based on the current coverage of this dataset. Furthermore, the addition of more taxa will require substantial field work in remote regions across the globe, which is logistically difficult and costly. The dataset I used represents most of the currently existing tissue samples of Vespertilioninae taxa from museums around the world. Future research will require collaboration of many researchers in other countries to add further taxonomic diversity.

Finally, other related research needs to be explored, leading to a more complete picture of Vespertilioninae evolution and creating real connections to ecological, behavioral, management, and conservation issues. First, phylogenetic research is still required to elucidate the intratribal relationships of Vespertilioninae taxa whose results would have direct impacts on species conservation and management (among other benefits previously addressed in Roehrs 2009: Chapter 1). Second, more effort needs to be focused on addressing disparities between various hypotheses of Vespertilioninae evolution based on different datasets (morphologic: dentition, skull, wing structure, baculum; cytogenetic; DNA sequence data). It needs to be determined if these divergent patterns are a result of the methods of analysis used (e.g. cladistic or phenetics) or convergent evolution of these traits and in so doing, elucidating which characters are cladistically informative and those that are ecologically informative (or to what degree they are both). We can then begin to develop a picture of what biotic and abiotic factors have governed the evolution of these taxa. Finally, it will be important to correlate divergent events with climatic and geological events by estimating divergence dates and

developing hypotheses about the evolutionary biogeographic patterns of Vespertilioninae taxa.

LITERATURE CITED

- BAKER, R. J., S. R. HOOFER, C. A. PORTER, AND R. A. VAN DEN BUSSCHE. 2003. Diversification among New World leaf-nosed bats: an evolutionary hypothesis and classification inferred from digenomic congruence of DNA sequence. Occasional Papers, Museum of Texas Tech University 230:1–32.
- BAURIAN, D., H. BRINKMANN, AND H. PHILIPPE. 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? *Molecular Biology and Evolution* 24:6–9.
- BRINKMANN, H., AND H. PHILIPPE. 2008. Animal phylogeny and large-scale sequencing: progress and pitfalls. *Journal of Systematics and Evolution* 46:274–286.
- HILL, J. E., AND D. L. HARRISON. 1987. The baculum in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. *Bulletin of the British Museum (Natural History), Zoology* 52:225–305.
- HOOFER, S. R., AND R. A. VAN DEN BUSSCHE. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica* 5(supplement):1–63.
- KOLENATI, F. A. 1856. Europa's Chiroptern. I. Synopsis der Europäischen Chiroptern. *Allgemeine deutsche Naturhistorische Zeitung* 2: 121–133.
- NATURHISTORISCHE ZEITUNG, N. F., 2 (4): 121–133. KOOPMAN, K. F. 1994. Chiroptera: systematics. *Handbook of zoology: a natural history of the phyla of the animal kingdom*. Walter de Gruyter, Berlin, Germany. 8(60):1–217.

- LARTILLOT, N., AND H. PHILIPPE. 2004. A Bayesian mixture model for across-site heterogeneities in the amino-acid replacement process. *Molecular Biology and Evolution* 21:1095–1109.
- MATTHEE, C. A., J. D. BURZLAFF, J. F. TAYLOR, AND S. K. DAVIS. 2001. Mining the mammalian genome for artiodactyl systematics. *Systematic Biology* 50:367–390.
- MATTHEE, C. A., AND S. K. DAVIS. 2001. Molecular insight into the evolution of the family Bovidae: a nuclear DNA perspective. *Molecular Biology and Evolution* 18:1220–1230.
- MATTHEE, C. A., G. EICK, S. WILLOWS-MUNRO, C. MONTGELARD, A. T. PARDINI, AND T. J. ROBINSON. 2007. Indel evolution of mammalian introns and the utility of non-coding nuclear markers in eutherian phylogenetics. *Molecular Phylogenetics and Evolution* 42:827–837.
- MATTHEE, C. A., B. J. VAN VUUREN, D. BELL, AND T. J. ROBINSON. 2004. A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. *Systematic Biology* 53:433–447.
- MCKENNA, M. C., AND S. K. BELL. 1997. Classification of mammals: above the species level. Columbia University Press, New York.
- MENU, H. 1984. Revision du statut de *Pipistrellus subflavus* (F. Cuvier, 1832). Proposition d'un taxon generique nouveau: *Perimyotis* nov. gen.. *Mammalia* 48:408–416.
- MILLER, G. S., JR. 1907. The families and genera of bats. *Bulletin of the United States National Museum* 57:1–282.

- MONTGELARD, C., E. FORTY, V. ARNAL, AND C. A. MATTHEE. 2008. Suprafamilial relationships among Rodentia and the phylogenetic effect of removing fast-evolving nucleotides in mitochondrial, exon and intron fragments. *BMC Evolutionary Biology* 8:321–337.
- PHILIPPE, H., AND M. J. TELFORD. 2006. Large-scale sequencing and the new animal phylogeny. *Trends in Ecology and Evolution* 21:614–620.
- RIDE, W. D. L., ET AL., EDS. 1999. International code of zoological nomenclature. 4th ed. International Trust for Zoological Nomenclature, London, United Kingdom.
<http://www.iczn.org/iczn/index.jsp>
- RODRÍGUEZ-EZPELETA, N., H. BRINKMANN, B. ROURE, N. LARTILLOT, B. F. LANG, AND H. PHILIPPE. 2007. Detecting and overcoming systematic errors in genome-scale phylogenies. *Systematic Biology* 56:389–399.
- ROEHRS, Z. P. 2009. Vespertilioninae systematics: using mitochondrial and nuclear markers to elucidate phylogenetics relationships. Ph.D. dissertation, Oklahoma State University, Stillwater, Oklahoma.
- SIMMONS, N. B. 2005. Order Chiroptera Pp. 312–529 in *Mammal species of the world: a taxonomic and geographic reference*. 3rd ed. (D. E. Wilson and D. M. Reeder, eds.). Johns Hopkins University Press, Baltimore, Maryland.
- SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. *Bulletin of the American Museum of Natural History* 85:i–xvi, 1–350.
- TATE, G. H. H. 1942. Results of the Archbold expeditions. No. 47: review of the Vespertilioninae bats, with special attention to genera and species of the Archbold collections. *Bulletin of the American Museum of Natural History* 80:221–296.

- TEELING, E. C., O. MADSEN, R. A. VAN DEN BUSSCHE, W. W. DE JONG, M. J. STANHOPE, AND M. S. SPRINGER. 2002. Microbat paraphyly and the convergent evolution of a key innovation in Old World rinolophoid microbats. *Proceedings of the National Academy of Sciences* 99:1431–1436.
- VAN DEN BUSSCHE, R. A., AND S. R. HOOFER. 2004. Phylogenetic relationships among recent chiropteran families and the importance of choosing appropriate out-group taxa. *Journal of Mammalogy* 85:321–330.
- VOLLETH, M., AND K. G. HELLER. 1994. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. *Zeitschrift für zoologische Systematik und Evolutionsforschung* 32:11–34.
- VOLLETH, M., K. G. HELLER, AND J. FAHR. 2006. Phylogenetic relationships of three “Nycticeiini” genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis. *Zeitschrift für Säugetierkunde* 71:1–12.

Table 5.1.—Classification of Vespertilioninae taxa examined in this dissertation.

Subfamily Vespertilioninae	Tribe Nycticeiini / Eptesicini ^f
Genus <i>Nycticeius</i> ^{a,b}	Genus <i>Arielulus</i>
Genus <i>Otonycteris</i> ^a	Genus <i>Lasionycteris</i>
Tribe Antrozoini ^a	Genus <i>Glauconycteris</i>
Genus <i>Antrozous</i>	Genus <i>Eptesicus</i>
Genus <i>Bauerus</i>	Genus <i>Scotomanes</i>
Genus <i>Baeodon</i> ^c	Tribe Hysugine Group ^g
Genus <i>Rhogeessa</i>	Genus <i>Chalinolobus</i>
Tribe Lasiurini ^a	Genus <i>Vespadelus</i>
Genus <i>Lasiurus</i>	Genus <i>Hypsugo</i> ^h
Tribe Plecotini ^{a,d}	Genus <i>Nycticeinops</i> ⁱ
Genus <i>Barbastella</i>	Genus <i>Neoromicia</i> ^j
Genus <i>Corynorhinus</i>	Genus <i>Laephotis</i> ^k
Genus <i>Euderma</i>	Tribe Vespertilionini
Genus <i>Idionycteris</i>	Genus <i>Pipistrellus</i> ^m
Genus <i>Plecotus</i>	Genus <i>Nyctalus</i> ^p
Tribe Scotophilini ^a	Genus <i>Scotoecus</i>
Genus <i>Scotophilus</i>	Genus <i>Tylonycteris</i> ^q
Tribe Perimyotine Group ^{a,e}	Genus <i>Vespertilio</i>
Genus <i>Parastrellus</i>	
Genus <i>Perimyotis</i>	

^a Positioned *incertae sedis* within Vespertilioninae.

^b Affinities of *Nycticeius* may lie with Eptesicini.

^c This position of *Baeodon* is supported by mtDNA and suggested by nDNA, but problematic in this study.

^d Plecotini was neither supported nor rejected by this study and is retained pending further study.

^e 'Perimyotini' would be suggested name since *Perimyotis* Menu, 1984 has priority.

^f Tentatively supported in nDNA gene tree and mtDNA Bayesian analysis, but unresolved in combined gene tree. If *Nycticeius* is found to be included in this clade most appropriate name would be Nycticeiini.

^g 'Hysugini' would be suggested name since *Hypsugo* Kolenati, 1856 has priority.

^h *Hypsugo* includes *H. cadornae* and *H. savii*.

ⁱ *Nycticeinops* includes *N. eisentrauti* and *H. schlieffeni*.

^j *Neoromicia* includes *N. nanus* formerly a member of *Hypsugo*.

^k *Laephotis* is tentitively retained.

^m *Pipistrellus* may be paraphyletic with respect to *Nyctalus*.

^p The genus *Nyctalus* is retained here pending futher study.

^q *Tylonycteris* includes specimen *E. dimissus* used in this study.

APPENDIX I

Taxonomic samples included in this study with voucher specimen catalog number, tissue collection number and general locality. 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, Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB, NK), Museum of Texas Tech University (TTU, TK), Natural History Museum Basel (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) Transvaal Museum of Natural History (TM), Universidad Autónoma Metropolitana - Iztapalapa (UAMI), Universidad Nacional Autónoma de México (UNAM), and University of Lausanne, Switzerland, Institut de Zoologie et d'Ecologie Animale (IZEA).

Appendix I. Taxonomic samples included in this study with voucher specimen catalog number, tissue collection number and general locality. 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, Genève (MHNG), Museum of Southwestern Biology at the University of New Mexico (MSB, NK), Museum of Texas Tech University (TTU, TK), Natural History Museum Basel (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) Transvaal Museum of Natural History (TM), Universidad Autónoma Metropolitana - Iztapalapa (UAMI), Universidad Nacional Autónoma de México (UNAM), and University of Lausanne, Switzerland, Institut de Zoologie et d'Ecologie Animale (IZEA).

Taxon	Museum Catalog Number	Tissue Collection Number	Locality
Vespertilionidae			
Kerivoulinae			
<i>Kerivoula hardwickii</i>	ROM 110829	F 44154	Vietnam: Đồng Nai Province
<i>Kerivoula lenis</i>	ROM 110850	F 44175	Vietnam: Đồng Nai Province
<i>Kerivoula pellucida</i>	ROM 102177	F 35987	Indonesia: East Kalimantan Province
Murinae			
<i>Harpiocephalus harpia</i>	CM 88159	TK 21258	Thailand: Uthai Thani Province
<i>Murina cyclotis</i>	MHNG 1826.033	M 1209	Laos: Phongsaly Province
<i>Murina huttoni</i>	ROM 107739	F 42722	Vietnam: Đắk Lắk Province
<i>Murina tubinaris</i>	MHNG 1926.034	M 1179	Laos: Phongsaly Province
Myotinae			
<i>Myotis albescens</i>	CM 77691	TK 17932	Suriname: Marowijne District
<i>Myotis bocagii</i>	FMNH 150075	FMNH 150075	Tanzania: Tanga Region
<i>Myotis browni</i>	FMNH 147067	FMNH 147067	Philippine Islands: Mindanao Island
<i>Myotis californicus</i>	TTU 79325	TK 78797	USA: Texas
<i>Myotis capaccinii</i>	TTU 40554	TK 25610	Jordan: Northern Province
<i>Myotis ciliolabrum</i>	TTU 78520	TK 83155	USA: Texas
<i>Myotis dominicensis</i>	***	TK 15613	Dominica: St. Joseph Parish
<i>Myotis fortidens</i>	UAMI	TK 43186	Mexico: Michoacán
<i>Myotis keaysi</i>	***	TK 13532	Mexico: Yucatán
<i>Myotis latirostris</i>	MHNG	M 606	Taiwan: Miao-Li County
<i>Myotis levis</i>	FMNH 141600	FMNH 141600	Brazil: São Paulo
<i>Myotis moluccarum</i>	TCWC	RLH 62	Australia
<i>Myotis myotis</i>	MHNG 1805.062	IZEA 3790	Switzerland: Canton of Berne
<i>Myotis nigricans</i>	FMNH 129210	FMNH 129210	Peru: Amazonas
<i>Myotis riparius</i>	AMNH 268591	AMNH 268591	French Guiana: Paracou
<i>Myotis septentrionalis</i>	ISUV 6454	DWS 608	USA: Indiana
<i>Myotis thysanodes</i>	TTU 79327	TK 78796	USA: Texas
<i>Myotis thysanodes</i>	TTU 79330	TK 78802	USA: Texas
<i>Myotis velifer</i>	TTU 78599	TK 79170	USA: Texas
<i>Myotis volans</i>	TTU 79545	TK 78980	USA: Texas
<i>Myotis welwitschii</i>	FMNH 144313	FMNH 144313	Uganda: Kasese District
<i>Myotis yumanensis</i>	TTU 43200	TK 28753	USA: Oklahoma
Vespertilioninae			
<i>Otonycteris hemprichii</i>	CM	SP 7882	Jordan: Ma'an Governorate
<i>Otonycteris hemprichii</i>	MBQ 1201	SP 7908	
<i>Otonycteris hemprichii</i>	MBQ 1226	SP 7933	
<i>Parastrellus hesperus</i>	TTU 79269	TK 78703	USA: Texas
<i>Perimyotis subflavus</i>	TTU 80684	TK 90671	USA: Texas

Appendix I. Continued.

Taxon	Museum Catalog Number	Tissue Collection Number	Locality
Antrozoini			
<i>Antrozous pallidus</i>	MSB 40576	NK 506	USA: California
<i>Antrozous pallidus</i>	MSB	NK 39195	USA: Arizona
<i>Antrozous pallidus</i>	TTU 71101	TK 49646	USA: Texas
<i>Baeodon alleni</i>	UNAM	TK 45023	Mexico: Michoacán
<i>Bauerus dubiaquercus</i>	ROM 97719	F 33200	Mexico: Campeche
<i>Rhogeessa aeneus</i>	TTU 40012	TK 20712	Belize: Belize District
<i>Rhogeessa mira</i>	UNAM	TK 45014	Mexico: Michoacán
<i>Rhogeessa parvula</i>	TTU 36633	TK 20653	Mexico: Sonora
<i>Rhogeessa tumida</i>	TTU 61231	TK 40186	Honduras: Valle Department
Lasiurini			
<i>Lasiurus atratus</i>	ROM 107228	F 39221	Guyana: Potaro-Siparuni
<i>Lasiurus blossevillii</i>	ROM 104285	F 38133	Panama: Chiriquí Province
<i>Lasiurus borealis</i>	TTU 71170	TK 49732	USA: Texas
<i>Lasiurus cinereus</i>	TTU	TK 78926	USA: Texas
<i>Lasiurus ega</i>	UNAM	TK 43132	Mexico: Michoacán
<i>Lasiurus intermedius</i>	TTU 36631	TK 20513	Mexico: Oaxaca
<i>Lasiurus intermedius</i>	TTU 80739	TK 84510	USA: Texas
<i>Lasiurus seminolus</i>	TTU 80699	TK 90686	USA: Texas
<i>Lasiurus xanthinus</i>	TTU 78296	TK 78704	USA: Texas
Nycticeiini			
<i>Arielulus aureocollaris</i>	ROM 106169	F 38447	Vietnam: Tuyen Quang Province
<i>Eptesicus brasiliensis</i>	CM 76812	TK 17809	Suriname: Nickerie District
<i>Eptesicus diminutus</i>	TTU 48154	TK 15033	Venezuela: Guárico
<i>Eptesicus dimissus</i>	MHNG 1926.053	M 1187	Laos: Phongsaly Province
<i>Eptesicus furinalis</i>	AMNH 268583	AMNH 268583	French Guiana: Paracou
<i>Eptesicus fuscus</i>	CM 102826	SP 844	USA: West Virginia
<i>Eptesicus hottentotus</i>	CM 89000 (type)	TK 33013	Kenya: Rift Valley Province
<i>Eptesicus macrotus</i>	CML 3230	OCGR 2301	Argentina: Neuquén Province
<i>Eptesicus macrotus</i>	FMNH 129207	FMNH 129207	Peru: Ancash Region
<i>Eptesicus macrotus</i>	OMNH 27925	OCGR 4227	Argentina: Salta Province
<i>Eptesicus macrotus</i>	OMNH 32879	OCGR 3806	Argentina: Catamarca Province
<i>Eptesicus magellanicus</i>	OMNH 23500	OCGR 2303	Argentina: Neuquén Province
<i>Eptesicus serotinus</i>	MHNG 1807.065	M 816	Greece
<i>Eptesicus serotinus</i>	TTU 70947	TK 40897	Tunisia: Sidi Bou Zid Governorate
<i>Glauconycteris argentatus</i>	FMNH 15119	FMNH 15119	Tanzania: Kilimanjaro Region
<i>Glauconycteris beatrix</i>	FMNH 149417	FMNH 149417	Zaire: Haute Zaire
<i>Glauconycteris egeria</i>	AMNH 109067	AMNH 109067	
<i>Glauconycteris egeria</i>	AMNH 268381	AMNH 268381	Central African Republic
<i>Glauconycteris variegatus</i>	CM 97983	TK 33545	Kenya: Western Province
<i>Lasionycteris noctivagans</i>	TTU 56255	TK 24216	USA: Texas
<i>Lasionycteris noctivagans</i>	***	TK 24889	USA: Oklahoma
<i>Nycticeius humeralis</i>	TTU 49536	TK 26380	USA: Texas
<i>Nycticeius humeralis</i>	TTU 80664	TK 90649	USA: Texas
<i>Scotomanes ornatus</i>	ROM 107594	F 42568	Vietnam: Tuyen Quang Province
Scotophilini			
<i>Scotophilus borbonicus</i>	CM 98041	TK 33267	Kenya: Coastal Province
<i>Scotophilus dinganii</i>	FMNH 147235	FMNH 147235	Tanzania: Tanga Region
<i>Scotophilus heathii</i>	ROM 107786	F 42769	Vietnam: Đắk Lắk Province
<i>Scotophilus kuhlii</i>	FMNH 145684	FMNH 145684	Philippine Islands: Sibuyan Island

Appendix I. Continued.

Taxon	Museum Catalog	Tissue	Locality
<i>Scotophilus leucogaster</i>	CM 98054	TK 33359	Kenya: Eastern Province
<i>Scotophilus nux</i>	***	TK 33484	Kenya: Western Province
<i>Scotophilus viridis</i>	FMNH 150084	FMNH 150084	Tanzania: Tanga Region
Pipistrellini			
<i>Nyctalus leisleri</i>	FMNH 140374	FMNH 140374	Pakistan: Malakand Division
<i>Nyctalus noctula</i>	NHMB 209/87	NHMB 209/87	Switzerland: Canton of Berne
<i>Nyctalus noctula</i>	IZEA	Nno1	Switzerland: Canton of Berne
<i>Pipistrellus coromandra</i>	FMNH 140377	FMNH 140377	Pakistan: Malakand Division
<i>Pipistrellus hesperidus</i>	DM 8013	DM 8013	South Africa: KwaZulu-Natal
<i>Pipistrellus javanicus</i>	FMNH 147069	FMNH 147069	Philippine Islands: Mindanao Island
<i>Pipistrellus nathusii</i>	MHNG 1806.003	IZEA 2830	Switzerland: Canton of Vaud
<i>Pipistrellus nathusii</i>	MHNG 1806.001	IZEA 3406	Switzerland: Canton of Vaud
<i>Pipistrellus nathusii</i>	***	TK 81167	
<i>Pipistrellus paterculus</i>	MHNG 1926.045	M 1181	Laos: Phôngsaly Province
<i>Pipistrellus pipistrellus</i>	MHNG 1956.031	M 1439	Switzerland: Canton of Genève
<i>Pipistrellus pygmaeus</i>	MHNG 1806.032	IZEA 3403	Spain: Barcelone Province
<i>Pipistrellus tenuis</i>	FMNH 137021	FMNH 137021	Philippine Islands: Sibuyan Island
<i>Scotoecus hirundo</i>	FMNH 151204	FMNH 151204	Tanzania: Kilimanjaro Region
Plecotini			
<i>Barbastella barbastellus</i>	MHNG 1804.094	IZEA 3590	Switzerland: Canton of Valais
<i>Corynorhinus mexicanus</i>	UAMI	TK 45849	Mexico: Michoacán
<i>Corynorhinus rafinesquii</i>	TTU 45380	TK 5959	USA: Arkansas
<i>Corynorhinus townsendii</i>	OSU 13099	OK 11530	USA: Oklahoma
<i>Corynorhinus townsendii</i>	TTU 78531	TK 83182	USA: Texas
<i>Euderma maculatum</i>	MSB 121373	NK 36260	USA: Utah
<i>Idionycteris phyllotis</i>	ACU 736	ACU 736	
<i>Idionycteris phyllotis</i>	MSB 120921	NK 36122	USA: Utah
<i>Plecotus auritus</i>	***	IZEA 2693	
<i>Plecotus auritus</i>	MHNG 1806.047	IZEA 2694	Switzerland: Canton of Valais
<i>Plecotus austriacus</i>	MHNG 1806.042	IZEA 3722	Switzerland: Canton of Valais
<i>Plecotus gaisleri</i>	MHNG 1806.051	IZEA 4780	Morocco: Meknés-Tafilalet
<i>Plecotus macrobullaris</i>	MHNG 1806.053	IZEA 4751	Switzerland: Canton of Valais
Vespertilionini			
<i>Chalinolobus gouldii</i>	TCWC	RLH 27	Australia
<i>Chalinolobus morio</i>	TCWC	05M3	Australia
<i>Hypsugo cadornae</i>	MHNG 1926.050	M 1183	Laos: Phôngsaly Province
<i>Hypsugo eisentrauti</i>	ROM 100532	F 34348	Côte d'Ivoire
<i>Hypsugo savii</i>	MHNG 1804.100	IZEA 3586	Switzerland: Canton of Valais
<i>Laephotis namibensis</i>	TM 37547	SP 4097	Namibia: Luderitz District
<i>Laephotis namibensis</i>	CM 93187	SP 4160	Namibia: Maltahöhe District
<i>Neoromicia brunneus</i>	CM 90802	TK 21501	Gabon: Estuaire Province
<i>Neoromicia capensis</i>	DM 8426	DM 8426	Swaziland: Lubombo
<i>Neoromicia nanus</i>	DM 7542	DM 7542	South Africa: KwaZulu-Natal
<i>Neoromicia rendalli</i>	CM 97977	TK 33238	Kenya: Coastal Province
<i>Neoromicia somalicus</i>	CM 97978	TK 33214	Kenya: Coastal Province
<i>Nycticeinops schlieffeni</i>	CM 97998	TK 33373	Kenya: Eastern Province
<i>Tylonycteris pachypus</i>	ROM 106164	F 38442	Vietnam: Tuyen Quang Province
<i>Tylonycteris robustula</i>	MHNG 1926.059	M 1203	Laos: Phôngsaly Province
<i>Vespadelus regulus</i>	TCWC	RLH 30	Australia
<i>Vespadelus vulturinus</i>	TCWC	RLH 16	Australia
<i>Vespertilio murinus</i>	MHNG 1808.017	IZEA 3599	Switzerland: Canton of Valais

*** undetermined voucher specimen location

VITA

Zachary P. Roehrs

Candidate for the Degree of

Doctor of Philosophy

Dissertation: VESPERTILIONINAE SYSTEMATICS: USING MITOCHONDRIAL AND NUCLEAR MARKERS TO ELUCIDATE PHYLOGENETIC RELATIONSHIPS

Major Field: Zoology

Biographical:

Personal Data: Born in Omaha, Nebraska on 1 January 1976, to William and JoAnn Roehrs.

Education: Graduated from Lincoln Southeast High School, Lincoln, Nebraska in May 1994; received Bachelor of Science degree in Fisheries and Wildlife in 1999, and Master of Science degrees in Museum Studies and in Natural Resource Sciences in 2003 and 2004 (respectively) from the University of Nebraska – Lincoln. Completed the requirements for the Doctor of Philosophy in Zoology at Oklahoma State University, Stillwater, Oklahoma in July 2009.

Experience: Teaching: Graduate Teaching Assistant / Associate: General Biology (1 semester), Ecology of the Great Plains (1), Human Heredity (3), Mammalogy (3), Vertebrate Zoology (2), and Interpersonal Skills (1); Guest Lecturer: Wildlife Management Techniques (3 years).
Research: Graduate Research Associate (1 semester, 5 summers); Contract Mammalogists, University of Nebraska State Museum (2 contracts), School Natural Resource Sciences, University of Nebraska (1), Nebraska Department of Health and Human Services (2).

Professional Memberships: American Association of Museums, American Society of Mammalogists, Central Plains Society of Mammalogists, Oklahoma Academy of Science, Sigma Xi, Society for the Preservation of Natural History Collections, Southwestern Association of Naturalists.

Name: Zachary P. Roehrs

Date of Degree: July 2009

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: VESPERTILIONINAE SYSTEMATICS: USING MITOCHONDRIAL AND NUCLEAR MARKERS TO ELUCIDATE PHYLOGENETIC RELATIONSHIPS

Pages in Study: 149

Candidate for the Degree of Doctor of Philosophy

Major Field: Zoology

Scope and Method of Study: Elucidating evolutionary relationships within Vespertilioninae has been challenging due to a paucity of useful morphological characters, convergent evolution of lineages, and potentially rapid radiation of the major clades in this subfamily of bats. Previous molecular studies of this subfamily using mitochondrial ribosomal DNA were unable to explicate deep phylogenetic relationships. I used 3 nuclear exons (APOB, DMP1, RAG2), 3 nuclear introns (PRKCI, STAT5A, THY), and existing mtDNA (12S rRNA, tRNA^{Val}, and 16S rRNA) gene regions in maximum parsimony and Bayesian phylogenetic analyses to generate hypotheses of the evolutionary relationship of Vespertilioninae and test previous hypotheses about inter- and intratribal relationships.

Findings and Conclusions: Analyses of >8 Kilo-bases of digenomic DNA were not able to fully resolve all deep phylogenetic relationships. However, gene trees using this dataset were more resolved than mtDNA alone and provided a more robust hypothesis of the evolution of Vespertilioninae. These results support the existence of 7 tribes within Vespertilioninae (Antrozoini, Lasiurini, Hypsugine group, Nycticeiini / Eptesicini, Perimyotine group, Scotophilini, and Vespertilionini). Three of these tribes (Hypsugine group, Perimyotine group, and Vespertilionini) constitute new hypotheses for the relationship of these bats and 2 are in need of formal description. The only intertribal relationship supported was a sister relationship between the Hypsugine group and Vespertilionini. The tribe Plecotini was neither supported nor rejected in these gene trees. Due to the remaining unresolved relationships generated by this relatively large dataset, which incorporated gene regions successfully used to resolve other similar mammalian phylogenetic questions, it is apparent that more DNA sequence data and implementation of new techniques to reduce systematic errors in large molecular datasets will be required to resolve these deep evolutionary relationships in Vespertilioninae.

ADVISER'S APPROVAL: Ronald A. Van Den Bussche
