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

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

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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—Hoofer and Van Den Bussche 2003; Miller-Butterworth et al. 2007; Teeling et al. 2005; Van Den

Bussche and Hoofer 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 Hoofer 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 Hoofer 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 (Hoofer 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: Hoofer 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 (Hoofer 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* Hoofer 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; Hoofer 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; Hoofer 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 Ärnbäck-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." Ärnbäck-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 (P^2) 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 Morison-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. Hoofer 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. Hoofer et al. 2003; Hoofer 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 (Avise 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 Hoofer and Van Den Bussche (2003).

5) Unresolved molecular phylogenetics.—Hoofer 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 Hoofer and Van Den Bussche (2003) suggested that the potential rapid diversification early in the cladogensis 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 though 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.

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Table 1.1.—Data from the International Union for Conservation of Natureand 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 Perimyotine group and the Hypsugine 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 I^2 size, anterior upper premolar, pelage color) has led to classifications incongruent with evolutionary history within Vespertilioninae (Ärnbäck-Christie-Linde 1909; Ellerman and Morrison-Scott 1951; Heller and Volleth 1984; Hill 1966; Hill and Harrison 1987; Hill and Topál 1973; Horáček and Zima 1978; Koopman 1975; Rosevear 1962; Tate 1942; Volleth and Heller 1994b; Zima and Horáček 1985). These limitations have led to ambiguity in our understanding of evolutionary relationships within this diverse subfamily, which has hindered development of a generally agreed upon classification.

Of particular interest in this study are supergeneric relationships of bats within Vespertilioninae. Although Miller (1907) set the foundation for our modern

classification of these bats (without downplaying work of his predecessors; 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 synonomized 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 (Hoofer 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; Hoofer 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; Hoofer and Van Den Bussche 2001; Hoofer 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 Palaearctic vespertilionids, Kawai et al. (2002) examined ND1, the nuclear exon vWF, and short interspersed elements (SINEs) of mainly Eastern Palaearctic bats, and Hoofer 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 Murininae were included as out groups to polarize character-state transformations. Tissue samples were provided by several natural history collections and most tissues are represented by voucher specimens (Ruedas et al. 2000) in the following institutions: Abilene Christian University, American Museum of Natural History, Carnegie Museum of Natural History, Field Museum of Natural History, Durban Natural Science Museum, Indiana State University Vertebrate Collection, Musèum d'Historie 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. Hoofer (Hoofer 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 μL of 10X buffer, 3.5 mM $MgCl_2$, 0.8 mg/mL of bovine serum albumin, and 0.15 μL of each primer in a 30μL total volume reaction. The general PCR thermal profile used for these reactions began with an initial 3 min denaturing of 94–95ºC, followed by 35–40 cycles of 94–95ºC for 30 s, 40–62ºC for 1.5 min, and 72ºC for 1 min (See Table 2.2 for individual primer annealing temperatures). Amplification ended with a final elongation at 72ºC 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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer et al. 2003; Lack et al. 2009; Van Den Bussche and Hoofer 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 Hoofer (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 X 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 ≥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 (Hoofer and Van Den Bussche 2003; Van Den Bussche and Hoofer 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 (Hoofer 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 ≥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 vulturnus* 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 ≥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 ≥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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer 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, Hoofer 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 (Hoofer 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; Hoofer 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 (Hoofer 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 (Hoofer and Van Den Bussche 2003) or 11 kb of nDNA (Miller-Butterworth et al. 2007). Hoofer and Van Den Bussche (2003) redefined Antrozoini by including *Rhogeessa* and *Baeodon* into the tribe. Their arrangement is 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 Hoofer 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 Hoofer 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 misidentifed *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; Hoofer 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* Hoofer 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 Hoofer 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 *Histiotus*), *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 *Histiotus* (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 (Hoofer and Van Den Bussche 2003). Hoofer and Van Den Bussche (2003) were unable to unambiguously support monophyly of the core Plecotini or their relationship to other previously proposed closely related genera. The combined 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).

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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	Hoofer and Van Den Bussche 2003	Simmons 2005
Vespertilioninae	Vespertilioninae	Vespertilioninae	Vespertilioninae		Vespertilioninae		
Myotini							
Myotini'		Myotini	Myotini	Myotinae	Myotini	Myotinae	Myotinae
Lasionycteris	*Lasionycteris*	*Lasionycteris*	*Lasionycteris*	*Myotis*	*Lasionycteris*	*Myotis*	*Lasionycteris*
Cistugo	*Cistugo*	*Myotis*	*Myotis*		*Myotis*	Vespertilioninae	*Cistugo*
Myotis	*Mytois*	**Cistugo*	**Cistugo*		**Cistugo*	*Otonycteris*†	*Myotis*
Pizonyx	**Pizonyx*	*Pizonyx*	**Pizonyx*		**Pizonyx*	*Parastrellus*†	
				Vespertilioninae		*Perimyotis*†	Vespertilioninae
Plecotini'		Plecotini	Plecotini	Plecotini	Plecotini	Plecotini†	Plecotini
Corynorhinus	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*	*Barbastella*
Euderma	*Euderma*	*Euderma*	*Euderma*	*Euderma*	*Euderma*	*Corynorhinus*	*Corynorhinus*
Idionycteris	*Idionycteris*	*Idionycteris*	*Plecotus*	*Idionycteris*	*Idionycteris*	*Euderma*	*Euderma*
Plecotus	*Plecotus*	*Plecotus*	**Corynorhinus*	*Plecotus*	*Plecotus*	*Idionycteris*	*Idionycteris*
	**Corynorhinus*	**Corynorhinus*	**Idionycteris*	**Corynorhinus*	**Corynorhinus*	*Plecotus*	*Otonycteris*
		Otonycteris		*Otonycteris*			*Plecotus*
		Baeodon		*Rhogeessa*			
		Rhogeessa		**Baeodon*			
		Nycticeius					
Lasiurini		Lasiurini	Lasiurini		Lasiurini	Lasiurini†	Lasiurini
Dasypterus	*Lasiurus*	*Lasiurus*	*Lasiurus*		*Lasiurus*	*Lasiurus*	*Lasiurus*
Lasiurus	**Dasypterus*	*Dasypterus*	**Dasypterus*		**Dasypterus*		
Nycticeini		Scotophilini	Nycticeini	Scotophilini	Nycticeini	Scotophilini†	Nycticeiini
Otonycteris	*Otonycteris*	*Scotomanes*	*Otonycteris*	*Scotophilus*	*Otonycteris*	*Scotophilus*	*Rhogeessa*
Baeodon	*Rhogeessa*	**Scoteinus*	*Rhogeessa*		*Rhogeessa*		**Baeodon*
Rhogeessa	**Baeodon*	*Scotophilus*	**Baeodon*		**Baeodon*		*Nycticeinops*
Nycticeius	*Nycticeius*		*Nycticeius*		*Nycticeius*		*Nycticeius*
Scoteinus	**Scoteinus*		**Nycticeinops*		**Nycticeinops*		*Scoteanax*
**Scoteanax*	**Scoteanax*		**Scoteanax*		**Scoteanax*		*Scotoecus*
**Scotorepens*	**Scotorepens*		**Scotorepens*		**Scotorepens*		*Scotomanes*
Scotoecus	**Scotoecus*		*Scotoecus*		*Scotoecus*		**Scoteinus*
Scotomanes	**Scotomanes*		*Scotomanes*		*Scotomanes*		*Scotophilus*
Scotophilus	*Scotophilus*		*Scotophilus*		**Scoteinus*		*Scotorepens*
					Scotophilus		
Pipistrellini		Vespertilionini	Vespertilionini		Vespertilionini		
Eudiscopus	*Eudiscopus*		*Chalinolobus*		*Chalinolobus*		
Eptesicoid			**Glauconycteris*	Eptesicini	**Glauconycteris*	Nycticeiini	Eptesicini
Eptesicus	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Eptesicus*	*Arielulus*
**Hypsugo*	**Hesperoptenus*	*Glauconycteris*	*Eudiscopus*	**Arielulus*	*Eudiscopus*	**Histiotus*	*Eptesicus*
**Vespadelus*	**Histiotus*	*Histiotus*	*Glischropus*	*Hesperoptenus*	*Glischropus*	*Glauconycteris*	*Hesperoptenus*
Histiotus	**Laephotis*	*Ia*	*Hesperoptenus*	*Histiotus*	*Hesperoptenus*	*Lasionycteris*	
Laephotis	**Mimetillus*	*Mimetillus*	*Histiotus*	*Ia*	*Histiotus*	*Nycticeius*	

Table 2.1.—Continued.

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

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)	GTTGCTCTCTTGTGATTTGCTGC		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)	GGATNTGCTTTCWGAACTGRAGG		Van Den Bussche et al. 2003
PRKCI	BatPKa (F)	CTTGTCAATGATGATGAGG	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)	TTTGTTATTGTTGGTGGCTATCAG		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)	GCAGCAWGTAATCCAGTAGC		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)	CCCATGTTGCTTCCAAACCATA		Stadelmann et al. 2007
	Myotis1458R (Ex - R)	TTGCTATCTTCACATGCTCATTGC		Stadelmann et al. 2007
STAT	BatSTATa (F)	CTGCTCATCAACAAGCCCGA	48 – 62	Eick et al. 2005
	BatSTATb (R)	GGCTTCAGGTTCCACAGGTTGC		Eick et al. 2005
	ArtiSTATa (F)	GAAGAAACATCACAAGCCCC	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)

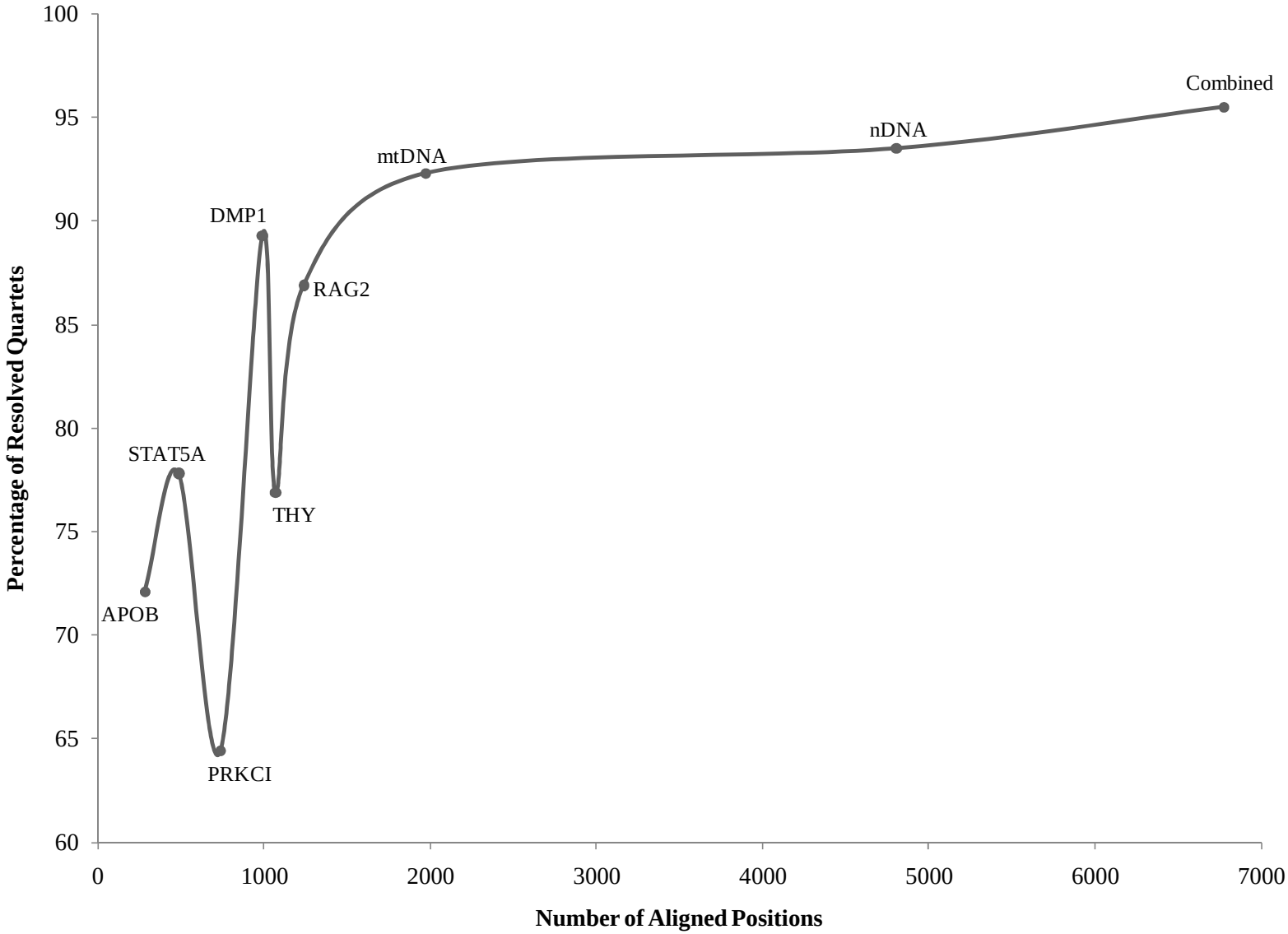
FIGURE LEGENDS

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 (≥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 (≥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 (≥70% bootstrap values) and Bayesian analysis (≥0.95 posterior probability) depicted. Symbols and abbreviations as in Fig. 2.2.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65
60
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2000
3000
4000
5000
6000
7000
Percentage of Resolved Quartets
Number of Aligned Positions
APOB
STAT5A
PRKCI
DMP1
THY
RAG2
mtDNA
nDNA
Combined


0.05
Ny. leisleri
Ny. noctula
P. hesperidus
P. pipistrellus
P. pygmaeus
P. nathusii
P. coromandra
P. tenuis
P. javanicus
P. paterculus
Scotoecus
Vespertilio
E. dimissus
T. pachypus
T. robustula
Ch. gouldi
Ch. morio
V. regulus
V. vulturnus
H. cadornae
H. savii
H. eisentrauti
Nycticeinops
N. nanus
N. brunneus
N. rendalli
N. somalicus
Laephotis
G. argentatus
G. egeria
G. beatrix
G. variegatus
Lasionycteris
Nycticeius
Arielulus
E. brasiliensis
E. furinalis
E. diminutus
E. macrotus (Arg. Ne.)
E. macrotus (Arg. Ca.)
E. macrotus (Arg. Sa.)
E. macrotus (Peru)
E. magellanicus
E. fuscus
E. hottentotus
E. serotinus (Eu.)
E. serotinus (Tu.)
Scotomanes
Parastrellus
Perimyotis
A. pallidus (CA)
A. pallidus (TX)
A. pallidus (AZ)
Bauerus
R. aeneus
R. tumida
R. mira
R. parvula
Baeodon
S. borbonicus
S. dinganii
S. leucogaster
S. viridis
S. heathi
S. nux
S. kuhlii
L. attratus
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L. borealis
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L. seminolus
L. cinereus
L. ega
L. xanthinus
C. mexicanus
C. townsendii
C. rafinesquii
Barbastellus
Otonycteris
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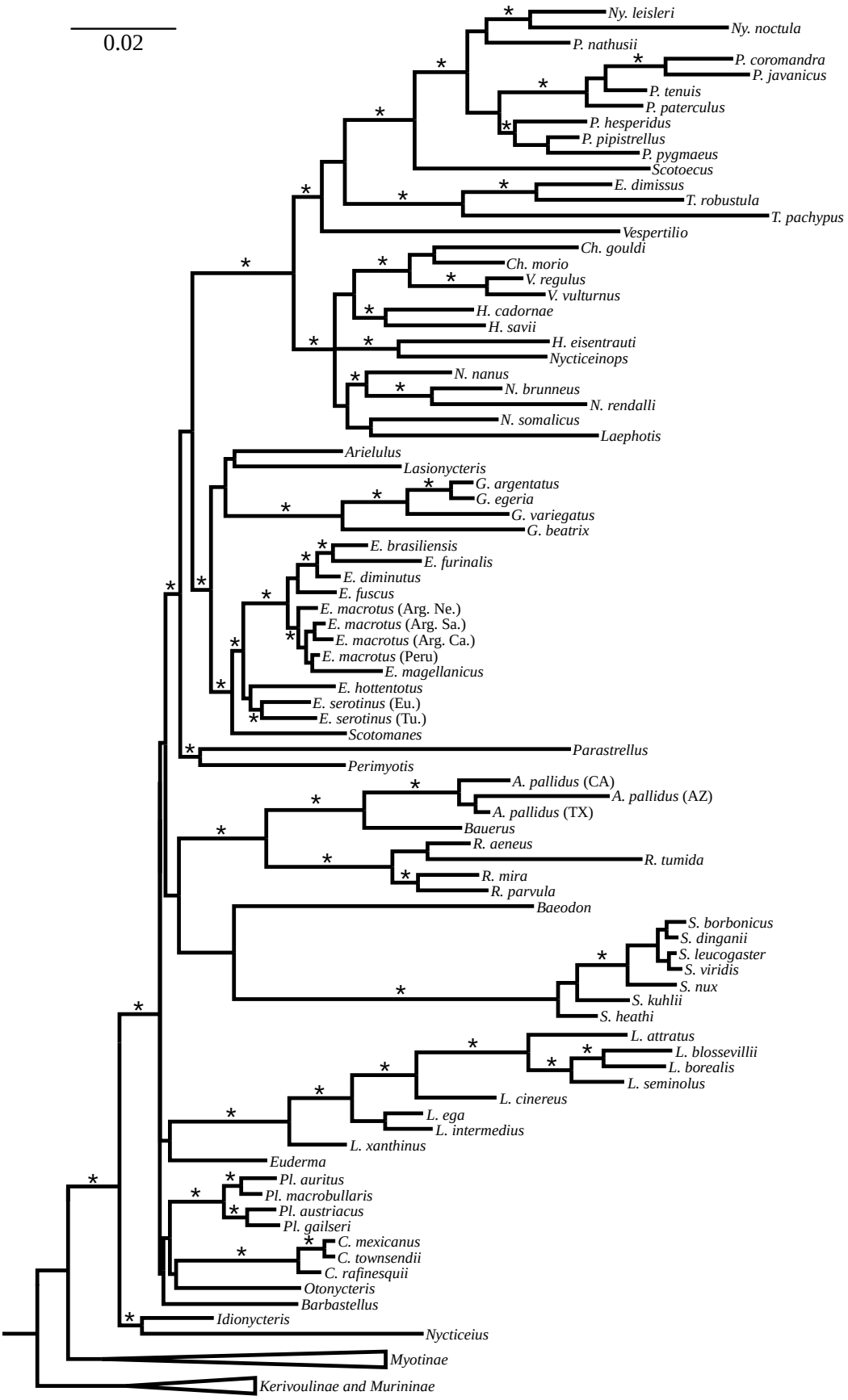
Pl. gailseri
Pl. macrobullaris
Euderma
Idionycteris
Myotinae
Kerivoulinae and Murininae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P. pygmaeus
Scotoecus
E. dimissus
T. robustula
T. pachypus
Vespertilio
Ch. gouldi
Ch. morio
V. regulus
V. vulturnus
H. cadornae
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G. beatrix
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E. diminutus
E. fuscus
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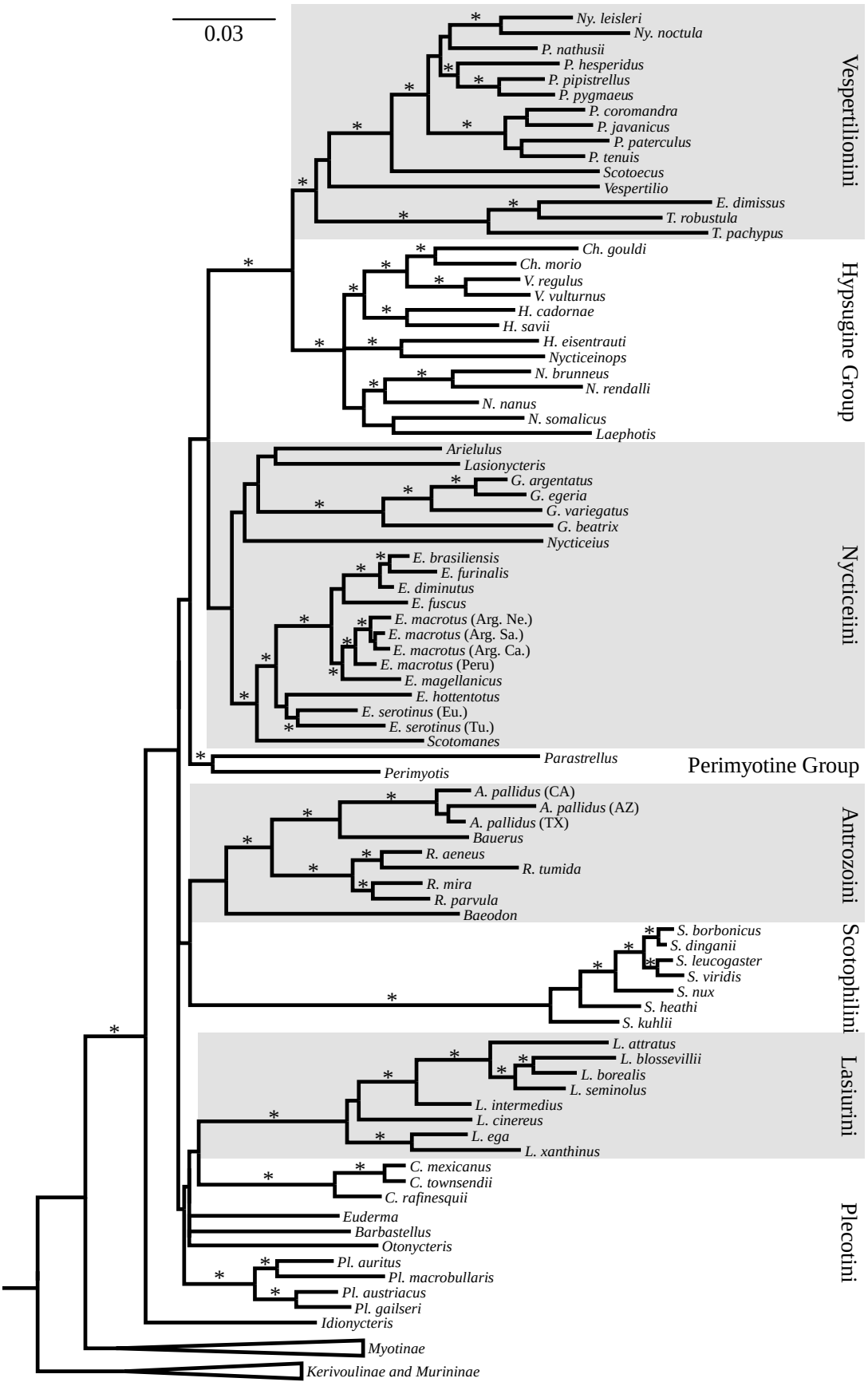
E. macrotus (Arg. Ca.)
E. macrotus (Peru)
E. magellanicus
E. hottentotus
E. serotinus (Eu.)
E. serotinus (Tu.)
Scotomanes
Parastrellus
Perimyotis
A. pallidus (CA)
A. pallidus (AZ)
A. pallidus (TX)
Bauerus
R. aeneus
R. tumida
R. mira
R. parvula
Baeodon
S. borbonicus
S. dinganii
S. leucogaster
S. viridis
S. nux
S. kuhlii
S. heathi
L. attratus
L. blossevillii
L. borealis
L. seminolus
L. cinereus
L. ega
L. intermedius
L. xanthinus
Euderma
Pl. auritus
Pl. macrobullaris
Pl. austriacus
Pl. gailseri
C. mexicanus
C. townsendii
C. rafinesquii
Otonycteris
Barbastellus
Idionycteris
Nycticeius
Myotinae
Kerivoulinae and Murininae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P. tenuis
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Vespertilio
E. dimissus
T. robustula
T. pachypus
Vespertilionini
Ch. gouldi
Ch. morio
V. regulus
V. vulturnus
H. cadornae
H. savii
H. eisentrauti
Nycticeinops
N. brunneus
N. rendalli
N. nanus
N. somalicus
Laephotis
Hypsugine Group
Arielulus
Lasionycteris
G. argentatus
G. egeria
G. variegatus
G. beatrix
Nycticeius
E. brasiliensis
E. furinalis
E. diminutus
E. fuscus
E. macrotus (Arg. Ne.)
E. macrotus (Arg. Sa.)
E. macrotus (Arg. Ca.)
E. macrotus (Peru)
E. magellanicus
E. hottentotus
E. serotinus (Eu.)
E. serotinus (Tu.)
Scotomanes
Nycticeiini
Parastrellus
Perimyotis
Perimyotine Group
A. pallidus (CA)
A. pallidus (AZ)
A. pallidus (TX)
Bauerus
R. aeneus
R. tumida
R. mira
R. parvula
Baeodon
Antrozoini
S. borbonicus
S. dinganii
S. leucogaster
S. viridis
S. nux
S. heathi
S. kuhlii
Scotophilini
L. attratus
L. blossevillii
L. borealis
L. seminolus
L. intermedius
L. cinereus
L. ega
L. xanthinus
Lasiurini
C. mexicanus
C. townsendii
C. rafinesquii
Euderma
Barbastellus
Otonycteris
Pl. auritus
Pl. macrobullaris
Pl. austriacus
Pl. gailseri
Plecotini
Idionycteris
Myotinae
Kerivoulinae and Murininae

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 Nycticeini [= 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 P^3 and I^3 and a reduction of I^2 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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer 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* (Hoofer and Van Den Bussche 2003). Hoofer 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 (*senus* 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 Hoofer 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 Hoofer 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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer et al. 2003; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2003). Using protocols outlined in Van Den Bussche and Hoofer (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 Hoofer 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 X 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, Genéve (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. Hoofer 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 Goverment, (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 tree having 3,776 steps, 21 supported clades (bootstrap values ≥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 1tree 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 ≥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* Hoofer 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* Hoofer 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* Hoofer 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 ≥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* Hoofer and Van Den Bussche 2003), a clade made up of Pleocotini, *Lasiurus*, and New Word 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 (Ärnbäck-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* Hoofer 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 (Hoofer 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 (Hoofer and Van Den Bussche 2003), but provided more resolution of deeper divergent clades. Results from the mtDNA gene region (and Hoofer and Van Den Bussche 2003) placed *Nycticeius* in a clade with *Arielulus, Eptesicus* (including *Histiotus*), *Glauconycteris, Lasionycteris*, and *Scotomanes* (their Nycticeiini). Hoofer 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* Hoofer 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*. Hoofer 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* Hoofer and Van Den Bussche 2003), but refutes Antrozoidae (Simmons 1998; Simmons and Geisler 1998) and Antrozinae (Miller 1897; Simmons 2005).

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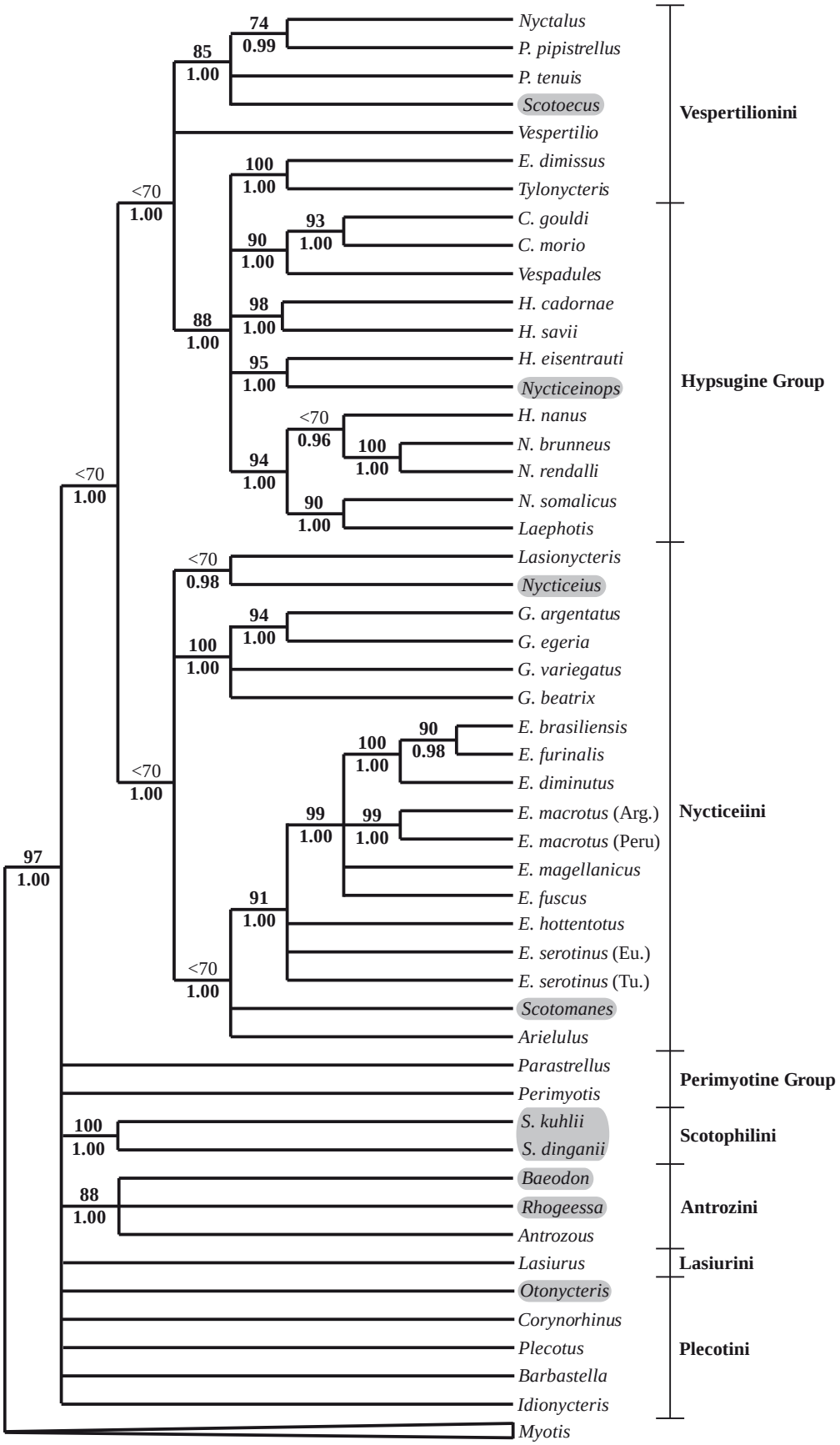
FIGURE LEGENDS

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 (≥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.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C. gouldi
C. morio
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H. cadornae
H. savii
H. eisentrauti
Nycticeinops
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N. brunneus
N. rendalli
N. somalicus
Laephotis
Lasionycteris
Nycticeius
G. argentatus
G. egeria
G. variegatus
G. beatrix
E. brasiliensis
E. furinalis
E. diminutus
E. macrotus (Arg.)
E. macrotus (Peru)
E. magellanicus
E. fuscus
E. hottentotus
E. serotinus (Eu.)
E. serotinus (Tu.)
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Arielulus
Parastrellus
Perimyotis
S. kuhlii
S. dinganii
Baeodon
Rhogeessa
Antrozous
Lasiurus
Otonycteris
Corynorhinus
Plecotus
Barbastella
Idionycteris
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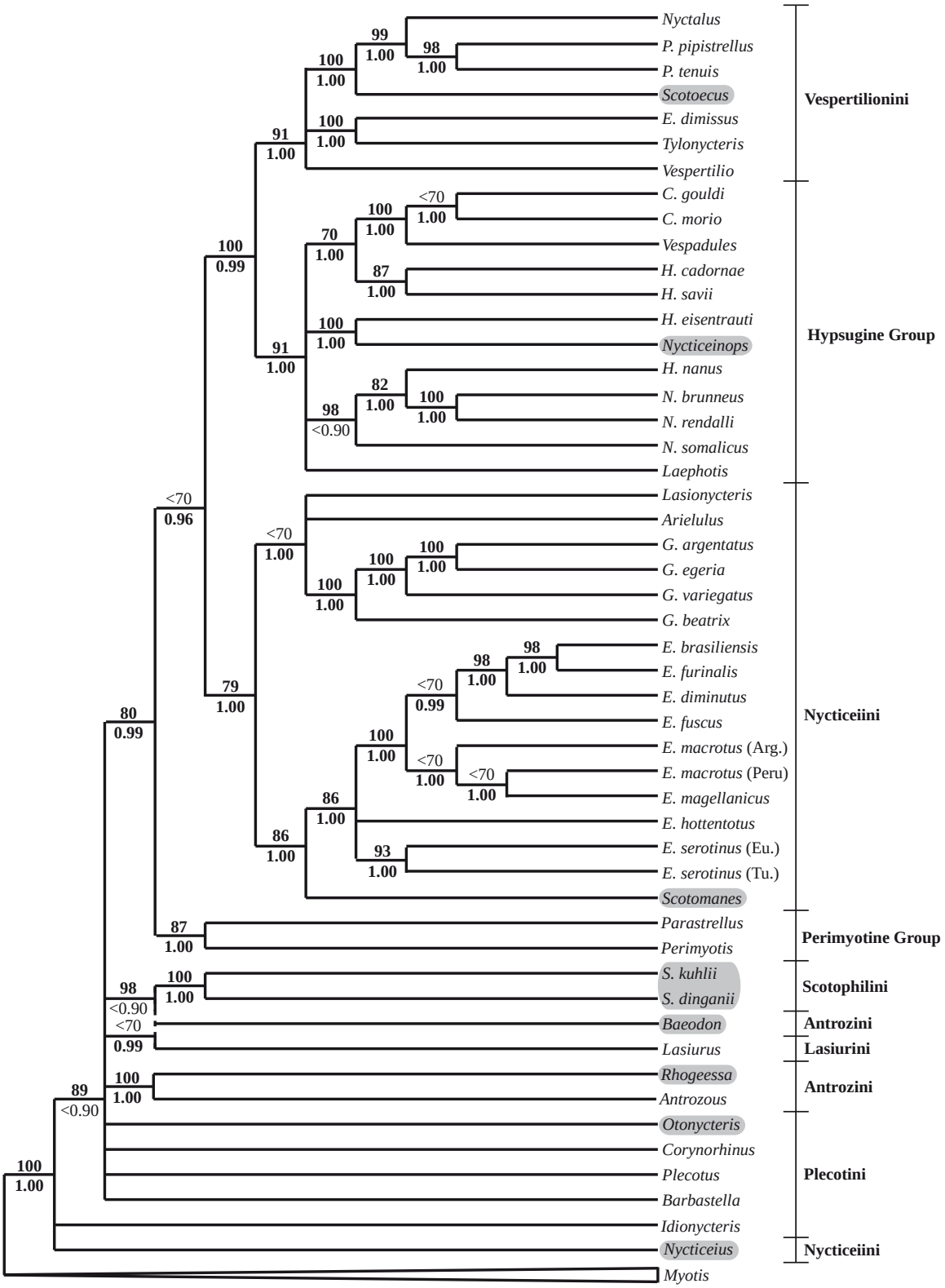
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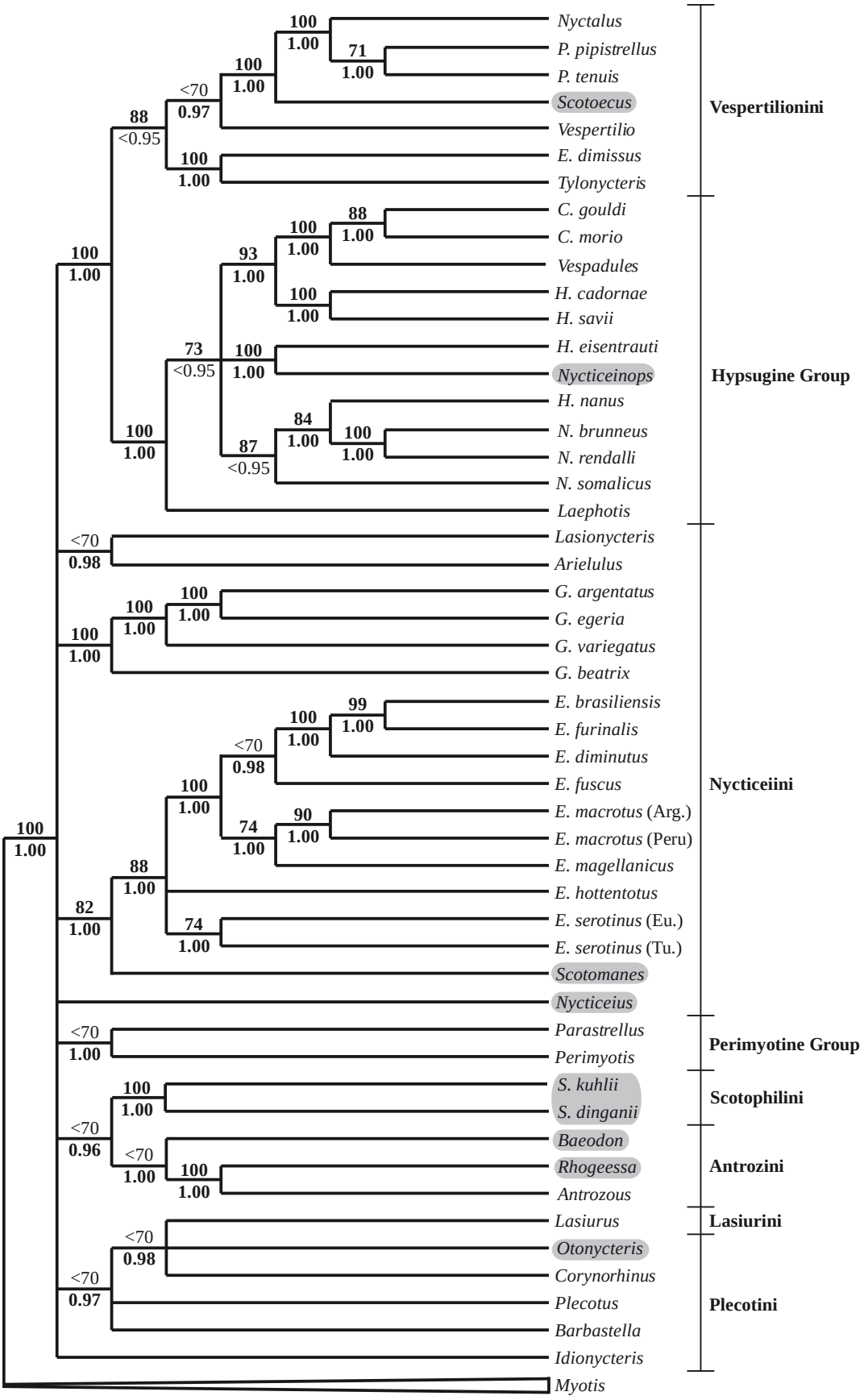
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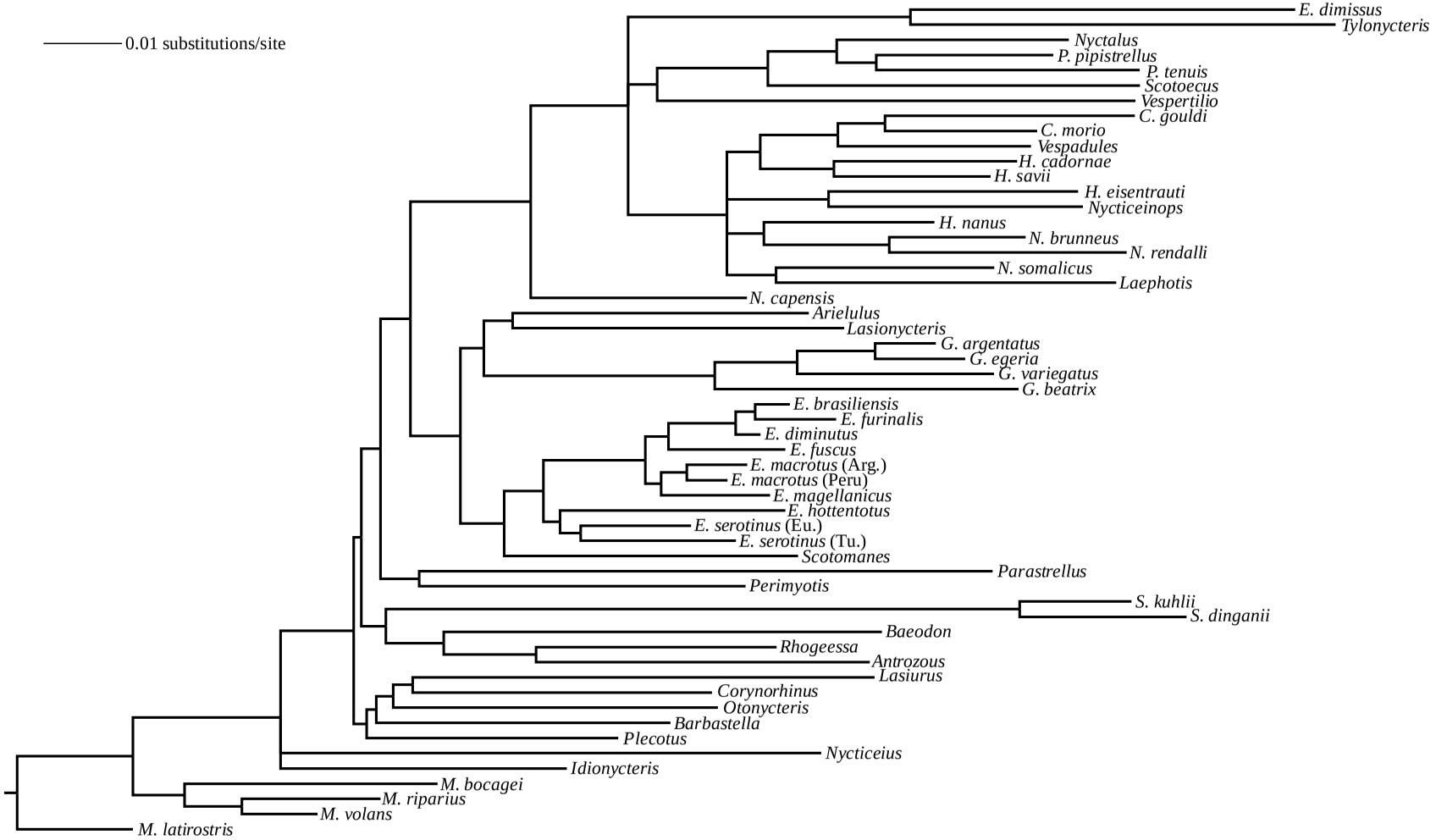
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Rhogeessa
Antrozous
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Otonycteris
Barbastella
Plecotus
Nycticeius
Idionycteris
M. bocagei
M. riparius
M. volans
M. latirostris

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 P^3 (shared with his "Nycticeini") and presence of I^2 (distinct from his "Nycticeini"). This group was further subdivided into 2 subgroups, Eptesicoid genera with P^2 absent and Pipistrelloid genera with P^2 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 (Ärnbäck-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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer 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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer et al. 2003; Van Den Bussche and Hoofer 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, Genéve (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-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; *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 vulturnus* (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 (Hoofer and Van Den Bussche 2001, 2003; Hoofer et al. 2003; Van Den Bussche and Hoofer 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 Hoofer 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 ≥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 X 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 (Hoofer 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 ≥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* Hoofer 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. vulturnus*, 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 ≥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* Hoofer 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 ≥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 (Ärnbäck-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 Hoofer 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 Hoofer 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 Hoofer 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 Hoofer 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 Hoofer 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 (Hoofer 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; Hoofer 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). Hoofer 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 (Hoofer 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 Hoofer 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 Hoofer 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).

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

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

Table 4.1.—Continued.

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

[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 *Chalinolobus*
Genus *Vespadelus*
Genus *Hypsugo*[b]
Genus *Nycticeinops*[c]
Genus *Neoromicia*[d]
Genus *Laephotis*[e]
Tribe Vespertilionini
Genus *Pipistrellus*[f]
Genus *Scotoecus*
Genus *Tylonycteris*[g]
Genus *Vespertilio*
Tribe Eptesicini[h]
Genus *Arielulus*
Genus *Lasionycteris*
Genus *Glauconycteris*
Genus *Eptesicus*
Genus *Scotomanes*
Tribe Unnamed Tribe[i]
Genus *Parastrellus*
Genus *Perimyotis*

[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 Nycticeiini.

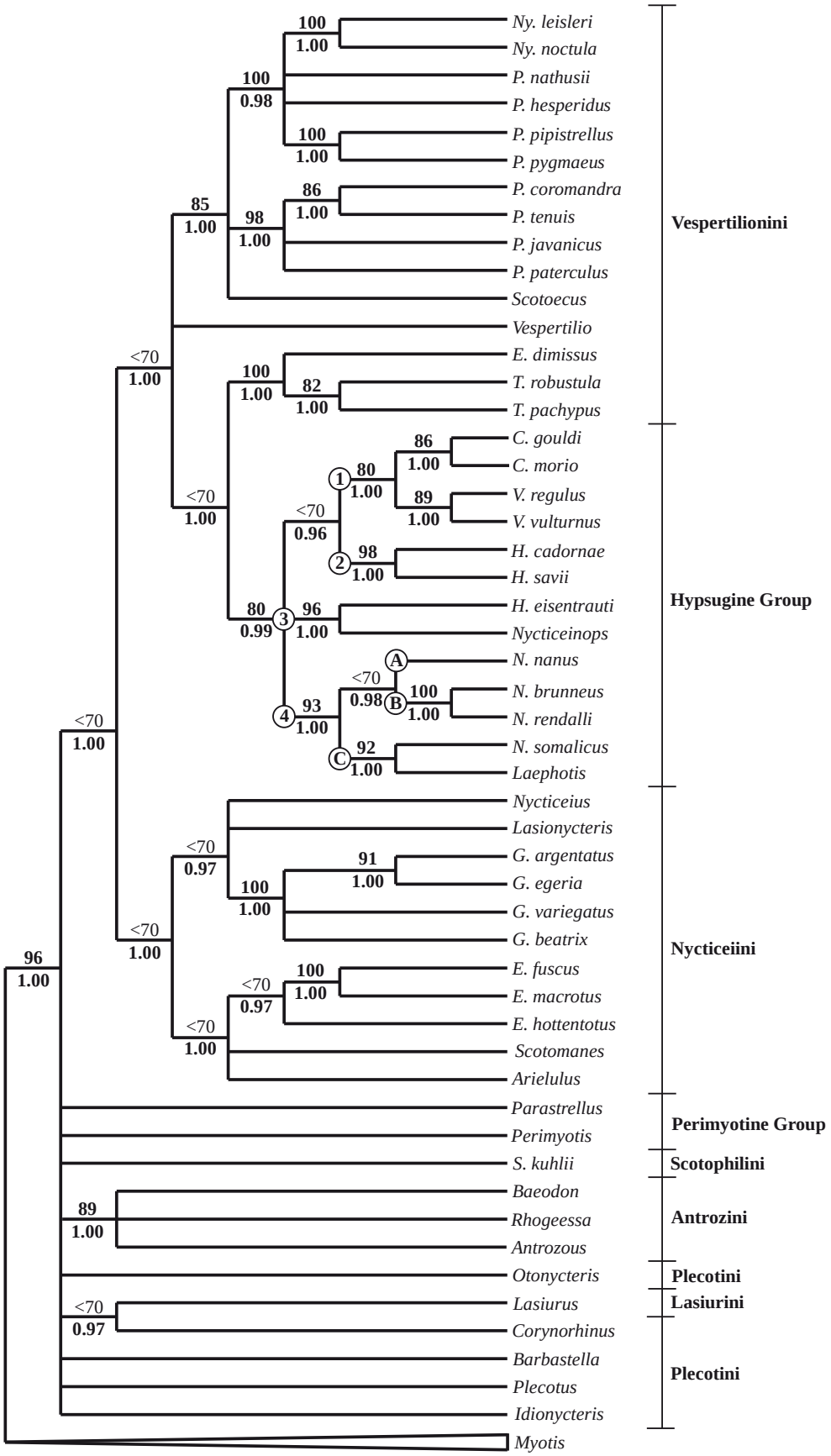
[i] 'Perimyotini' would be suggested name since *Perimyotis* Menu, 1984 has priority.

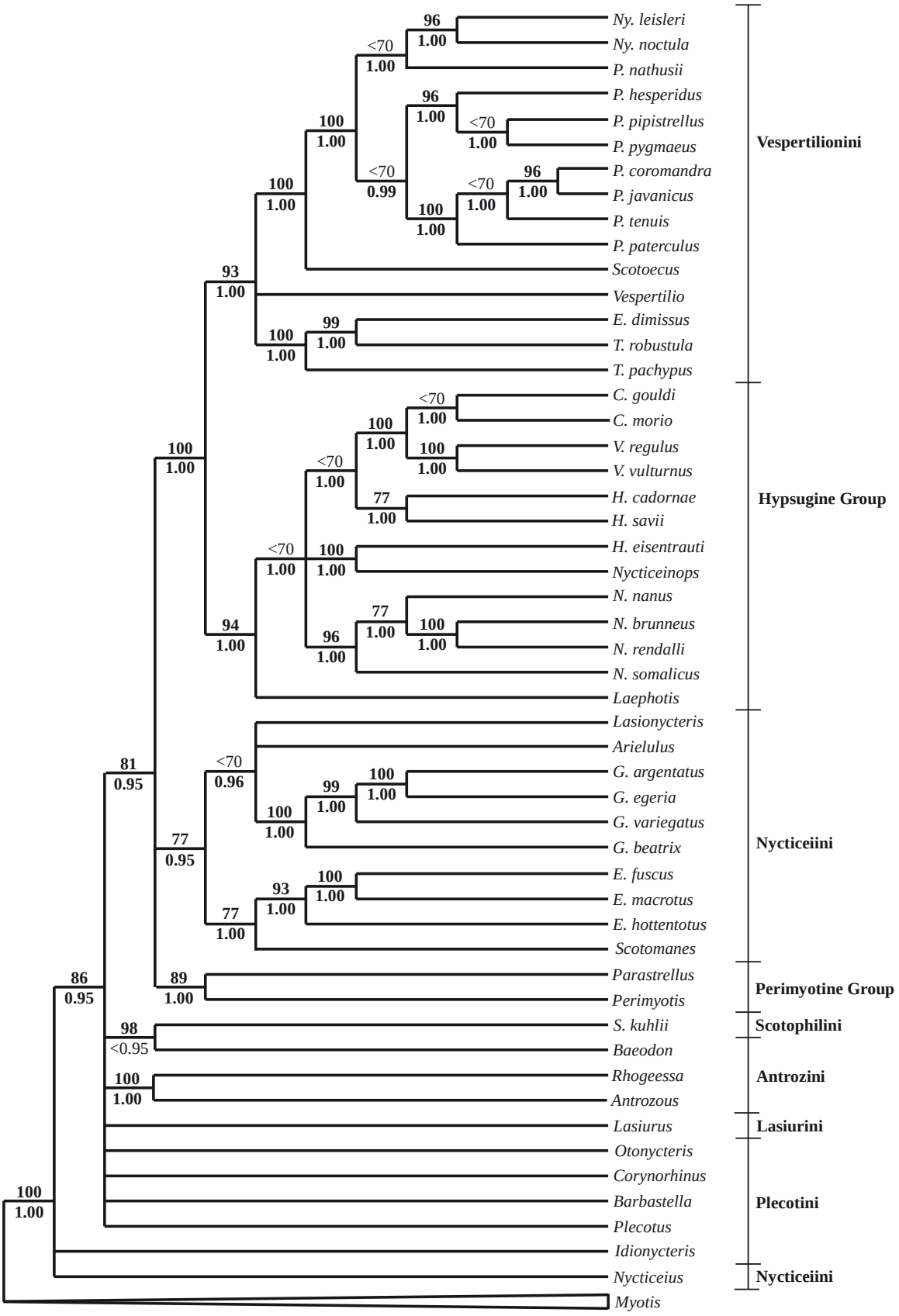
FIGURE LEGENDS

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 (≥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.


Ny. leisleri
Ny. noctula
P. nathusii
P. hesperidus
P. pipistrellus
P. pygmaeus
P. coromandra
P. tenuis
P. javanicus
P. paterculus
Scotoecus
Vespertilio
E. dimissus
T. robustula
T. pachypus
C. gouldi
C. morio
V. regulus
V. vulturnus
H. cadornae
H. savii
H. eisentrauti
Nycticeinops
N. nanus
N. brunneus
N. rendalli
N. somalicus
Laephotis
Nycticeius
Lasionycteris
G. argentatus
G. egeria
G. variegatus
G. beatrix
E. fuscus
E. macrotus
E. hottentotus
Scotomanes
Arielulus
Parastrellus
Perimyotis
S. kuhlii
Baeodon
Rhogeessa
Antrozous
Otonycteris
Lasiurus
Corynorhinus
Barbastella
Plecotus
Idionycteris
Myotis
Vespertilionini
Hypsugine Group
Nycticeiini
Perimyotine Group
Scotophilini
Antrozini
Plecotini
Lasiurini
Plecotini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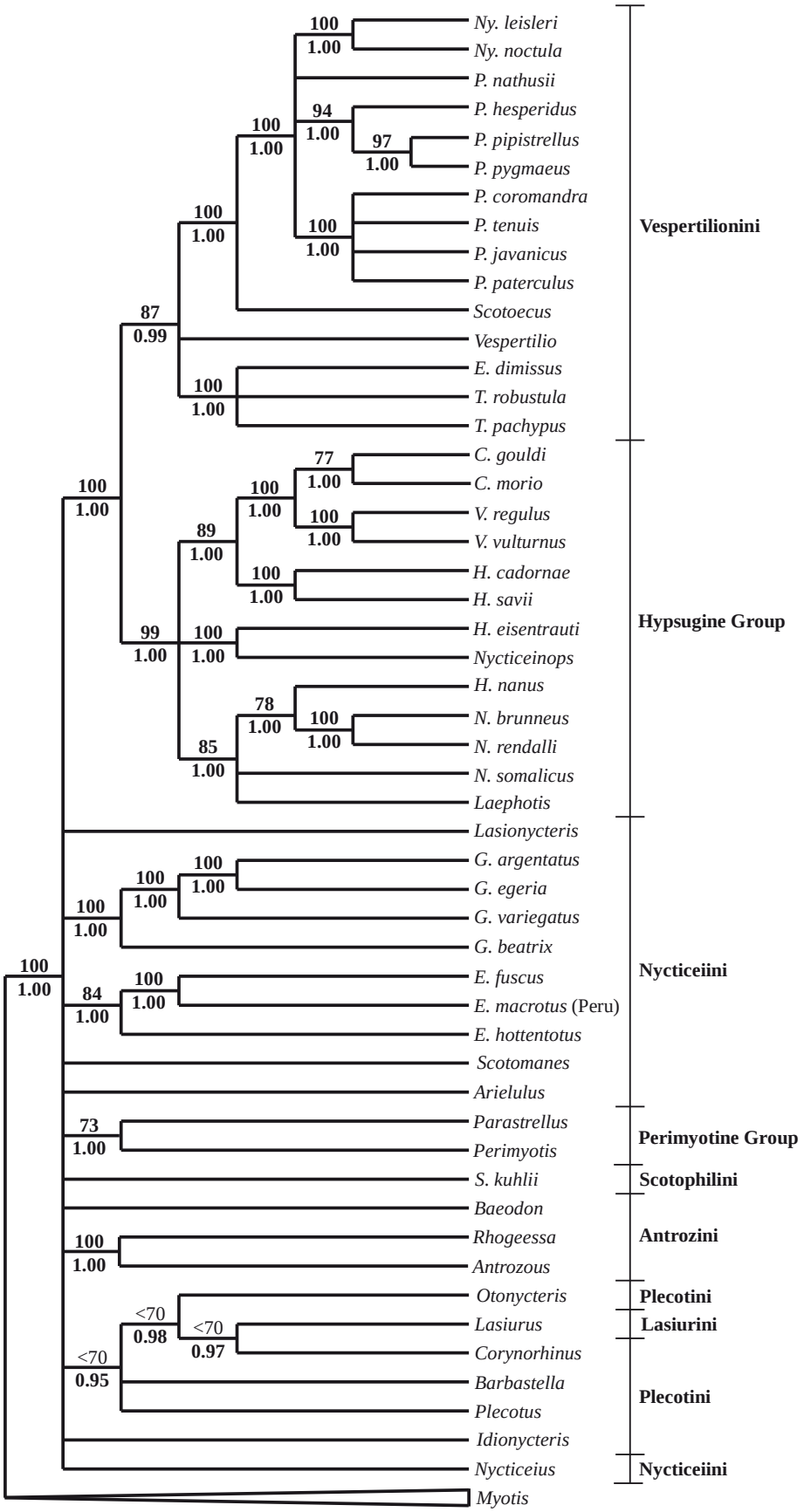
Ny. noctula
P. nathusii
P. hesperidus
P. pipistrellus
P. pygmaeus
P. coromandra
P. javanicus
P. tenuis
P. paterculus
Scotoecus
Vespertilio
E. dimissus
T. robustula
T. pachypus
C. gouldi
C. morio
V. regulus
V. vulturnus
H. cadornae
H. savii
H. eisentrauti
Nycticeinops
N. nanus
N. brunneus
N. rendalli
N. somalicus
Laephotis
Lasionycteris
Arielulus
G. argentatus
G. egeria
G. variegatus
G. beatrix
E. fuscus
E. macrotus
E. hottentotus
Scotomanes
Parastrellus
Perimyotis
S. kuhlii
Baeodon
Rhogeessa
Antrozous
Lasiurus
Otonycteris
Corynorhinus
Barbastella
Plecotus
Idionycteris
Nycticeius
Myotis
Vespertilionini
Hypsugine Group
Nycticeiini
Perimyotine Group
Scotophilini
Antrozini
Lasiurini
Plecotini
Nycticeiini


Ny. leisleri
Ny. noctula
P. nathusii
P. hesperidus
P. pipistrellus
P. pygmaeus
P. coromandra
P. tenuis
P. javanicus
P. paterculus
Scotoecus
Vespertilio
E. dimissus
T. robustula
T. pachypus
C. gouldi
C. morio
V. regulus
V. vulturnus
H. cadornae
H. savii
H. eisentrauti
Nycticeinops
H. nanus
N. brunneus
N. rendalli
N. somalicus
Laephotis
Lasionycteris
G. argentatus
G. egeria
G. variegatus
G. beatrix
E. fuscus
E. macrotus (Peru)
E. hottentotus
Scotomanes
Arielulus
Parastrellus
Perimyotis
S. kuhlii
Baeodon
Rhogeessa
Antrozous
Otonycteris
Lasiurus
Corynorhinus
Barbastella
Plecotus
Idionycteris
Nycticeius
Myotis
Vespertilionini
Hypsugine Group
Nycticeiini
Perimyotine Group
Scotophilini
Antrozini
Plecotini
Lasiurini
Plecotini
Nycticeiini

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 (Hoofer 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* Hoofer and Van Den Busshce 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*; Hoofer 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* Hoofer 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 Hoofer 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; $\overline{X}$ = 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 Hoofer 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; Hoofer 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.

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

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

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

Appendix I. Continued.

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

Appendix I. Continued.

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

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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.

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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