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PHYLOGENETIC RELATIONSHIPS AMONG RECENT CHIROPTERAN FAMILIES AND THE IMPORTANCE OF CHOOSING APPROPRIATE OUT-GROUP TAXA

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Results of recent molecular studies cast doubt on the validity of the superorder Archonta, suborders Megachiroptera and Microchiroptera, and infraorder Yinochiroptera and has even led some to consider novel alternatives for the evolution of flight and echolocation in mammals. At present, higher-level relationships within Chiroptera still is without consensus, and much of this controversy is related to how bats are related to other mammals and also to relationships among family-level lineages within Chiroptera. Although this controversy superficially manifests itself as differences in the relative merits of morphologic versus molecular data, both classes of data are themselves conflicting. We contend that much of the discrepancy among these studies is due to improper choice of out-group, limited taxonomic sampling, or both. We examined approximately 3 kb of mitochondrial DNA from 104 bats representing the taxonomic, geographic, and morphologic diversity within all families (except the monotypic Craseonycteridae) and 58 additional taxa representing 12 other orders of mammals. Results of our analyses strongly support other recent work indicating that Archonta is not a natural assemblage and that the sister taxon to Chiroptera may include Cetartiodactyla, Perissodactyla, Carnivora, and possibly Pholidota. Using representatives of these taxa as out-groups to evaluate interfamilial relationships within Chiroptera, we detected strong support for recognition of the suborders Yinpterochiroptera and Yangochiroptera. Within Yangochiroptera, our analyses strongly support expansion of the superfamily Noctilionoidea to include the New World Thyropteridae and Furipteridae.

Key words: Archonta, Chiroptera, Laurasiatheria, molecular phylogenetics, Yinpterochiroptera

Although higher-level relationships of bats (Mammalia: Chiroptera) have been debated without consensus for centuries, relationships suggested by Smith (1976) represent the generally accepted view of chiropteran evolution since the 17th century. Since the 1970s, investigations of new types of characters (morphological and molecular), coupled with technological advancements in data management and explicit phylogenetic analysis, have provided new insight into chiropteran evolution and questioned the validity of the superorder Archonta, order Chiroptera, suborders Megachiroptera and Microchiroptera, and infraorder Yinochiroptera (Hutcheon et al. 1998; Kirsch 1996; Kirsch and Pettigrew 1998; Lin and Penny 2001; Madsen et al. 2001; Murphy et al. 2001a, 2001b; Nikaido et al. 2000; Pettigrew and Kirsch, 1998; Smith and Madkour 1980; Teeling et al. 2000, 2002, 2003). Molecular evidence even has led some to consider novel alternatives for the evolution of echolocation and flight in mammals (Teeling et al. 2000). At present, higher-level systematics of Chiroptera still is without consensus and is complicated by conflicting results among studies that have employed different data sources, taxonomic sampling schemes, or analytical methods (Hoofer et al. 2003; Jones et al. 2002; Kawai et al. 2002; Lin and Penny 2001; Nikaido et al. 2000; Simmons 1998, 2000; Simmons and Geisler 1998, Teeling et al. 2000, 2002, 2003; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2003; Volleth et al. 2002). This has resulted in several discordant higher-level classifications (Hoofer et al. 2003; Koopman 1994; Simmons 1998; Teeling et al. 2002).

Most of the controversy is related to 2 general questions regarding how bats are related to other mammals (Lin and Penny 2001; Madsen et al. 2001; Miyamoto et al. 2000; Murphy et al. 2001a, 2001b; Nikaido et al. 2000; Novacek 1994; Novacek and Wyss 1986) and monophyly of, and relationships among, family-level lineages within Chiroptera (Gopalakrishna and Karim 1980; Hoofer and Van Den Bussche 2003; Hoofer et al. 2003; Jones et al. 2002; Kennedy et al. 1999; Kirsch 1996;

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Simmons 1998; Simmons and Geisler 1998; Teeling et al. 2002, 2003; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2002, 2003). In each case, the controversy superficially manifests itself as difference in the relative merits of morphologic versus molecular data despite the fact that both classes of data are themselves conflicting.

Although bat biologists have generated a vast amount of data that could profitably be interpreted in a higher-level phylogenetic framework, the lack of a well-resolved and well-supported higher-level phylogeny has hampered phylogenetic interpretation of these data. Moreover, interpretation of diverse ecological, morphological, and behavioral data based on a poorly supported phylogeny may be hindering our attempts at understanding the adaptive radiation of bats. Therefore, the objective of this study is to evaluate the phylogenetic relationships among Recent chiropteran families using statistical methodologies that extract from the data the maximum amount of information (Lewis 2001; Whelan et al. 2001). It is our contention that much of the discrepancy among previous higher-level phylogenetic studies of Chiroptera is due to improper choice of out-group, limited taxonomic sampling, or both. To account for the 1st potential problem (improper choice of out-group), we performed a phylogenetic analysis of 16 orders that include several exemplars of Archonta and Laurasiatheria, taxa proposed to be closely related to Chiroptera (Gregory 1910; Lin and Penny 2001; Madsen et al. 2001; Murphy et al. 2001a, 2001b; Nikaido et al. 2000; Novacek 1994; Novacek and Wyss 1996; Simmons 1998). Based on perceived close relationships and suggested strong morphological evidence (Novacek 1992; Novacek and Wyss 1996), Chiroptera has been placed in superorder Archonta, along with orders Dermoptera, Primates, and Scandentia (Gregory 1910). However, recent molecular studies provide strong support (in terms of parametric and nonparametric bootstrapping and Bayesian posterior probabilities) for Chiroptera belonging to Laurasiatheria along with orders Cetartiodactyla, Perissodactyla, Carnivora, Pholidota, and Eulipothypla (Lin and Penny 2001; Madsen et al. 2001; Murphy et al. 2001a, 2001b; Nikaido et al. 2000), and significant differences exist in resulting phylogenetic hypotheses depending on the choice of out-group (Hulva and Horecek 2002; Simmons 1998; Teeling et al. 2002). Therefore, we evaluate the effect of choice of out-group on resulting chiropteran interfamilial relationships. Because we have generated the most taxonomically diverse chiropteran data set to date, examining interfamilial relationships based on alternative out-groups allows an evaluation of whether choice of out-group is the primary factor for discrepancies among previous studies or, alternatively, whether morphologic and molecular data truly are in conflict.

Once the most appropriate out-group is determined, we assess the phylogenetic relationships among chiropteran families by including 104 exemplars of Chiroptera to better represent the morphological, ecological, and biogeographic diversity than has been used in previous studies. Specifically, we are interested in evaluating the validity of the subordinal grouping of Megachiroptera and Microchiroptera compared to Yinpterochiroptera and Yangochiroptera (sensu Teeling et al. 2002), Nataloidea (sensu Simmons 1998), and Noctilionoidea (sensu Hoofer et al. 2003).

Materials and Methods

Standard methods were used to extract total cellular DNA from skeletal muscle or organ tissue samples (Longmire et al. 1997) and to amplify by PCR and to sequence the 12S rRNA, $tRNA^{Val}$, and 16S rRNA genes (Van Den Bussche and Hoofer 2000) from 34 species of bats. All 3 genes were sequenced entirely in both directions using a combination of internal and flanking primers (Van Den Bussche and Hoofer 2000). Additionally, we obtained from GenBank 12S rRNA, $tRNA^{Val}$, and 16S rRNA sequences for 58 taxa representing 15 mammalian orders plus 70 additional representatives of Chiroptera, most of which we generated in previous studies (Hoofer and Van Den Bussche 2001; Hoofer et al. 2003; Lee et al. 2002; Van Den Bussche and Hoofer 2000, 2001; Van Den Bussche et al. 2002). This taxonomic sampling represents all families of Chiroptera (except Craseonycteridae) and proposed sister groups to Chiroptera (Appendix I).

Because several parameters can affect alignment of multiple DNA sequences and hence the resulting phylogenetic tree(s), the most critical appearing to be subjective assignment of cost ratios for opening and extending gaps (DeSalle et al. 1994; Hickson et al. 2000; Morrison and Ellis 1997; Simon et al. 1994; Wheeler 1994, 1995), we performed 2 multiple sequence alignments using CLUSTALX software (Thompson et al. 1997), 1 with the default gap cost ratio (16.6:6) and the 2nd with a smaller gap cost ratio (5:4). Prior to phylogenetic analysis, both alignments were refined by eye, and hypervariable regions for which the assumption of positional homology was questionable were identified and excluded from subsequent phylogenetic analyses.

To address the objectives of this study, we analyzed several taxonomic subsets using Bayesian phylogenetics with the computer program MrBayes (Huelsenbeck and Ronquist 2001). The GTR + Γ + I model of sequence evolution, as determined via the computer program Modeltest (Posada and Crandall 1998), was used for all Bayesian analyses; however, we did not define values for model parameters a priori but treated them as unknown variables with uniform priors to be estimated in each analysis. All analyses were conducted with random starting trees without constraints, 4 simultaneous Markov chains were run for 1,600,000 generations with trees sampled every 10 generations, and temperature was set to 0.02. Burn-in values were empirically determined based on evaluation of likelihood scores converging on stable values (= stationarity). For each data set, 3 independent analyses of MrBayes were conducted to ensure that final trees converged on the same topology.

With exception of the evaluation of interordinal relationships, results from Bayesian analyses were evaluated further by performing 100 maximum likelihood bootstrap analyses with nearest neighbor interchange branch swapping using the computer program PAUP (Swofford 2000). We used the GTR + Γ + I model of sequence evolution with model parameters determined via the computer program Modeltest (Posada and Crandall 1998). Because of the large number of taxa included in each analysis (115–121), maximum likelihood analyses were performed using constrained trees, as described in the following.

Evaluation of the sister group to Chiroptera.—To evaluate the sister group to Chiroptera, 162 taxa representing orders Artiodactyla (n = 19), Carnivora (n = 2), Cetacea (n = 3), Chiroptera (n = 104), Dermoptera (n = 1), Didelphimorphia (n = 1), Eulipotyphla (n = 5), Lagomorpha (n = 2), Perissodactyla (n = 4), Pholidota (n = 1), Pinnipedia (n = 2), Primates (n = 7), Rodentia (n = 7), Scandentia (n = 2), Sirenia (n = 1), and Tubulidentata (n = 1) were included with

character-state changes polarized using *Didelphis virginianus* as the out-group.

Chiropteran interfamilial relationships.—To evaluate interfamilial relationships within Chiroptera, 104 chiropteran sequences plus 16 sequences representing Artiodactyla ($n = 9$), Cetacea ($n = 3$), and Perissodactyla ($n = 4$) were aligned as described previously. Out-group taxa were chosen on the basis of our results of mammalian interordinal relationships (see "Results"). Parameters for the GTR + Γ + I model of sequence evolution for alignment with gap cost ratio of 5:4 for maximum likelihood analysis were base frequencies = 0.402, 0.181, 0.183, 0.234; r-matrix = 4.873, 14.040, 3.072, 0.531, 40.644; shape parameter (α) of the gamma distribution = 0.6384; and proportion of invariant sites = 0.4056. Parameters for the GTR + Γ + I model of sequence evolution for default alignment were base frequencies = 0.359, 0.180, 0.202, 0.259; r-matrix = 5.110, 15.437, 2.461, 0.474, 38.728; α = 0.6575; and proportion of invariant sites = 0.5074. Each chiropteran family except Vespertilionidae and Rhinolophidae were constrained to be monophyletic. For Vespertilionidae, all taxa except the 2 representatives of *Miniopterus* were constrained to be monophyletic, as results from Bayesian analyses were interpreted as indicating that *Miniopterus* may represent a lineage independent from the remainder of Vespertilionidae (see "Results"). Because of recent data questioning monophyly of Rhinolophidae (Bogdanowicz and Owen 1998; Hand and Kirsch 1998; Hulva and Horecek 2002; Volleth et al. 2002), we chose not to constrain Rhinolophidae to be monophyletic.

To evaluate the effect of choice of out-group on chiropteran interfamilial relationships, 104 chiropteran sequences were aligned with sequences representing Dermoptera ($n = 1$), Primates ($n = 7$), and Scandentia ($n = 2$). We evaluated robustness of the results from Bayesian analysis by comparison with results from maximum likelihood analysis with the constraints described previously. Model parameters of the GTR + Γ + I model of sequence evolution for alignment with an opening-to-extending gap cost ratio of 5:4 were base frequencies = 0.396, 0.212, 0.174, 0.218; r-matrix = 4.276, 14.433, 3.574, 0.590, 39.736; α = 0.6102; and proportion of invariant sites = 0.3879. Model parameters for the GTR + Γ + I model of sequence evolution for alignment based on default parameters were base frequencies = 0.393, 0.200, 0.192, 0.216; r-matrix = 4.515, 13.084, 3.692, 0.539, 44.930; α = 0.5870; and proportion of invariant sites = 0.4337.

Results

Mitochondrial 12S rRNA, $tRNA^{Val}$, and 16S rRNA sequences from 34 taxa generated in this study were deposited in GenBank (accession nos. AY395835–AY395868). For each question outlined in this paper, we performed 2 alignments with different opening-to-extending gap cost ratios. In all cases, topologies produced by 2 alignments were identical in branching order, but in concordance with results from other studies (Hickson et al. 2000; Hoofer et al. 2003; Wheeler 1994), results based on smaller opening-to-extending gap cost ratio produced more statistically significant clades (in terms of posteriori probabilities and bootstrap support). Thus, only results from alignment with smaller opening-to-extending gap cost ratio are presented. For all Bayesian analyses, topologies, posterior probabilities, and model parameters were in excellent agreement among all 3 independent analyses. All alignments with designated hypervariable regions excluded from phylogenetic analysis, priors for the Bayesian analyses, and trees depicting relationships of all species can be obtained from the 1st author.

Evaluation of the sister group to Chiroptera.—Alignment of 162 mitochondrial ribosomal sequences based on an opening-to-extending gap cost ratio of 5:4 resulted in 3,019 aligned sites, of which 865 hypervariable sites were excluded from the analysis. Bayesian likelihoods reached stationarity at 100,000 generations (burn-in = 10,000), reducing this to 150,000 sampled trees. Although monophyly of all orders, Cetartiodactyla (Cetacea plus Artiodactyla), and Carnivora and Pinnipedia were supported with Bayesian posterior probabilities of ≥0.99, few clades documenting interordinal relationships were supported with posterior probabilities ≥0.95 (Fig. 1). These analyses failed to support Archonta (which includes Chiroptera, Primates, Scandentia, and Dermoptera) as a natural assemblage (posterior probability = 0) by documenting that Chiroptera shares closer phylogenetic affinities with Laurasiatheria (Pholidota, Cetartiodactyla Perissodactyla, Pinnipedia, and Carnivora), and this was supported with a Bayesian posterior probability of 0.96 (Fig. 1).

Chiropteran interfamilial relationships.—Based on our analysis to determine the sister group of Chiroptera (Fig. 1), representatives of Cetartiodactyla and Perissodactyla were used as out-groups in assessment of chiropteran interfamilial relationships. Alignment of 121 taxa with an opening-to-extending gap cost ratio of 5:4 resulted in 2,958 positions, of which 933 hypervariable sites were excluded from the analysis. Bayesian analysis reached stationarity at 52,000 generations (burn-in = 5,200), reducing the amount of data available for determining clade composition to 154,800 trees. Fig. 2 depicts phylogenetic relationships among all families along with Bayesian posterior probabilities and maximum likelihood bootstrap support ≥50%. The following clades received strong support (posterior probabilities ≥0.95; bootstrap support ≥70%) documenting monophyly of Chiroptera; a sister-group relationship of Rhinolophinae and Hipposiderinae; a clade consisting of Megadermatidae, Rhinopomatidae, and Rhinolophidae; monophyly of Yangochiroptera (sensu Teeling et al. 2002); a clade containing the New World Noctilionoidea, Furipteridae, and Thyropteridae, plus the New Zealand endemic Mystacinidae; a sister-group relationship between Furipteridae and Noctilionidae; and a sister-group relationship between Mormoopidae and Phyllostomidae.

Alignment of 115 sequences, representing the traditionally recognized Archonta (Chiroptera, Dermoptera, Primates, and Scandentia), based on an opening-to-extending gap cost ratio of 5:4 resulted in 2,964 aligned sites, of which 827 hypervariable sites were excluded from phylogenetic analysis. Bayesian analysis reached stationarity at 70,000 generations, reducing the amount of data available for determining clade composition to 153,000 trees. Fig. 3 depicts phylogenetic relationships among chiropteran families when representatives of Dermoptera, Primates, and Scandentia are used as out-groups. Although not receiving strong statistical support, this analysis reveals monophyly of Microchiroptera. Clades receiving strong statistical support (posterior probabilities ≥0.95; bootstrap support ≥70%) document monophyly of Chiroptera; sister-group relationship between Hipposiderinae and Rhinolophinae; monophyly of a clade comprising Megadermatidae, Rhinolophidae,

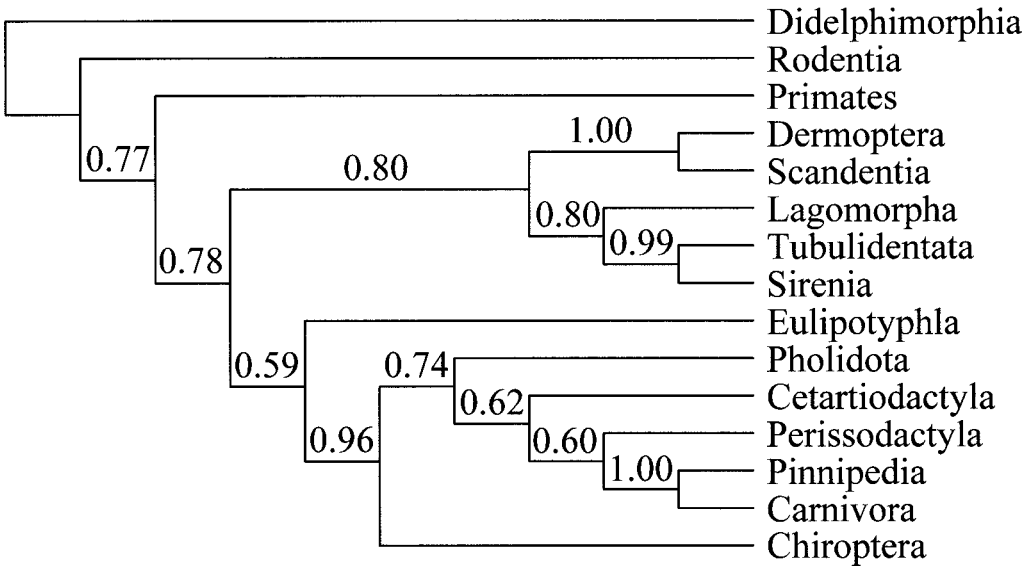

FIG. 1.— Phylogenetic relationships among mammalian orders as shown by results of a Bayesian phylogenetic analysis under the GTR + Γ + I model of DNA evolution. Numbers above internal lineages reflect Bayesian posterior probabilities.

and Rhinopomatidae; monophyly of Yangochiroptera (sensu Teeling et al. 2002); and sister-group relationships of Furipteridae and Noctilionidae as well as Mormoopidae and Phyllostomidae.

DISCUSSION

Sister group to Chiroptera.—Over the past 2 decades, the assumption that Chiroptera is most closely related to Dermoptera, Primates, and Scandentia has been repeatedly questioned (Cartmill and MacPhee 1980; Luckett 1980; Stanhope et al. 1992) but discounted because of apparent strong morphological evidence supporting this grouping or because such studies were based on poor taxonomic sampling (Novacek 1994; Novacek and Wyss 1986; Simmons 1998). Recently, phylogenetic analysis of large quantities of DNA sequence data provided strong support for Chiroptera belonging to Laurasiatheria along with Cetartiodactyla, Perissodactyla, Carnivora, Pholidota, and Eulipothyphla, whereas Dermoptera, Primates, and Scandentia belong to Euarchontoglires, along with Lagomorpha and Rodentia (Lin and Penny 2001; Madsen et al. 2001; Murphy et al. 2001a, 2001b; Nikaido et al. 2000). Similar to results of these recent molecular studies, results of our study support Chiroptera belonging to Laurasiatheria (Fig. 1).

Chiropteran interfamilial relationships.—Based primarily on morphological data and polarizing character-state changes with representatives of Archonta, Chiroptera has been divided into the suborders Megachiroptera, containing the Old World Pteropodidae, and Microchiroptera, containing all other families of bats. Microchiropteran paraphyly was 1st proposed based on studies of DNA hybridization (Hutcheon et al. 1998; Kirsch 1996; Kirsch and Hutcheon 1997; Kirsch et al. 1998; Pettigrew and Kirsch 1995) and satellite DNA (Baker et al. 1997). However, because microchiropteran monophyly appeared to be strongly supported based on morphologic data, Kirsch (1996) and Kirsch and Pettigrew (1998) suggested that patterns observed in the molecular data may be due to base compositional bias and suggested that the association of microchiropteran rhinolophoids with megachiropteran pteropodids may represent a case where molecular data are misleading, a conclusion supported by Simmons (1998). It is important to note that the apparent strong morphological evidence supporting Archonta monophyly is based on 2 characters that are variable in Archonta (Novacek and Wyss 1986). As summarized by Novacek and Wyss (1986), eutherian males have an external penis that in most species is enclosed in a sheathlike genital pouch that forms a sling in close attachment to the abdomen. In Primates, scandentians, dermopterans, and chiropterans, attachment of this pouch shows a posterior reduction and a pendulous penis. However, this characteristic varies within archontans. For example, while the penis is pendulous in dermopterans, it is less so in some primates and tupaiids, and in some bats the penis reverts to the more generalized abdominal form. Similarly, the 2nd character that apparently unites archontans, a suite of characters of the proximal tarsus, is also variable (Novacek and Wyss 1986). Therefore, the apparent strong morphological support for archontan monophyly may be just that—apparent.

To address the question of microchiropteran paraphyly, Teeling et al. (2002) examined approximately 7 kb of nuclear DNA sequence data from representatives of 11 chiropteran families and 8 additional mammalian orders. Phylogenetic analyses using several optimality criteria and algorithms resulted in strong support for the traditionally recognized microchiropteran families Rhinolophidae, Megadermatidae, and Rhinopomatidae being most closely related to the megachiropteran Pteropodidae. Teeling et al. (2002) proposed the suborder Yinpterochiroptera, containing the superfamilies Pteropodoidea (consisting of Pteropodidae) and Rhinolophoidea (consisting of Rhinolophidae, Rhinopomatidae, and Megaderamtidae), and the suborder Yangochiroptera, containing all remaining families of Chiroptera.

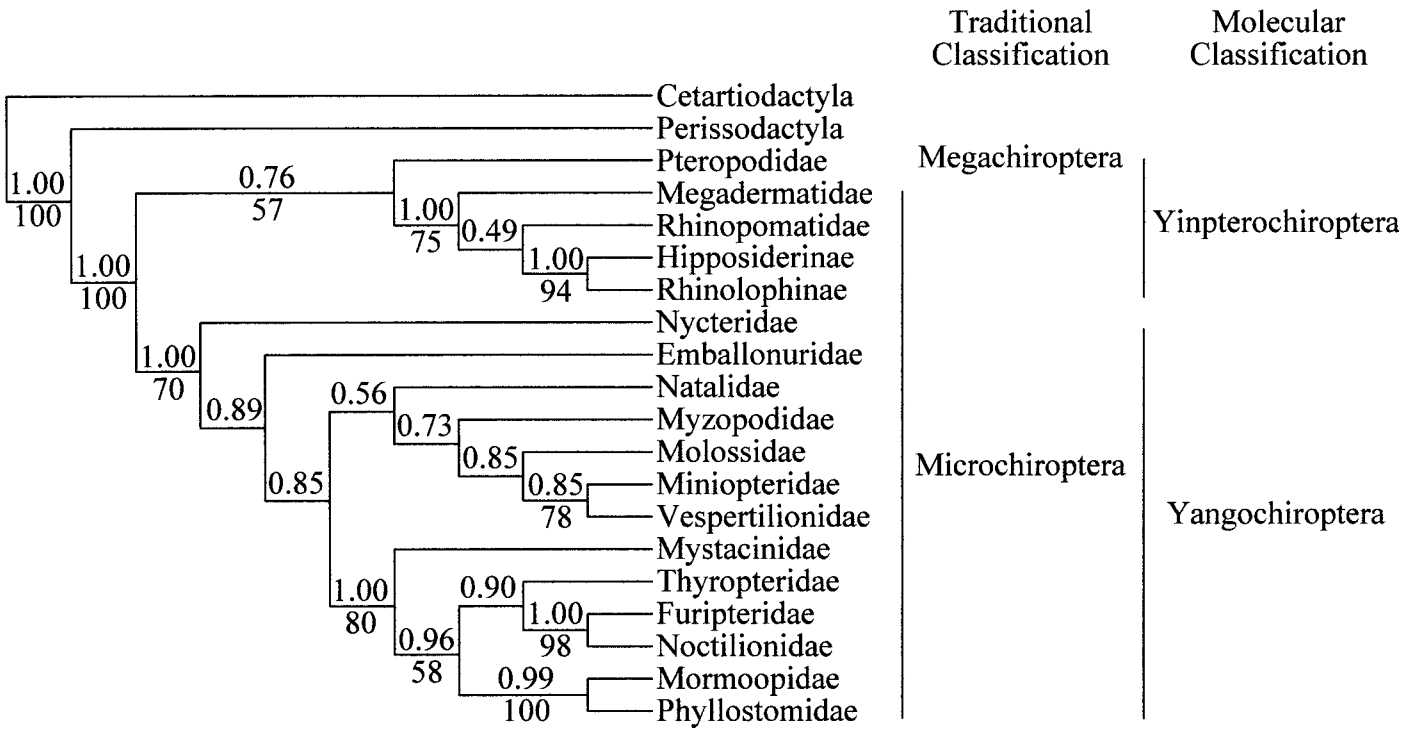

FIG. 2.— Higher-level relationships within Chiroptera resulting from unconstrained Bayesian phylogenetics and maximum likelihood analyses, along with traditional and molecular (Teeling et al. 2002) subordinal classification. Numbers above lineages are Bayesian posterior probabilities; numbers below lineages are percentage of 100 maximum likelihood bootstrap iterations documenting the formation of the specific clade in ≥50% of the bootstrap iterations.

Although not unambiguous, our data support the findings and taxonomic recommendations of Teeling et al. (2002). Using representatives of Laurasiatheria as out-groups, our optimal tree documents that Rhinolophidae, Rhinopomatidae, and Megadermatidae share a more recent ancestry with Pteropodidae than with the remaining chiropteran families (Fig. 2). Moreover, our data unambiguously document that Nycteridae shares closer phylogenetic affinities with the remaining chiropteran families. These same phylogenetic affinities have also recently been detected in a study of approximately 400 bp of the mitochondrial cytochrome-*b* gene (Hulva and Horacek 2002). Although our grouping of Yinpterochiroptera received low statistical support, grouping Rhinolophidae, Rhinopomatidae, and Megadermatidae sister to the remaining chiropteran families with Pteropodidae as the most basal lineage of Chiroptera received even lower statistical support (posterior probability = 0.21; bootstrap support = 13%).

We further evaluated the hypotheses of Megachiroptera and Microchiroptera as opposed to Yinpterochiroptera and Yangochiroptera using the Kishino and Hasegawa (1989) and Shimodaira and Hasegawa (1999) tests. Although statistical hypothesis testing in phylogenetics has been criticized when the optimal tree is compared a posteriori with other trees (Goldman et al. 2000), we deemed it appropriate to use in this case because we compared our tree with a priori hypotheses derived independent of our data. The length of our optimal tree, which supports subordinal groupings of Yinpterochiroptera and Yangochiroptera is –39402.22, whereas the length of the alternative topology, supporting Megachiroptera and Microchiroptera, is –39445.77. Resulting *P*-values for length differences between these topologies based on Kishino-Hasegawa (1989) and Shimodaira-Hasegawa (1999) tests, respectively, are 0.00 and 0.04, providing further statistical support for division of Chiroptera into the suborders Yinpterochiroptera and Yangochiroptera (sensu Teeling et al. 2002).

Because Chiroptera has traditionally been placed within Archonta, coupled with significant difference in subordinal composition of taxa between studies that used representatives of Archonta as out-groups relative to more recent molecular studies that polarized character-state changes with representatives of Laurasiatheria, we evaluated chiropteran interfamilial relationships under the assumption of archontan monophyly. The most significant taxonomic difference between results from this analysis (Fig. 3) and results of our phylogenetic analysis that used representatives of Laurasiatheria as out-groups (Fig. 2) regards the subordinal arrangement within Chiroptera. Under the assumption of Archonta monophyly, our optimal Bayesian and maximum likelihood tree reveals that Megadermatidae, Rhinopomatidae, and Rhinolophidae are the most basal members of a clade containing all families of Chiroptera except Pteropodidae. Thus, although weakly supported (posterior probability = 0.72; bootstrap support = 56%), based on the perceived strong morphological evidence for this relationship (Novacek 1994; Novacek and Wyss 1986), if we had not performed our 1st phylogenetic analysis to evaluate the sister group to Chiroptera, we would have concluded that our data

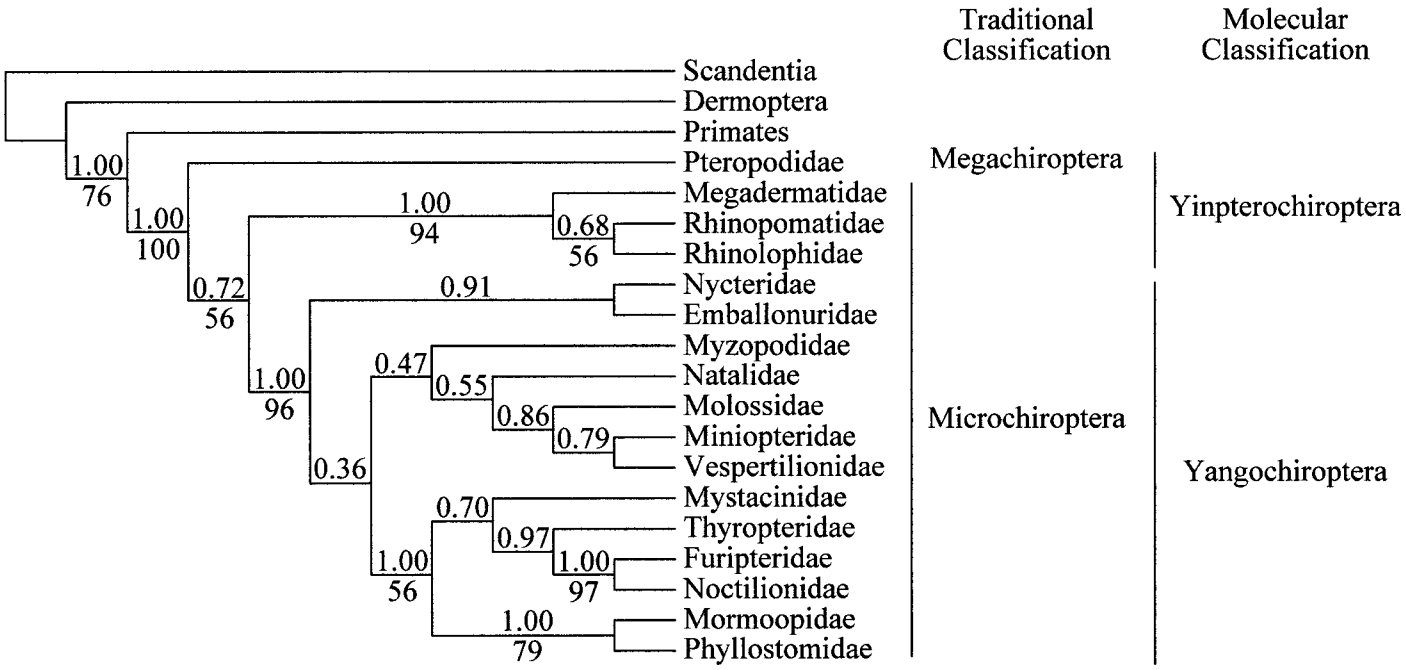

FIG. 3.— Phylogenetic relationships among chiropteran families under the assumption of Archonta monophyly, recovered with Bayesian phylogenetics and maximum likelihood analyses along with traditional and molecular (Teeling et al. 2002) subordinal classification. Numbers above lineages are Bayesian posterior probabilities; numbers below are percentage of 100 maximum likelihood bootstrap iterations of the specific clade in $\geq$50% of the bootstrap iterations.

support the traditional subordinal arrangement of Megachiroptera and Microchiroptera. However, based on our data and other independent molecular studies (Lin and Penny 2001; Madsen et al. 2001; Murphy et al. 2001a, 2001b; Nikaido et al. 2000), Chiroptera shares its closest phylogenetic affinities with Laurasiatheria, and therefore choice of out-group appears to be the primary factor for apparent discrepancies between recent higher-level phylogenetic studies of Chiroptera based on morphological and molecular data (Hulva and Horecek 2002; Simmons 1998, 2000; Simmons and Geisler 1998; Teeling et al. 2002).

Although Hipposideridae has been relegated to a subfamily of Rhinolophidae in most recent classifications (Koopman 1994; Simmons, 1998, 2000; Simmons and Geisler 1998), because several authors suggest that hipposiderids represent a family distinct from Rhinolophidae (Bogdanowicz and Owen 1998; Hand and Kirsch 1998; Hulva and Horacek 2002; Volleth et al. 2002), we did not constrain the maximum likelihood analysis to force monophyly of Rhinolophidae (sensu Koopman 1994). Results of our analyses provide strong support (posterior probability = 1.00; bootstrap support = 94%) that these taxa shared a most recent common ancestry after diverging from the remainder of Chiroptera. Unfortunately, our analysis was not of broad enough taxonomic scope to evaluate the validity of whether they each deserve familial status.

The 2nd large clade in Fig. 2 includes all remaining families of Chiroptera (posterior probability = 1.00; bootstrap support = 70%) and supports recognition of the suborder Yangochiroptera (sensu Teeling et al. 2002). Moreover, the strong statistical support for this clade supports the conclusion of Teeling et al. (2002) and Hulva and Horacek (2002) that Nycteridae does not share closer phylogenetic affinities with members of Rhinolophoidea (sensu Teeling et al. 2002) and thus refutes grouping Nycteridae with Rhinolophidae (including Hipposiderinae), Rhinopomatidae, and Megadermatidae (Jones et al. 2002; Simmons 1998; 2000; Simmons and Geisler 1998).

Results of our analysis detected strong support (posterior probability = 1.0; boostrap support = 80%) for Furipteridae, Mormoopidae, Mystacinidae, Noctilionidae, Phyllostomidae, and Thyropteridae sharing a most recent common ancestry after diverging from the remainder of chiropteran families (Fig. 2). With the exception of Furipteridae and Thyropteridae, a close association of the New World Mormoopidae, Noctilionidae, and Phyllostomidae and the New Zealand endemic Mystacinidae has been repeatedly detected (Hoofer et al. 2003; Kirsch et al. 1998; Pierson et al. 1986; Simmons and Conway 2001; Teeling et al. 2003; Van Den Bussche and Hoofer 2000, 2001). Although Furipteridae and Thyropteridae have been considered closely related to each other (Simmons 1998; Simmons and Geisler 1998) and to the vespertilionids and molossids (Koopman 1994), additional support for the close association of Furipteridae and Thyropteridae with Noctilionoidea comes from the concatenation of DNA sequence data of the mitochondrial ribosomal and nuclear RAG2 genes (Hoofer et al. 2003). These authors found strong support for these 6 families sharing a recent common ancestry after diverging from the remainder of the yangochiropteran families and suggested expansion of Noctilionoidea to include Furipteridae and Thyropteridae. Although our data strongly support sister-group relationships between Mormoopidae and Phyllostomidae and between Noctilionidae

and Furipteridae, relationships of these groups to each other and to Thyropteridae and Mystacinidae remain unresolved, and additional independent data are required to better elucidate the branching order within this superfamily.

In her reappraisal of higher-level relationships within Chiroptera, which resulted in her proposing a new classification, Simmons (1998) detected high bootstrap support (92%) for the close association of Natalidae with Furipteridae, Thyropteridae, and Myzopodidae and proposed the superfamily Nataloidea to reflect these close phylogenetic relationships. Neither this study (posterior probability = 0.00; boostrap support = 0%) nor the studies by Van Den Bussche et al. (2003) or Hoofer et al. (2003) supported recognition of Nataloidea.

The final taxonomic conclusion that can be made from our data regards the traditionally recognized vespertilionid subfamily Miniopterinae. Although most studies have regarded the 11 species of *Miniopterus* as representing a distinct subfamily within Vespertilionidae (Kawai et al. 2002; Koopman 1994; Simmons 1998), recent studies suggest that *Miniopterus* may represent a lineage distinct from the remaining vespertilionids (Gopalakrishna and Karim 1980; Hoofer and Van Den Bussche 2003; Mein and Tupinier 1977). Whether we analyzed our data without constraints (Bayesian analyses) or constrained Vespertilionidae to be monophyletic to the exclusion of *Miniopterus* (maximum likelihood analyses), we detected no significant statistical support for inclusion of *Miniopterus* within Vespertilionidae and concur with other studies that Miniopterinae should be accorded familial status (Miniopteridae).

Our data were unable to provide statistically significant support for us to confidently make conclusions regarding the phylogenetic affinities of the remaining 7 families of Yangochiroptera (Emballonuridae, Miniopteridae, Molossidae, Myzopodidae, Natalidae, Nycteridae, and Vespertilionidae). Recent molecular studies using several nuclear and mitochondrial protein–coding genes (Hoofer et al. 2003; Hulva and Horecek 2002; Teeling et al. 2002, 2003; Van Den Bussche et al. 2002, 2003) have provided resolution for the phylogenetic affinities of some of these taxa; however, none of these studies included all families of Yangochiroptera, and all have limited taxonomic sampling within each family. We are currently generating data from these nuclear protein–coding genes for all taxa used in this study and will defer additional conclusions regarding their phylogenetic affinities until more thorough phylogenetic analyses are conducted with these new data.

It is important to note that Craseonycteridae is missing from our study, and this family may be critical to our understanding of the phylogenetic relationships among chiropteran families and hence adaptive radiation in bats. The reason this taxon may be critical and desperately needs to be examined is that 3 of the 5 families within Simmons's (1998) Yinochiroptera are now placed within the suborder Yinpterochiroptera, as they share closer phylogenetic affinities with Pteropodidae, and, as discussed by Teeling et al. (2002), this subordinal arrangement has considerable implication for our understanding of the evolution of echolocation in bats. Strong support also exists for Nycteridae, a 4th member of Yinochiroptera, being placed within Yangochiroptera. The final representative of Simmons' (1998) Yinochiroptera is Craseonycteridae. Simmons (1998) and Simmons and Geisler (1998) place Craseonycteridae as sister to Rhinopomatidae within Rhinopomatoidea, whereas Koopman (1994) placed Craseonycteridae within Emballonuroidea, along with Emballonuridae and Rhinopomatidae. Most recently, based on DNA sequence variation of a portion of the mitochondrial cytochrome-*b* gene and using representatives of Laurasiatheria to root their phylogenetic tree, Hulva and Horecek (2002) concluded that Craseonycteridae was a member of Yinpterochiroptera. Thus, without having tissue samples for our analysis, it is unclear whether Craseonycteridae shares closer phylogenetic affinities with representatives of Yinpterochiroptera or Emballonuridae, a yangochiropteran.

Conclusions

Higher-level relationships within Chiroptera have been debated for centuries, and it is our contention that much of the recent controversy is due to improper choice of out-group. When our data are analyzed under the assumption of Archonta monophyly, a hypothesis not supported by our data, or other recent molecular studies, support is detected for the division of Chiroptera into the suborders Megachiroptera and Microchiroptera. However, if the data are analyzed with more appropriate out-groups (Laurasiatheria), support is detected for the subordinal groupings of Yinpterochiroptera and Yangochiroptera. Based on the totality of molecular evidence, it is no longer valid to assume that Archonta is a monophyletic group based on 2 apparent morphologic synapomorphies (Novacek and Wyss 1986).

It is now the challenge of morphologists to prove that Chiroptera has not shared a most recent common ancestry with members of Laurasiatheria. With regards to the evolutionary implications of Yinpterochiroptera, Simmons (2000:22) states, ''Implications of microchiropteran paraphyly are profound; among other things, such an arrangement implies either convergent evolution of echolocation in 2 lineages (in Rhinolophoidea and again in the lineage leading to the remaining microchiropteran), or loss of echolocation in Pteropodidae (an unlikely possibility, see discussion in Simmons and Geisler 1998). In either case, much of what we surmise about bat evolution would require rethinking if Microchiroptera were paraphyletic.'' Based on the strong support for Yinpterochiroptera and Yangochiroptera and the resurgence in recoupling functional morphology, ecology, and evolution (Adams and Pedersen 2000), if we are to understand the adaptive radiation of bats, it is time to reevaluate the data on chiropteran evolution in light of these new phylogenetic hypotheses.

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APPENDIX I

Specimens examined.—Following is a list of taxa included in this study along with their assigned GenBank accession numbers. Didelphimorphia: *Didelphis virginiana* (NC001610); Tubulidentata: *Orycteropus afer* (NC002078); Sirenia: *Dugong dugon* (NC003314); Pholidota: *Manis tetradactyla* (AJ421454); Primates: *Gorilla gorilla* (X93347), *Homo sapiens* (X93334), *Hylobates lar* (X99256), *Pan paniscus* (D38116), *P. troglodytes* (D38113), *Papio hamadryas* (NC001992), *Pongo pygmaeus* (X97707); Dermoptera: *Cynocephalus variegates* (NC004031); Scandentia: *Tupaia belangeri* (AJ421453), *T. tana* (AF038021, AF203727); Insectivora: *Erinaceus concolor* (AY012099, AY011132), *Scalopus aquaticus* (AF069539), *Solenodon paradoxus* (AF076646), *Sorex palustris* (U97343), *Talpa europaea* (Y19192); Rodentia: *Cavia tschudii* (AY012121, AY011153), *Glaucomys volans* (AF038020), *Hydrochaeris hydrochaeris* (AY012122, AY011154), *Mus musculus* (V00711), *Myoxus glis* (AJ001562), *Rattus norvegicus* (X14848), *Sciurus vulgaris* (NC002369); Lagomorpha: *Ochotona princeps* (AF390540), *Oryctolagus cuniculus* (AJ001588); Pinnipedia: *Halichoerus grypus* (X72004), *Phoca vitulina* (NC001325); Carnivora: *Canis familiaris* (AB048590), *Felis domesticus* (NC001700); Perissodactyla: *Equus asinus* (NC001788), *E. caballus* (NC001640), *Ceratotherium simum* (Y07726), *Rhinoceros unicornis* (NC001779); Cetacea: *Balaenoptera musculus* (X72204), *B. physalus* (X61145), *Physeter catodon* (NC002503); Artiodactyla: *Aepyceros melampus* (M86496), *Antilocapra americana* (M55540), *Boselaphus tragocamelus* (M86494), *Bos taurus* (J01394), *Camelus bactrianus* (Y08808; AJ010814), *Capra hircus* (M55541), *Cervus unicolor* (M35875), *Damaliscus dorcas* (M86499), *Gazella thomsonii* (M86501), *Hippopotamus amphibius* (AJ010957), *Kobus ellipsiprymnus* (M86497), *Lama pacos* (NC002504), *Muntiacus reevesi* (M35877), *Odocoileus virginianus* (M35874), *Oryx gazella* (M86500), *Ovis aries* (NC001941), *Sus scrofa* (AF034253), *Tragelaphus imberbis* (M86493), *Tragulus napu* (M55539); Chiroptera: Emballonuridae: *Balantiopteryx plicata* (AY395847); *Cormura brevirostris* (AY395848), *Diclidurus scutatus* (AY141031), *Emballonura atrata* (AF203773), *Peropteryx kappleri* (AY395849), *P. macrotis* (AY395850), *Rhynchonycteris naso* (AY395851), *Saccopteryx bilineata* (AF263213), *S. leptura* (AY395852), *Taphozous nudiventis* (AY395853); Furipteridae: *Furipterus horrens* (AF345921); Megadermatidae: *Macroderma gigas* (AY395854), *Megaderma lyra* (AF069538), Molossidae: *Eumops auripendulus* (AF263214), *Molossops abrasus* (AY395862), *Molossus molossus* (AF263215), *M. rufus* (AF263216), *M. sinaloae* (U93053, AF203739), *Nyctinomops macrotis* (AF263217), *Promops centralis* (AF263218), *Tadarida brasiliensis* (AF203774); Mormoopidae: *Mormoops blainvilli* (AF407172), *M. megalophylla* (AF263220), *Pteronotus davyi* (AF407176), *P. gymnonotus* (AF407177), *P. macleayii* (AF407178), *P. parnellii* (AF263221), *P. personatus* (AF407182), *P. quadridens* (AF407179); Mystacinidae: *Mystacina tuberculata* (AF263222); Myzopodidae: *Myzopoda aurita* (AF345926); Natalidae: *Natalus micropus* (AF345925), *N. stramineus* (AF345924); Noctilionidae: *Noctilio albiventris* (AF263223), *N. leporinus* (AF263224); Nycteridae: *Nycteris arge* (AY395860), *N. grandis* (AY395861); Phyllostomidae: *Anoura cauidfer* (AY395835), *Artibeus jamaicensis* (AF263226), *Carollia perspicillata* (AY395836), *Centurio senex* (AF263227), *Chiroderma salivini* (AY395807), *Enchisthenes hartii* (TK55331), *Erophylla sezerkorni* (AY395839), *Glossophaga soricina* (AY395840), *Glyphonycteris sylvestris* (AY395841), *Lonchophylla thomasi* (AY395842), *Lonchorhina aurita* (AY395843), *Lophostoma evotis* (AF411529), *Macrophyllum macrophyllum* (AF411540), *Macrotus waterhousii* (AF263229), *Micronycteris schmidtorum* (AF411535), *Musonycteris harrisoni* (AY395844), *Sturnira magna* (AY395845), *Vampyrodes caraccioli* (AY395846), *Vampyrum spectrum* (AF411537), Pteropodidae: *Acerodon celebensis* (U93071, AF293641), *Aproteles bulmerae* (U93066, AF293645), *Cynopterus brachyotis* (U93068, AF203740), *Dobsonia mollucensis* (U93065, AF179290), *Eidolon helvum* (U93058, AF293648), *Eonycteris spelaea* (U93059, AF203743), *Epomophorus wahlbergi* (U93064, AF203744), *Lissonycteris angolensis* (U93063, AF044612), *Macroglossus minimus* (U93062, AF293649), *Megaloglossus woermanni* (U93055, AF203741), *Melonycteris fardoulisi* (U93056, AF293644), *Notopteris macdonaldi* (U93057, AF293642), *Nyctimene albiventer* (U61077, AF293640), *Nyctimene robinsoni* (U93061, AF069536), *Pteralopex atrata* (U93069, AF293643), *Pteropus hypomelanus* (U93073, AF069537), *Rousettus amplexicaudatus* (U93070, AF203742), *Syconycteris australis* (U93060, AF293650), *Thoopterus nigescens* (U93067, AF293646); Rhinolophidae: *Hipposideros abae* (AY395855), *H. cyclops* (AY395857), *H. commersoni* (AY395856), *Rhinolophus alcyone* (AY395858), R. *ferrumquinum* (AY395859); Rhinopomatidae: *Rhinopoma hardwickei* (AF263231); Thyropteridae: *Thyroptera discifera* (AF345923), *T. tricolor* (AF263233); Vespertilionidae: *Antrozous pallidus* (AF326088), *Bauereus dubiaquercus* (AY395863), *Corynorhinus rafinesquii* (AF326091), *C. townsendii* (AF263238), *Eptesicus furinalis* (AF263234), *Harpiocephalus harpia* (AF263235), *Kerivoula hardwicki* (AF345928), *Lasionycteris noctivagans* (AF326095), *Miniopterus australis* (AY395864), *M. schrebersi* (AY395865), *Myotis myotis* (AF326098), *M. riparius* (AF263236), *M. velifer* (AF263237), *Nycticeius humeralis* (AF326102), *Nyctophilus gouldi* (AY395868), *Pipistrellus nathusii* (AF326104), *P. pipistrellus* (AF326105), *Plecotus austriacus* (AF326107), *Rhogeessa tumida* (AF326110), *Scotophilus leucogaster* (AY395867), *Vespertilio murinus* (AY395866).