A Family Matter: Conclusive Resolution of the Taxonomic Position of the Long-Fingered Bats, *Miniopterus*

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The long-fingered bats (*Miniopterus* sp.) are among the most widely distributed mammals in the world. However, despite recent focus on the systematics of these bats, their taxonomic position has not been resolved. Traditionally, they are considered to be sole members of Miniopterinae, 1 of 5 subfamilies within the largest family of bats, the Vespertilionidae. However, this classification has increasingly been called into question. Miniopterines differ extensively from other vespertilionids in numerous aspects of morphology, embryology, immunology, and, most recently, genetics. Recent molecular studies have proposed that the miniopterines are sufficiently distinct from vespertilionids that Miniopterinae should be elevated to full familial status. However, controversy remains regarding the relationship of the putative family, Miniopteridae to existing Vespertilionidae and to the closely related free-tailed bats, the Molossidae. We report here the first conclusive analysis of the taxonomic position of *Miniopterus* relative to all other bat families. We generated one of the largest chiropteran data sets to date, incorporating ~11 kb of sequence data from 16 nuclear genes, from representatives of all bat families and 2 *Miniopterus* species. Our data confirm the distinctiveness of *Miniopterus*, and we support previous recommendations to elevate these bats to full familial status. We estimate that they diverged from all other bat species approximately 49–38 MYA, which is comparable to most other bat families. Furthermore, we find very strong support from all phylogenetic methods for a sister group relationship between Miniopteridae and Vespertilionidae. The Molossidae diverged from these lineages approximately 54–43 MYA and form a sister group to the Miniopteridae–Vespertilionidae clade.

Introduction

The long-fingered bats, genus *Miniopterus* (Bonaparte 1837), are among the most widely distributed mammals in the world (Richarz and Limbrunner 1993; Hutson et al. 2001). Their range extends through the majority of the Afrotropic (sub-Saharan Africa), Palearctic (north Africa and Eurasia), Indomalayan (southern and southeastern Asia) and Australasian (including Australia, New Guinea and neighboring islands) ecozones (Nowak 1994; Simmons 2005). These small (typically <20 g), highly gregarious insectivores are characterized by an elongated third finger, the second phalanx of which is about 3 times longer than the first (Nowak 1994). The lengthened terminal part of the wing folds back on itself when the bat is at rest, such that the wings have a "bent" appearance, hence the alternative common name for this group: the bent-wing bats. In flight, the elongated digit gives the wings a narrow, pointed shape, with high aspect ratio (Findley et al. 1972; Norberg and Rayner 1987) and thus greater aerodynamic efficiency (Norberg and Rayner 1987). This enables fast flight (~16 ms^{-1}) in open areas with relatively low maneuverability (McDonald et al. 1990; Jacobs 1999). Long, narrow wings are also an adaptation for long-distance migration (Findley et al. 1972), and several members of this genus are known to undertake seasonal migrations over hundreds of kilometers (Dwyer 1966; van der Merwe 1975; Palmeirim and Rodrigues 1995; Miller-Butterworth et al. 2003; Jones and Teeling 2006).

Key words: *Miniopterus*, long-fingered bats, Vespertilionidae, Molossidae, Chiroptera, phylogeny, molecular dating.

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Despite its abundance and wide distribution, the taxonomy and phylogeny of *Miniopterus* remains a source of considerable debate. It is the sole genus within the subfamily Miniopterinae, but the number of species recognized worldwide varies widely, with between 11 and 20 designated by various authors over the last 2 decades (Maeda 1982; Skinner and Smithers 1990; Koopman 1994; Nowak 1994; Simmons 2005). Most members of the genus resemble one another closely in both size and pelage coloration, and despite considerable overlap, many species have been classified solely on the basis of differences in size or coloration (Hayman and Hill 1971; Skinner and Smithers 1990).

The subfamily designation of this group (fig. 1*a*) within the family Vespertilionidae or plain-faced bats has a long history (Dobson 1875; Miller 1907) and is based primarily on morphology (Simmons and Geisler 1998; Gunnell and Simmons 2005; Simmons 2005). However, this classification has increasingly been called into question on the basis of both molecular and morphological data, which support full familial status for this subfamily (Agrawal and Sinha 1973; Mein and Tupinier 1977; Gopalakrishna and Chari 1983; Pierson 1986; Tiunov 1989; Krutzsch and Crichton 1990; Reep and Bhatnagar 2000; Bhatnagar et al. 2001; Kawai et al. 2002; Hoofer and Van Den Bussche 2003; Hutcheon and Kirsch 2004; Van Den Bussche and Hoofer 2004; Eick et al. 2005).

In addition to the uniquely elongated second phalanx of the third digit as discussed above, a number of immunological, anatomical, and embryological apomorphies distinguish miniopterines from vespertilionids (Agrawal and Sinha 1973; Mein and Tupinier 1977; Gopalakrishna and Chari 1983; Pierson 1986; Tiunov 1989; Krutzsch and Crichton 1990; Reep and Bhatnagar 2000; Bhatnagar et al. 2001). Their distinctiveness is also supported by

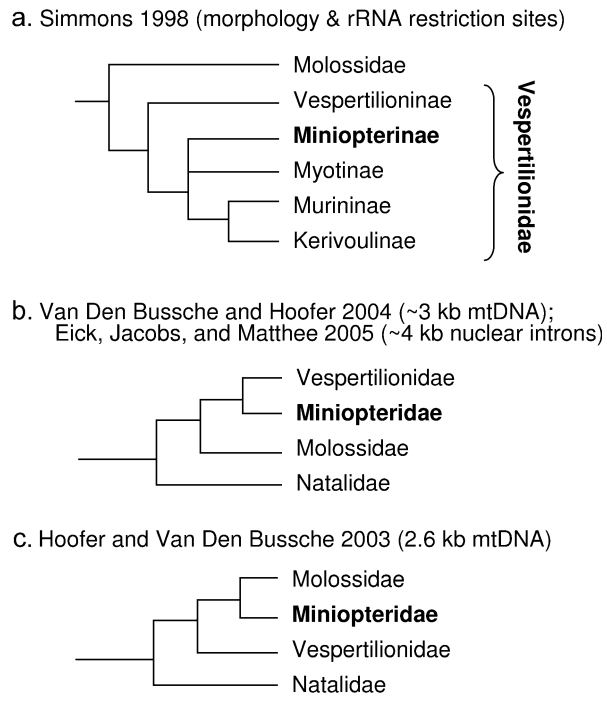

FIG 1.—Bats of the genus *Miniopterus* have traditionally been classified as members of Miniopterinae (*a*) 1 of the 5 subfamilies within the family Vespertilionidae (Simmons 1998). However, recent molecular studies suggest miniopterines are sufficiently distinct to be considered members of their own family, Miniopteridae, although it remains uncertain whether this family is more closely related (*b*) to the Vespertilionidae (Van Den Bussche and Hoofer 2004; Eick et al. 2005) or (*c*) to the free-tailed bats, family Molossidae (Hoofer and Van Den Bussche 2003).

a handful of molecular phylogenetic analyses. These molecular studies suggest that *Miniopterus* is sufficiently distinct from Vespertilionidae to warrant elevating Miniopterinae to familial status as Miniopteridae (Kawai et al. 2002; Hoofer and Van Den Bussche 2003; Hutcheon and Kirsch 2004; Van Den Bussche and Hoofer 2004; Eick et al. 2005). However, beyond this agreement, the molecular phylogenetic results have been contradictory and weakly supported. To date, none of the studies has been able to determine conclusively whether the putative Miniopteridae form a sister group to the vespertilionids (fig. 1*b*) or whether they are in fact more closely related to the free-tailed bats, the Molossidae (fig. 1*c*). In each study, all possible branching orders were obtained or there was only weak to moderate support for any single topology. None of these studies could convincingly reject one topology over another, and thus, none has been able to resolve the *Miniopterus*—Vespertilionidae—Molossidae trichotomy with any certainty.

Miniopterus represents a taxon of exceptional scientific interest, not merely because of its anatomical and genetic divergence from vespertilionids. Bats of this genus employ unusual reproductive strategies, such as delayed implantation (Bernard 1980; Bernard et al. 1996), as well as remarkable dispersal and migratory behaviors (Miller-Butterworth et al. 2003). They are distributed throughout most of the Old World, suggesting an extraordinary ability to exploit a wide range of global environmental conditions. However, *Miniopterus natalensis*, one of only a handful of mammalian species known to exhibit strong philopatry in both sexes, also displays strikingly high levels of genetic differentiation for such a highly migratory species, suggesting these bats can also become highly adapted and thus restricted to local ecological biomes (Miller-Butterworth et al. 2003). Furthermore, *Miniopterus schreibersii* has the smallest genome reported for any mammal. Mammalian *C* values average 3.5 pg or 3423 Mb (Gregory et al. 2007), whereas the *M. schreibersii* genome (C-value = 1.73 pg or 1692 Mb) is approximately half that size (Capanna and Manfredi Romanini 1971; Gregory 2006; Gregory et al. 2007). This makes this species of considerable interest for future evolutionary, genome mapping, and comparative genomics studies. However, such studies will enjoy little success if the phylogeny of the genus remains uncertain.

To resolve the taxonomic position of *Miniopterus*, we generated and examined one of the largest chiropteran data sets to date, incorporating ~11 kb of sequence data from portions of 16 nuclear genes. We included representatives of all bat families, totaling 33 genera, including 2 *Miniopterus* species, 4 other vespertilionids, and 3 molossids. We used Bayesian dating analyses, incorporating key fossil constraints to date the basal divergence between *Miniopterus* and other taxa. Our study confirms the distinctiveness of *Miniopterus*, and we support previous recommendations to elevate this taxon to familial status. Our analysis also conclusively resolves, with very strong support from all phylogenetic methods, that *Miniopterus* constitutes a sister taxon to Vespertilionidae and that Molossidae is a sister taxon to this clade.

Materials and Methods

Genes and Taxa

We expanded the data set of Teeling et al. (2005) where possible by including 2 species of *Miniopterus* and 1 additional basal vespertilionid. Our data set included 4 pteropodids (*Cynopterus*, *Nyctimene*, *Pteropus*, and *Rousettus*), 2 megadermatids (*Megaderma* and *Macroderma*), 2 rhinolophids (*Rhinolophus* and *Hipposideros*), 1 rhinopomatid (*Rhinopoma*), 1 craseonycterid (*Craseonycteris*), 1 nycterid (*Nycteris*), 3 emballonurids (*Emballonura*, *Taphozous*, and *Rhynchonycteris*), 1 natalid (*Natalus*), 2 molossids (*Tadarida* and *Eumops*), 6 vespertilionids from 3 of the 5 traditionally recognized subfamilies (*Myotis* [subfamily Myotinae], *Rhogeessa*, *Antrozous* [Although *Antrozous* has been recognized as an independent family (Simmons and Geisler 1998), evidence from large molecular data sets indicates that it is indeed a member of the Vespertilionidae (Teeling et al. 2002)], and *Eptesicus* [subfamily Vespertilioninae], *Miniopterus natalensis* and *Miniopterus fraterculus* [subfamily Miniopterinae]), 1 myzopodid (*Myzopoda*), 1 mystacinid (*Mystacina*), 1 furipterid (*Furipterus*), 1 thyropterid (*Thyroptera*), 1 noctilionid (*Noctilio*), 1 mormoopid (*Pteronotus*), and 4 phyllostomids (*Desmodus*, *Anoura*, *Tonatia*, and *Artibeus*), sensu Simmons (2005), Teeling et al. (2002), Miller-Butterworth et al. (2005). We included representatives of 4 laurasiatherian orders as outgroup taxa (supplementary table S1, Supplementary Material online),

namely Carnivora (*Felis catus* and *Panthera onca*), Cetartiodactyla (*Tragelaphus eurycerus* and *Bos taurus*), Eulipotyphla (*Condylura cristata*, *Talpa europaea* and *Scalopus aquaticus*) and Perissodactyla (*Ceratotherium simum* and *Equus caballus*).

Amplification conditions have been described previously (Teeling et al. 2005). The sequences were aligned using ClustalX, incorporating default settings (Thompson et al. 1997), and modified in Se-Al (Rambaut 1996). Regions of alignment ambiguities due to repeats were removed from the noncoding 3' untranslated regions of the genes *APP*, *BMI1*, *CREM*, and *PLCB4*. It was not possible to amplify *ADRA2B* and *VWF* in either *Miniopterus* species; therefore, these gene segments were not included in our analyses. This final data set totaled approximately 11 kb of nuclear sequence data for 16 nuclear genes and includes representatives of all bat families. Additional sequences have been deposited in GenBank (accession numbers: EF397701–EF397742; supplementary table S1, Supplementary Material online).

Phylogenetic Analyses

An evaluation of data set incongruence with a bootstrap support (BSS)/conflict criterion of 90% (De Queiroz 1993) revealed no conflicting nodes. We therefore performed phylogenetic analyses on the concatenated data set. Maximum likelihood (ML) and maximum parsimony (MP) analyses were performed with PAUP 4.0b10 (Swofford 2003). ML analyses were performed using the GTR (general time reversible) + Γ (gamma distribution of rates) + I (proportion of invariant sites) sequence evolution with the following parameters settings estimated by Modeltest (Posada and Crandall 1998): R-matrix = (1.2959 4.5548 0.5513 1.4600 5.4839); base frequencies = (0.2714 0.2463 0.2412); proportion of invariant sites = 0.3308; and shape parameter of gamma distribution = 0.7790. In all ML analyses, starting trees were obtained via Neighbor-Joining (NJ). A single ML heuristic search was completed using tree bisection and reconnection branch swapping (TBR). ML bootstrap analyses were performed using nearest-neighbor interchange branch swapping. In MP analyses, we used stepwise addition with 10 randomized input orders. Nucleotide positions were unweighted and gaps were coded as missing data. Bootstrap analyses included 100 replicates for ML and 500 replicates for MP. We used TBR-based heuristic searches in all analyses except in ML bootstrap replicates.

Bayesian analyses were completed with MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2005). MrBayes 3.1.1 concurrently executes 2 Metropolis coupled Markov Chain Monte Carlo (MCMC) runs. Convergence was indicated when the standard deviation (SD) of split frequencies was less than 0.01 (Ronquist et al. 2005). Four simultaneous chains were run, 3 hot and 1 cold. Analyses were run for as many generations as was required for the average SD of split frequencies to be less than 0.01. Chains were sampled every 1,000 generations. Starting trees were random, and the prior indicated that all trees were equally probable.

Statistical Analyses

Eick et al. (2005) reported a sister group relationship between Vespertilionidae and Miniopteridae, whereas Hoofer and Van Den Bussche (2003) reported a sister group relationship between Molossidae and Miniopteridae. The Kishino and Hasegawa (KH) test (1989) and the Shimodaira –Hasegawa (SH) test (1999) with resampling of estimated log-likelihoods optimization and 1,000 bootstrap replicates were used to compare the statistical significance of the independent molecular a priori hypotheses regarding the phylogenetic position of the putative Miniopteridae in relation to Vespertilionidae and Molossidae.

Dating Analyses

Branch Length Estimation

Branch lengths were estimated with ESTBRANCHES for the concatenated data set and for each data partition discussed above (Thorne et al. 1998). The mole was chosen as the outgroup (supplementary table S1, Supplementary Material online). The ML topology (fig. 2) was incorporated in the analyses. We used Felsenstein's (1984) model of sequence evolution with an allowance for a gamma distribution of rates with 4 discrete rate categories. The estimates of the rate categories for the gamma distribution, base frequencies, and the transition/transversion parameter were calculated in PAUP 4.0b (Swofford 2003) for the entire data set.

Divergence Time Estimations

DIVTIME5B was used to estimate the divergence times (Thorne et al. 1998; Kishino et al. 2001). The DIVTIME5B program utilized the estimated branch length for the entire data set and incorporated MCMC analyses, which were run for 1 million generations and sampled every 100 generations. We incorporated an estimate for the mean ingroup prior: 65 MYA, following a strict interpretation of the Explosive model of placental diversification, placing the root at or near the K–T boundary (Archibald and Deutschman 2001). Six fossil constraints (supplementary material, Supplementary Material online) were incorporated in the analyses, as described in Teeling et al. (2005):

1. A maximum of 34 MYA for the base of the family Phyllostomidae.
2. A minimum of 30 MYA for the Mormoopidae/Phyllostomidae split.
3. A minimum of 37 MYA for the split between Vespertilionidae/Molossidae.
4. A minimum of 37 MYA for the base of Emballonuridae.
5. A minimum of 37 MYA for the base of Rhinolophidae.
6. A maximum of 55 MYA for the base of Rhinolophoidea.

Results

Phylogenetic Analyses

The molecular tree is congruent with that of Teeling et al. (2005) and is strongly supported (fig. 2, table 1). The association of the Pteropodidae and the

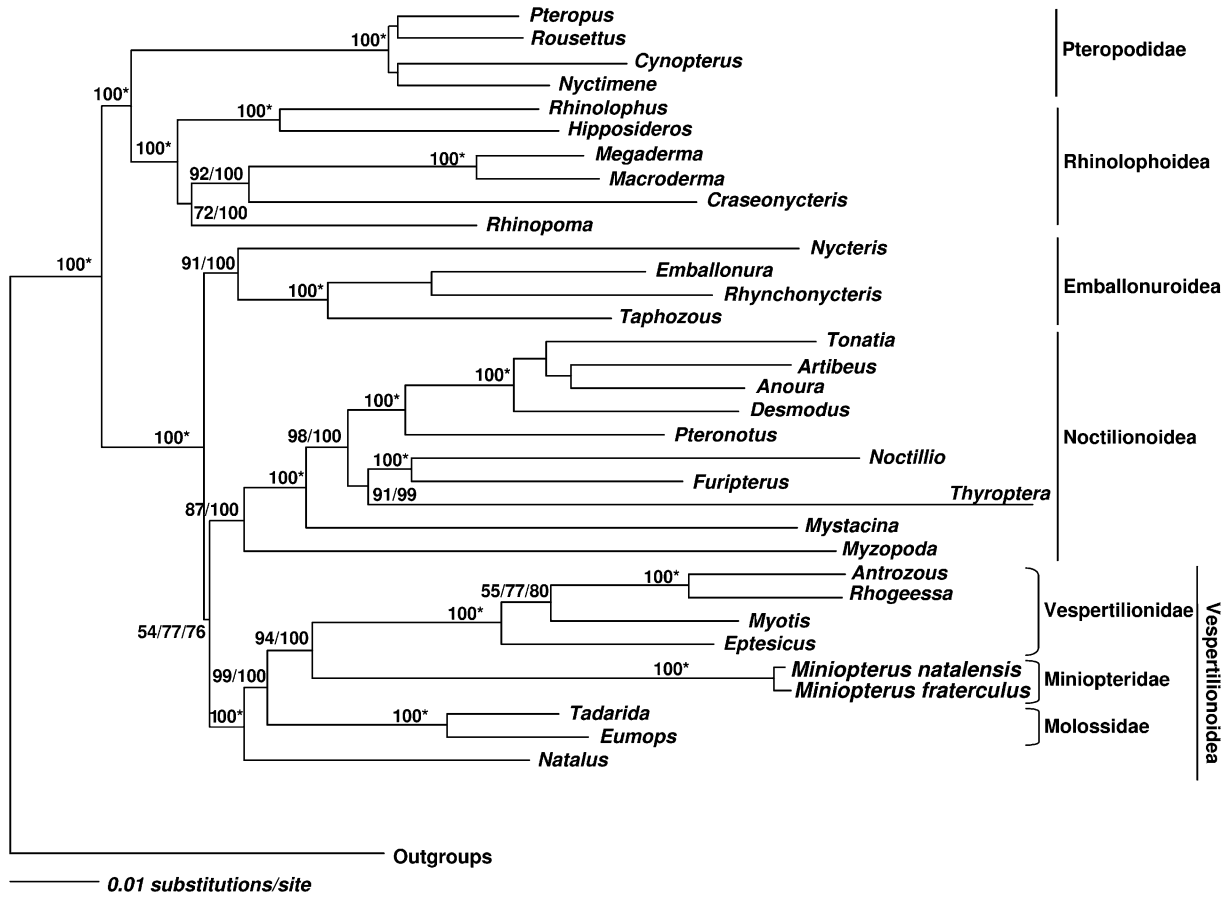

FIG 2.—The ML tree (−ln likelihood = 77679.61) for the concatenated data set under the GTR + Γ + I model of sequence evolution. Numbers at the nodes are the ML bootstrap values/BPP from both analyses, shown as percentages. In cases where posterior probabilities generated by the 2 Bayesian analyses differed, both values are presented, in addition to the ML bootstrap value. The 100* indicates clades that received 100% BSS in all analyses and had posterior probabilities of 1.000.

Rhinolophoidea within the suborder Yinpterochiroptera received 93–100% BSS and had a Bayesian posterior probability (BPP) of 1.00. All other chiropteran families grouped together with 100% BSS and BPP 1.00 within the suborder Yangochiroptera. The monophyly of the 4 superfamilial groups including Rhinolophoidea, Emballonuroidea, Noctilionoidea and Vespertilionoidea (sensu Teeling et al. 2005) were highly supported by ML and Bayesian analyses (87–100% BSS and BPP 1.00; table 1 and fig. 2). Our data support a basal position for *Natalus* within the superfamily Vespertilionoidea (100% BSS and BPP 1.00; table 1 and fig. 2), and place *Antrozous* within the family Vespertilionidae (100% BSS and BPP 1.00; fig. 2).

Miniopteridae grouped within the superfamily Vespertilionoidea with 100% ML BSS and BPP 1.00 (fig. 2). All phylogenetic methods strongly supported the monophyly of the Vespertilionidae (BSS 100%; BPP 1.00), with the exclusion of *M. natalensis* and *M. fraterculus* (table 1, fig. 2). Both species of *Miniopterus* formed a monophyletic clade (Miniopteridae), which received BSS 100%, BPP 1.00 from all methods. Miniopteridae grouped as the sister taxon to Vespertilionidae and was strongly supported by all phylogenetic methods (ML BSS 94–100%; BPP 1.00). The Molossidae formed a monophyletic clade (100% BSS; BPP 1.00) and were sister taxa to the Vespertilionidae—Miniopteridae association (91–100% BSS; BPP 1.00).

Statistical Testing

The SH and KH tests rejected a sister group relationship between Miniopteridae and Molossidae (KH: $P < 0.0001$ and SH: $P = 0.017$) over a sister group relationship between Vespertilionidae and Miniopteridae (table 2).

Molecular Dating

Crown group bats began to originate and diverge approximately 64 MYA (95% credibility interval (CI): 70–58 MYA). All superfamilies of bats diverged approximately 52 MYA (fig. 3). Vespertilionidae and Miniopteridae last shared a common ancestor approximately 43 MYA (CI: 49–38 MYA). This is in accordance with all other recognized bat families, which originated, on average ~43 MYA (range 24–52 MYA). The Molossidae diverged from the last common ancestor of Vespertilionidae and Miniopteridae about 48 MYA (CI: 54–43 MYA, fig. 3). *Miniopterus natalensis* and *M. fraterculus* diverged less than 2 MYA (CI: 1.9–0.6 MYA; fig. 3).

Table 1
Bootstrap Values for the Various Clades in Figure 2

	ML	MP	Bayes 1	Bayes 2
Pteropodidae	100	100	100	100
Rhinolophidae	100	100	100	100
Megadermatidae	100	100	100	100
Megadermatidae + Craseonycteridae	92	100	100	100
Megadermatidae + Craseonycteridae + Rhinopomatidae	72	74	100	100
Rhinolophoidea	100	100	100	100
Yinpterochiroptera	100	98	100	100
Yangochiroptera	100	100	100	100
Emballonuridae	100	100	100	100
Emballonuroidea	91	60	100	100
Phyllostomidae	100	100	100	100
Phyllostomidae + Mormoopidae	100	100	100	100
Noctilionidae + Furipteridae	100	53	100	100
Noctilionidae + Furipteridae + Thyropteridae	91	44	99	99
Monophyly of South American families	98	49	100	100
Basal position for Myzopodidae in Noctilionoidea	100	58	100	100
Noctilionoidea	87	58	100	100
Vespertilionidae (minus Miniopteridae)	100	100	100	100
Vespertilionidae + Miniopteridae	94	94	100	100
Miniopteridae	100	100	100	100
Molossidae	100	100	100	100
Molossidae + Miniopteridae + Vespertilionidae	99	92	100	100
Vespertilionoidea	100	95	100	100

NOTE.—Bayesian posterior probabilities are shown as percentages. ML = ML with nearest-neighbor branch swapping; Bayes 1 & 2 = Bayesian analyses.

Discussion

Our expanded 11-kb data set was able to resolve the *Miniopterus*–Vespertilionidae–Molossidae trichotomy conclusively for the first time. We found strong support for the family Miniopteridae and for its sister relationship to Vespertilionidae rather than Molossidae (fig. 2). Previous molecular studies of *Miniopterus* have produced inconsistent and contradictory results. Although some of these phylogenetics studies have alluded to the distinctiveness of *Miniopterus* relative to vespertilionids and molossids, they have been unable to resolve the topology of this trichotomy conclusively.

Volleth and Heller (1994) examined chromosome-banding patterns of Old World representatives of over 20 genera of the family Vespertilionidae. They found support for vespertilionid monophyly, with the subfamily Miniopterinae occupying the most basal branch in their family tree containing subfamilies Vespertilionidae, Myotinae, Murininae, and Kerivoulinae. In contrast, DNA–DNA hybridization suggests that *Miniopterus* is distinct from Vespertilionidae, but weakly supports a sister relationship either between miniopterines and molossids or between *Miniopterus* and Vespertilionidae, depending on how many taxa are included in the analysis (Hutcheon and Kirsch 2004).

Kawai et al. (2002) identified a short interspersed element insertion unique to *Miniopterus* and concluded that the Vespertilionidae are monophyletic to the exclusion of Miniopterinae. This was moderately supported (70%) by a composite ML phylogeny based on the mitochondrial gene *NDI* and the nuclear gene *vWF*. However, they were unable to resolve conclusively whether *Miniopterus* formed the sister group to a clade containing Molossidae and Emballonuridae or instead was located basal to a clade containing the remaining Vespertilionidae species.

Larger mitochondrial DNA sequence data sets similarly support the exclusion of the subfamily Miniopterinae from Vespertilionidae and indicate that *Miniopterus* represents a sufficiently divergent lineage from vespertilionids that it should be recognized in its own family, Miniopteridae (Hoofer and Van Den Bussche 2003; Van Den Bussche and Hoofer 2004). However, despite the inclusion of 2.6 kb of sequence data from representatives of most vespertilionid genera and all subfamilies, Hoofer and Van Den Bussche (2003) were unable to resolve the *Miniopterus*–Vespertilionidae–Molossidae trichotomy, with all possible branching orders being obtained in their various Bayesian analyses, none with substantial support. Parsimony analysis suggested a sister relationship between *Miniopterus* and the molossids (fig. 1*c*), but with only moderate support (66%). In contrast, their subsequent study (Van Den Bussche and Hoofer 2004) placed the putative Miniopteridae as a sister family to Vespertilionidae rather than Molossidae (fig. 1*b*), but once again, with only moderate Bayesian and weak to moderate likelihood support. This latter topology is consistent with that reported by Eick et al. (2005), based on a nuclear intron data set of ~4 kb. They identified an indel event which was unique to *Miniopterus* and found that *Miniopterus* was the sister group to Vespertilionidae, whereas Molossidae formed a sister family to the vespers and miniopterids. However, as with all the molecular studies conducted to date, these taxonomic groupings received poor statistical support, and these authors could not reject the alternative hypothesis that miniopterids are not the sister taxon to vespers but instead are more closely related to molossids.

Our results are consistent with those of Van Den Bussche and Hoofer (2004) and of Eick et al. (2005) but

Table 2
Statistical Comparison of Phylogenetic Hypotheses by Means of Topology Tests

Comparison of Phylogenetic Hypotheses	Log Likelihood Score (a) Eick	Log Likelihood Score (b) Hoofer	Δ in −ln likelihood	*P* Values for KH Tests	*P* Values for SH Tests
(a) Miniopteridae + Vespertilionidae (Eick et al. 2005) versus (b) Miniopteridae + Molossidae (Hoofer and Van Den Bussche 2003)	77679.61	77697.14	17.52	<0.0001*	=0.017*

* Indicates hypotheses that were rejected at $P < 0.01$ in pairwise comparisons of −ln likelihood values.

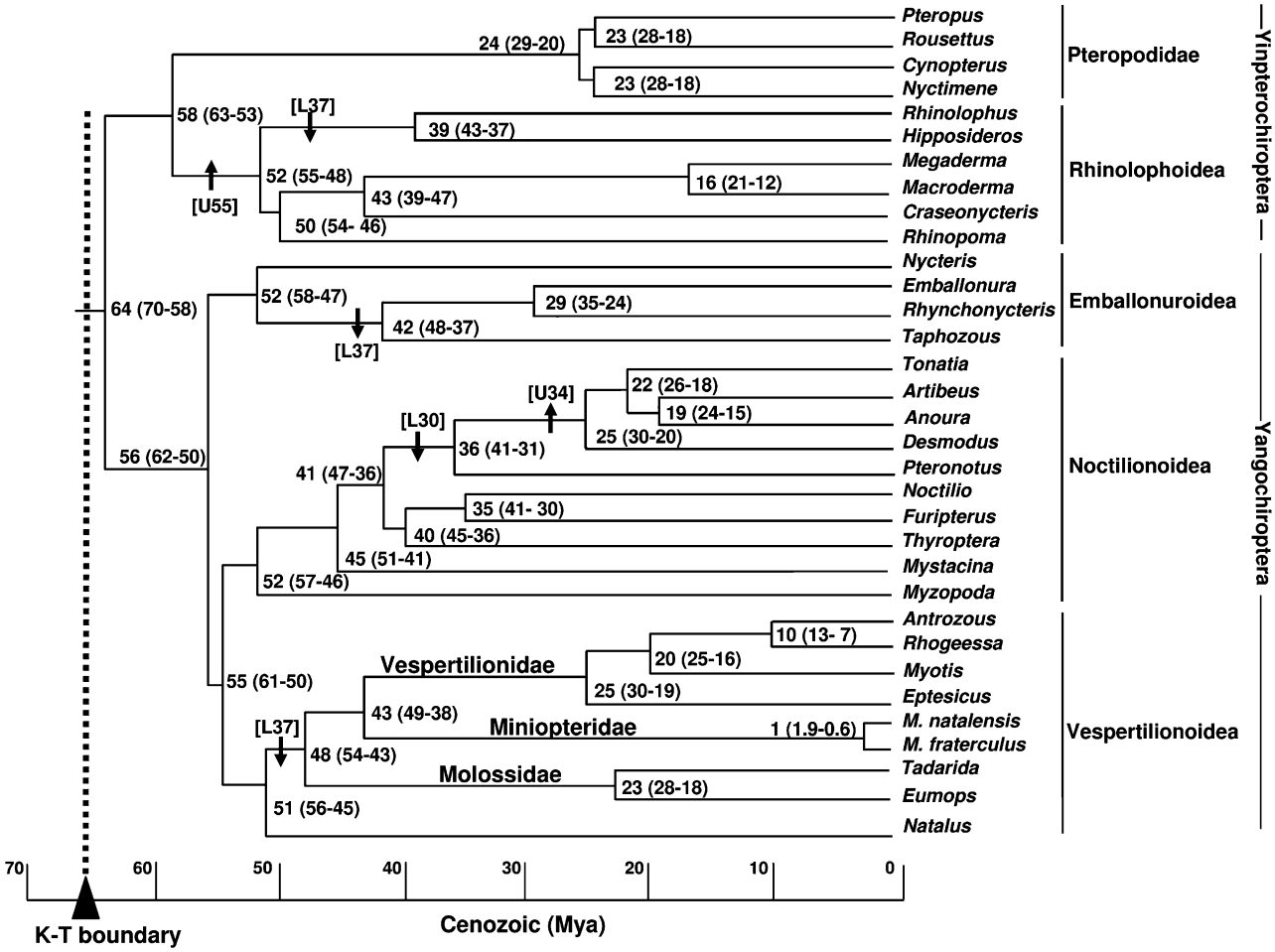

FIG 3.—Molecular timescale for the order Chiroptera based on the DIVTIME5B analyses, using the ML topology depicted in figure 2, 6 fossil constraints and a mean prior of 65 MYA for the base of the in-group root. Numbers at the nodes are the molecular dates in millions of years, values in parentheses are the 95% credibility intervals. Numbers in the square brackets along the branches (indicated by arrows) refer to the fossil constraint age (MYA) imposed on that particular node. U refers to an upper bound and L refers to a lower bound constraint.

provide much stronger support for this topology. We were able confidently to reject the alternative hypothesis that Miniopteridae is a sister taxon to Molossidae ($P < 0.01$, table 2). Our data suggest that the molossid divergence from vesper bats and miniopterids (~ 48 MYA) predates that between the family Mystacinidae, which is endemic to New Zealand, and the clade containing the Neotropical noctilionoid families (Teeling et al. 2005). These data are consistent with the divergence times proposed by Eick et al. (2005) for Miniopteridae and Vespertilionidae (~45 MYA), and between these sister taxa and Molossidae (~48 MYA). Our data further indicate that the split between Miniopteridae and Vespertilionidae is at least as ancient as that between Megadermatidae (*Macroderma*) and Craseonycteridae (*Craseonycteris*) or between Noctilionidae (*Noctilio*) and Phyllostomidae (*Desmodus*), all recognized as distinct families (this study; Simmons and Geisler 1998; Teeling et al. 2005). This provides further support for the proposal to elevate the subfamily Miniopterinae to full familial status.

In contrast to Eick et al. (2005), however, we found strong ML and Bayesian support for placing *Myzopoda* basal to the clade containing families Phyllostomidae, Mormoopidae, Noctilionidae, Furipteridae, Thyropteridae, and Mystacinidae (table 1), whereas they found the myzopodids to be basal to the vespertilionoids, although with weak support. Furthermore, in contrast to Simmons (2005) and Simmons and Geisler (1998), our phylogeny places *Antrozous* within the family Vespertilionidae. This is consistent with traditional classification schemes (e.g., Koopman 1994) and with recent molecular studies (Teeling et al. 2002, 2005; Hoofer and Van Den Bussche 2003).

A limitation of the present study is that our sampling of the Vespertilionidae does not include members of 2 of its subfamilies, namely Murininae and Kerivoulinae, whose classification within Vespertilionidae has not been tested with large molecular data sets (e.g., Teeling et al. 2002, 2005). However, recent morphological studies and smaller molecular data sets have placed both these subfamilies within Vespertilionidae with strong support (Simmons and Geisler 1998; Kawai et al. 2002; Simmons 2005). In particular, Hoofer and Van Den Bussche (2003), using 2.6 kb of mitochondrial sequence data, reported a well-supported basal split within Vespertilionidae between Vespertilionidae and a Myotinae–Kerivoulinae–Murininae clade. We have sampled this deep divergence within Vespertilionidae taxonomically and, therefore, feel confident of

our results. However, future analyses should include representatives of Murininae and Kerivoulinae to verify the mtDNA results and to corroborate our findings.

Our molecular results suggest that there is a long stretch of fossil history missing between stem and extant miniopterids. Approximately 42 Myr of evolutionary history separate stem from crown group miniopterids. The oldest fossil *Miniopterus* is Early Miocene in age (McKenna and Bell 1997; Jones et al. 2005), which indicates that there are approximately 18 Myr of missing fossil data. Furthermore, this fossil *Miniopterus* has not been identified to the species level.

The familial status of *Miniopterus* is also supported by many unique, nonmolecular features, which distinguish these bats from Vespertilionidae (see Hoofer and Van Den Bussche 2003 for review). *Miniopterus* differs from vespertilionids in its dental formula, having an additional vestigial tooth between the upper canine and the first premolar (Mein and Tupinier 1977). In further contrast to vesper bats, *Miniopterus* lacks a digital tendon locking mechanism in its feet (Simmons 1998), has prominent rostral and sylvian sulci in the brain (Reep and Bhatnagar 2000), and possesses a well-developed vomeronasal organ, which is absent or rudimentary in all vespertilionids examined to date (Cooper and Bhatnagar 1976; Reep and Bhatnagar 2000; Bhatnagar et al. 2001).

Additional anatomical differences in male *Miniopterus* include the absence of a baculum, the supporting bone in the penis (Agrawal and Sinha 1973), the presence of urethral glands, which are absent in vespertilionids (Tiunov 1989), the greater length and anterior rather than posterior location of the ducts of Cowper's glands (Tiunov 1989), and the morphology of their sperm, which have a very large acrosome and are much longer (9 mm) than those of other vespertilionids (4–5.5 μm) studied (Breed and Inns 1985). These morphological differences are supported by immunological studies. Transferrin immunological distances between *Miniopterus* and molossids are lower than between miniopterines and vespertilionids, whereas albumin immunodiffusion experiments suggest *Miniopterus* is equidistant from both Molossidae and Vespertilionidae (Pierson 1986).

Miniopterus also differs from vespertilionids in that hibernating bats of this genus synchronize male and female gamete production (Bernard et al. 1996; Racey and Entwistle 2000). Sperm production and insemination coincide with ovulation and conception, which is followed by a period of delayed implantation of the blastocyst until after the hibernation period (van der Merwe 1986; Krutzsch and Crichton 1990). Male *Miniopterus* therefore differ from vesper bats in that their accessory sex gland activity declines after this synchronous fall breeding season (Krutzsch and Crichton 1990). Delayed implantation has been recorded in *M. schreibersii* (Dwyer 1963; Richardson 1977; Krutzsch and Crichton 1990), *M. natalensis* (van der Merwe 1986; Bernard et al. 1996), *M. fraterculus* (Bernard 1980), *Miniopterus minor* (McWilliam 1988), and *Miniopterus australis* (Richardson 1977) but not in any nonminiopterid, hibernating species (Richardson 1977; Strahan 1998). All other hibernating vespertilionids have dysynchronous male and female gametic cycles, during which sperm production and insemination generally occur in late summer, females store sperm during the winter hibernation period, and ovulation and conception occur the following spring (Krutzsch and Crichton 1990; Racey and Entwistle 2000). In addition to delayed implantation of the blastocyst, miniopterid embryology differs from that of vesper bats in a number of ways. These include, inter alia, the site of blastocyst attachment, the structure of the chorioallantoic placenta, and the development of the roof of the amniotic cavity without cavitation (Gopalakrishna and Chari 1983).

In conclusion, this study supports previous recommendations (Hoofer and Van Den Bussche 2003; Van Den Bussche and Hoofer 2004; Eick et al. 2005) to recognize bats of the genus *Miniopterus* as members of their own family, Miniopteridae. Furthermore, these data conclusively resolve the controversy over the classification of Miniopteridae in relation to other closely related bat families. We provide incontrovertible support for this family to be considered a sister taxon to the Vespertilionidae and find that these families diverged between 38 and 49 MYA. The third member of the controversial trichotomy, the Molossidae, itself forms a sister group to the Miniopteridae–Vespertilionidae association, having diverged from these lineages 43–54 MYA. This information is essential to inform future comparative genomic studies, which will investigate the genomic structure and organization of this unusual and ancient bat family.

Supplementary Material

Supplementary materials and Supplementary table S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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