

## Science Supporting Online Material

# A Molecular Phylogeny for Bats Illuminates Biogeography and the Fossil Record

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## Materials and Methods

### *Genes and taxon sampling*

We expanded the Teeling *et al.* (*S1*) data set by generating 6.1 kb of new nuclear sequence data from 13 gene segments for 30 bat genera, using previous methods (*S2, S3*). Two new primer pairs spanning two segments of exon 18 of the *TITIN* gene are described below. Additional sequences are from Murphy *et al.* (*S4*), necessitating some chimeric OTUs (table S6). Our data set included four pteropodids (*Cynopterus*, *Nyctimene*, *Pteropus*, *Rousettus*) two megadermatids (*Megaderma*, *Macroderma*), two rhinolophids (*Rhinolophus*, *Hipposideros*), one rhinopomatid (*Rhinopoma*), one craseonycterid (*Craseonycteris*), one nycterid (*Nycteris*), three emballonurids (*Emballonura*, *Taphozous*, *Rhynchonycteris*), one natalid (*Natalus*), two molossids (*Tadarida*, *Eumops*), two vespertilionids (*Rhogeessa*, *Myotis*), one antrozoid (*Antrozous*), one myzopodid (*Myzopoda*), one mystacinid (*Mystacina*), one furipterid (*Furipterus*), one thyropterid (*Thyroptera*), one noctilionid (*Noctilio*), one mormoopid (*Pteronotus*), four phyllostomids (*Desmodus*, *Anoura*, *Tonatia*, *Artibeus*) *sensu* Simmons and Geisler (*S5*). We included four laurasiatherians as outgroup taxa. The sequences were aligned using Clustal-X, incorporating default settings (*S6*), and modified in Se-Al (*S7*). Regions of alignment ambiguities due to repeats were removed from the noncoding 3' UTRs *APP* (313 bp), *BMI1* (65 bp), *CREM* (133 bp), and *PLCB4* (156 bp). The repetitive regions were also removed from *ADRA2B* (87 bp) prior to analyses. This final data set totaled ~13 kb of nuclear sequence data for 18 nuclear genes and includes representative of all bat families. Alignments files that detail regions of ambiguity are posted online at <http://home.ncifcrf.gov/CCR/lgd/>. GenBank accession numbers for all sequences included in this study are in table S6.

### *TITIN primer sequence*

**TTNF1** CACCTCTTGTGACAATG; **TTNR2** CCTTTGGAGGATCAGGTTATC;  
**TNNF3** GGATGATGTCACCAGAACAGTG; **TTNR3** GCCTGGTTCTTGAGGGATATT;  
**TTNF6** TGTGATCCTGTGTTCAAACCT; **TTNR6** GCATTACAGACTTGGATTCAAGC;  
**TTNF7** TTCCCCACCAGGAAAGGT; **TTNR7** TGGTCCAGGTTCTTAAATGGAT.

### *Phylogenetic analyses*

An evaluation of data set incongruence with a bootstrap support/conflict criterion of 90% (*S8*) revealed no conflicting nodes, thus we performed phylogenetic analyses on the concatenated data set. Maximum likelihood (ML), minimum evolution (ME) and maximum parsimony (MP) analyses were performed with PAUP 4.0b10 (*S9*). ML analyses were performed using the GTR (general time reversible) +  $\Gamma$  (gamma

distribution of rates) + I (proportion of invariant sites) sequence evolution with the following parameters settings estimated by Modeltest (S10): R-Matrix = (1.2302 4.6545 0.6304 1.4023 5.5530); base frequencies = (0.2585 0.2592 0.2503 0.2320); proportion of invariant sites = 0.3240; and shape parameter of gamma distribution = 0.7628. In all ML analyses, starting trees were obtained via neighbor-joining (NJ). Owing to computational demands, we used the following taxonomic constraint in ML bootstrap analyses with tree-bisection and reconnection (TBR) branch swapping: (*Pteropus*, *Cynopterus*, *Rousettus*, *Nyctimene*), (*Rhinolophus*, *Hipposideros*), (*Megaderma*, *Macroderma*), *Nycteris*, (*Emballonura*, *Taphozous*, *Rhynchonycteris*), (*Tonatia*, *Artibeus*, *Desmodus*, *Anoura*), (*Tadarida*, *Eumops*), (*Antrozous*, *Rhogeessa*, *Myotis*), *Rhinopoma*, *Noctilio*, *Myzopoda*, *Pteronotus*, *Thyroptera*, *Mystacina*, *Furipterus*, *Natalus*, *Craseonycteris*). All clades constrained received 100% bootstrap support in most other analyses and are highly supported by independent data sets (S1, S4, S11, S12). ML bootstrap analyses were also performed without this molecular constraint using nearest-neighbor interchange branch swapping. ME analyses were performed with ML distances implementing a GTR +  $\Gamma$  + I model of evolution. Starting trees were obtained using NJ. In MP analyses, we used stepwise addition with 1000 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 ME and MP. We used TBR based heuristic searches in all analyses except unconstrained ML bootstrap replicates.

Bayesian analyses were completed with MrBayes 3.0 (S13). Two kinds of analyses were performed: The first incorporated a single model of sequence evolution for the entire data set (GTR +  $\Gamma$  + I); the second incorporated an independent model of sequence evolution for each data partition. The data set was divided into 17 partitions corresponding to either gene type or genomic linkage: *ADRA2B*; *ADORA3*; *ADRB2*; *APP*; *ATP7A*; *BDNF*; *BMI1*; *BRCA1*; *CREM*; *EDG1*; *PLCB4*; *PNOC*; *RAG1+RAG2*; *TTN*; *TYR*; *VWF*; *ZFX*. We used the Akaike Information Criterion incorporated in Modeltest (S10) to assess the optimal model for each partition. GTR +  $\Gamma$  + I was ascertained to be the optimal model for all partitions except *APP*, *BRCA1*, and *ATP7A* where GTR +  $\Gamma$  was the optimal model, HKY85+ $\Gamma$  was optimal for *CREM* and GTR + I was optimal for *BMI1*. Flat priors were used, starting trees were random, and phylogenetic constraints were not incorporated. Four simultaneous Markov chains were run for 1,000,000 generations, burn in values were set at 50,000 generations (based on empirical values of stabilizing likelihoods), and trees were sampled every 100 generations. Each Bayesian run was repeated to test for convergence.

#### *Statistical testing*

We used the Kishino and Hasegawa (S14) test with RELL optimization and 1000 bootstrap replicates to compare the statistical significance of eight *a priori* hypotheses regarding interfamilial relationships: (a) Yinpterochiroptera (S15); (b) microbat monophyly (5); (c) sistergroup relationship between Noctilionidae and Mystacinidae (S16); (d) basal position for Mystacinidae within the noctilionid + mormoopid + phyllostomid clade (S17); (e) monophyly of the superfamily Nataloidea *sensu* Simmons and Geisler (S5); (f) association of thyropterids + furipterids with the exclusion of the natalids (S18); (g) monophyly of the superfamily Rhinolophoidea *sensu* Koopman (S19); (h) monophyly of the superfamily Rhinolophoidea *sensu* Teeling *et al.* (1).

### *Molecular dating branch length estimation*

Branch lengths were estimated with ESTBRANCHES for the concatenated data set and for each data partition discussed above (S20). The mole was chosen as the outgroup. The maximum likelihood topology (fig. S2) was incorporated in the analyses. We used Felsenstein's 1984 model of sequence evolution with an allowance for a gamma distribution of rates with four discrete rate categories (S21). The estimates of the rate categories for the gamma distribution, base frequencies and the transition/transversion parameter were calculated in PAUP 4.0b (S9) for the entire data set and for each data partition.

### *Divergence time estimations*

Two programs were used to estimate the divergence times: DIVTIME5B and MULTIDIVTIME (S20, S22). The DIVTIME5B program utilized the estimated branch length for the entire dataset, whereas MULTIDIVTIME utilized the estimated branch lengths for each data partition. *PNOc* was not incorporated in the MULTIDIVTIME analyses due to missing outgroup data. Both programs incorporated Markov Chain Monte Carlo analyses that were run for 1 million generations and sampled every 100 generations. In the both analyses we incorporated two estimates 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 (S23), and 56 Mya based on the oldest fossil bat (S5). Six fossil constraints were incorporated in the analyses.

- (1) A maximum of 34 Mya for the base of the family Phyllostomidae. The oldest verified phyllostomid fossils have been found in the Laventan (13.8-11.8 Mya) deposits of La Venta, Colombia (S24). The oldest suspected, but unconfirmed, and possible stem phyllostomids, are from the Whitneyan (32-30 Mya) I-75 formation from Florida (S25). Therefore we constrained the maximum divergence date for this clade at the Eocene / Oligocene boundary.
- (2) A minimum of 30 Mya for the Mormoopidae / Phyllstomidae split. The oldest fossils in this clade are mormoopids found in the Whitneyan (32-30 Mya) land deposits in Florida (S26).
- (3) A minimum of 37 Mya for the split between Vespertilionidae / Molossidae. Verified vespertilionid and molossid fossils have been found from the middle Eocene (S27).
- (4) A minimum of 37 Mya for the base of Emballonuridae. The oldest crown group emballonurid, *Tachypteron franzeni*, is found in Germany from middle Eocene deposits (S28).
- (5) A minimum of 37 Mya for the base of Rhinolophidae as crown group rhinolophids have been reported from the middle Eocene of Europe (S5, S27).
- (6) A maximum of 55 Mya for the base of Rhinolophoidea (S27). There are no early Eocene rhinolophoids so we constrained the maximum age for this split at the Paleocene/Eocene boundary.

To ascertain the sensitivity of our analyses to the fossil constraints each constraint was systematically removed in the DIVTIME5B analyses.

### *Missing fossil record estimation*

To estimate the fraction of missing data from the fossil record we collated the oldest definitive fossil for every chiropteran lineage on the tree (fig S3; table S5; S24–S31). We

aimed to identify unequivocal fossils that consisted of more than a single tooth (table S5). A lineage includes any taxon that may fall on that particular branch and any "off-shoots" from that branch (30 terminal branches, 28 internal branches). The date of basal divergence between two sister-lineages was estimated as the first fossil occurrence to represent either lineage, and was assessed for all lineages from tip to root (ghost-lineage). The age of first fossil occurrences for each lineage was compared with the estimated Bayesian molecular dates of first occurrences, (fig. S3, table S5) to identify the "unrepresented basal branch length" or UBBL. When both the molecular and fossil age are in agreement, or when the oldest fossil predated the molecular age (in cases where the either the lineage in question was not taxonomically sampled at its most basal divergence; was a paraphyletic assemblage; or the fossils are incorrectly identified), we concluded that there was no missing data for that lineage. When the oldest fossil was younger than the molecular age we subtracted that the age of the fossil from the molecular date to estimate the UBBL, and hence missing data for that lineage. Where no fossil information was available for the genera sampled, we used the oldest fossil age of the closest relative ("off-shoot" from the branch), and where appropriate, relatedness was based on previous intergeneric phylogenetic analyses (S11, S12, S32–S34). We used the midpoint dates of an epoch or land mammal age to assign an age to a fossil (S24–S31). The percentage of the "total missing" and the average of the "percentage missing" per lineage were calculated. Note the following worked example, see fig. S3, table S5.

#### Pteropodidae -Branches 1-7

Lineage 1, oldest fossil is *Pteropus* at 0.89- molecular age = 23, missing = 22.11,  
percentage missing = 96%

Lineage 2, oldest fossil is *Rousettus* at 0.13- molecular age = 23, missing = 22.87,  
percentage missing = 99%

Lineage 3, no fossil available, the time interval for this internal branch is 24-23 = 1,  
missing = 1, percentage missing = 100%

Lineage 4, oldest fossil is *Cynopterus* at 0.005-molecular age 22, missing = 21.99,  
percentage missing = 100%

Lineage 5, oldest fossil is *Nyctimene* at 0.005-molecular age 22, missing = 21.99.  
percentage missing = 100%

Lineage 6, no fossil available, the time interval for this internal branch is 24-22 = 2,  
missing = 2, percentage missing = 100%

Lineage 7, oldest representative is indet. pteropodid. at 26.25, the time interval for this  
internal branch is 58-24 = 34, amount of missing data is 58-26.45 = 31.85, percentage  
missing is 94%

Sum of age of lineages for Pteropodidae:                    $23+23+1+22+22+2+34 = 127$   
Sum of missing:    $22.11+22.87+1+21.99+21.99+2+31.85 = 123.82$

Therefore, percentage total missing:                            $123.82/127 = 98\%$

Average of percentage missing per lineage:  $96 + 99 + 100 + 100 + 100 + 100 + 94 / 7 = 98\%$

#### Geographic center of origin reconstruction

To assess the evolutionary relationships of the four Eocene fossil taxa, *Icaronycteris*, *Archaeonycteris*, *Hassianycteris*, *Paleochiropteryx* in light of our molecular phylogeny,

we reanalyzed the data set of Simmons and Geisler [(S5); which includes 195 morphological characters, scored for representatives of every extant bat family and for the four extinct fossil lineages], by incorporating a molecular scaffold that constrained all extant lineages as depicted in figure 1 in a parsimony framework. The fossil taxa were not constrained by the molecular scaffold. We used unweighted MP with 1,000 random input orders. The topology of the outgroup laurasiatherian orders is taken from Murphy *et al.* (S4). It has been suggested the early Eocene Australian fossil (*Australonycteris*) is most similar to the Eocene taxa *Hassianycteris*, *Archaeonycteris* and *Palaeochiropteryx* (S35), however the exact phylogenetic position of *Australonycteris* cannot be assessed due to insufficient data. Therefore, we placed *Australonycteris* as a possible sister-taxon to each of the above lineages, and to *Icaronycteris*, in each of the most parsimonious trees. Parsimony reconstructions of ancestral character states were obtained using MACCLADE (S36). Both delayed transformations and accelerated transformations were performed. The geographic characters and states are described in table S7. Geographic distributions were mapped onto each of the most parsimonious trees in two ways: (a) The earliest occurrence of each lineage in either the Laurasian or Gondwanan landmasses was assessed and mapped onto the trees (S4, S27, S30); (b) The current global distribution for each lineage was assessed and defined by nine character states (S37); Europe (East of the Ural mountains); Asia (West of the Ural mountains); Africa; Madagascar; Australia; New Zealand; North America; South America (includes central America); West Indies. If any fossils were found outside of the current range, this was also recorded eg. *Mystacina* is currently found only in New Zealand, however the oldest fossil is found in Australia, therefore the geographic distribution of *Mystacina* has two states, New Zealand and Australia (S27).

## SOM Text

Systematically removing each of the fossil constraints had minimal impact on the dates, always being within 1-2 million years of those shown in Fig. 2. Likewise, employing a mean prior of 56 Mya had a negligible effect on the above dates (fig. S2; table S3). When the maximum constraint of 55 Mya at the base of the rhinolophoid radiation was relaxed, the divergence date for the base of Chiroptera increased from ~ 64 Mya to ~72 Mya. Similarly, the date of basal divergence of all clades increased from 1 to 8 million years; however, these older point estimates fell within the credibility intervals of all other analyses (fig. S2; table S3).

<i>Pteropus</i>	AGAATAGAACTGAATAAGCAGAACCTCCATGCT	ACATTATGAATATTG
<i>Cynopterus</i>	AGAATAAAACTGAATAAGCAGAACCTCCATGCT	ACATTTGAATATTA
<i>Rousettus</i>	AGAATAGAACTAAATAAGCAGAACCTCCATGCT	ACATTTGAATATTG
<i>Nyctimene</i>	AGAATAGAACTGAATAAGCAGAACCTCCATGCT	AGATTCGAATATTG
<i>Rhinolophus</i>	AGAAAAGAACTGAATAAGCAGAACCTCCATGCT	ACATTTGAATATTG
<i>Hipposideros</i>	AGAAAAGAACTGAATAAGCAGAACCTCCATGCT	ACATTTGAATATTG
<i>Megaderma</i>	AGAAAAGAACTGAATAATCAGAACCTCTATGCT	ACATTTGAATACTG
<i>Macroderma</i>	AGAAAAGAACTGAATAATCAGAACCTCTATGCT	ACATTTGAATATTG
<i>Craseonycteris</i>	AGAGAAGAACTGAGTAAGCAGAACCTCTATGCT	ACATTTGAATATTG
<i>Rhinopoma</i>	AGAAAAGAACTGAGTAAGCATAAACCTGCATGCT	ACATTTGAATATTG
<i>Nycteris</i>	AGACAAGAA-----CTGCCATGCT	ACAT-----ACTG
<i>Emballonura</i>	AGAAGAGAA-----CCTCCATGCT	ACAT-----ATTG
<i>Taphozous</i>	AGAAAAGAA-----CCTCCATACT	ACAT-----ATTG
<i>Rhynchonycteris</i>	AGAAAAGAA-----CCTCCATGCT	ACAT-----ATTG
<i>Tonatia</i>	AGGGAAAGAA-----TCTCCATCCT	ACAT-----ACTG
<i>Artibeus</i>	AGGAGAGAA-----TCTCCTTCCT	ANGT-----ATTG
<i>Desmodus</i>	AGAAGAGAA-----TCTCCATCCT	ACAT-----ATTG
<i>Anoura</i>	AGAAAAGAA-----TCCCCTTCT	ACAT-----ACTG
<i>Noctilio</i>	AGAAGAGCT-----TCTCCATGCT	ACAT-----ATTG
<i>Antrozous</i>	GGAAAAGAA-----CTTCCACGCT	ACAT-----ATTG
<i>Rhogeessa</i>	AGAAAAGAA-----CTTCCACGCT	ACAT-----ATTG
<i>Myotis</i>	GAAAAAGAA-----CTTCCACGCT	ACAT-----ATTG
<i>Myzopoda</i>	AGAAAAGAA-----CATCCATGCG	ACAT-----TA
<i>Pteronotus</i>	AGAGAAGAA-----TCTCCATGCT	ACAT-----ACTG
<i>Thyroptera</i>	AGAAAAGAA-----TCTCCATGCT	ACAT-----ATTG
<i>Mystacina</i>	AGAACCGAA-----TCTCCATGCT	ACAT-----ATTG
<i>Furipteris</i>	AGAAAAGAA-----TCTCCATGCT	ACAT-----ATAG
<i>Natalus</i>	AGAAAAGAA-----CCTCCATGCT	ACAT-----ACTG
<i>Tadarida</i>	AGAAAAGAA-----CCTCCAGGCT	ACAT-----ATTG
<i>Eumops</i>	AGAAAAGAA-----CCTCCAGGCT	ACAT-----ATTG
cat	AGAAAA---CTGAGTAAGCAGAACCTCCATGCT	ACATTTGAGTATTG
bovine	AGAAAAGAACTGCGTAAGCAGAACCTGCATGCC	ACATTCTGAACGCTA
mole	AGAAAAGAACTGAATAAGCAGAACCTCCATGCT	ATATTTGAATTTG
horse	AGAGAAGAACTGAATAAGCAGAACCTCCACGCT	ACATCTTGAATCTTG

**Yinpterochiroptera**

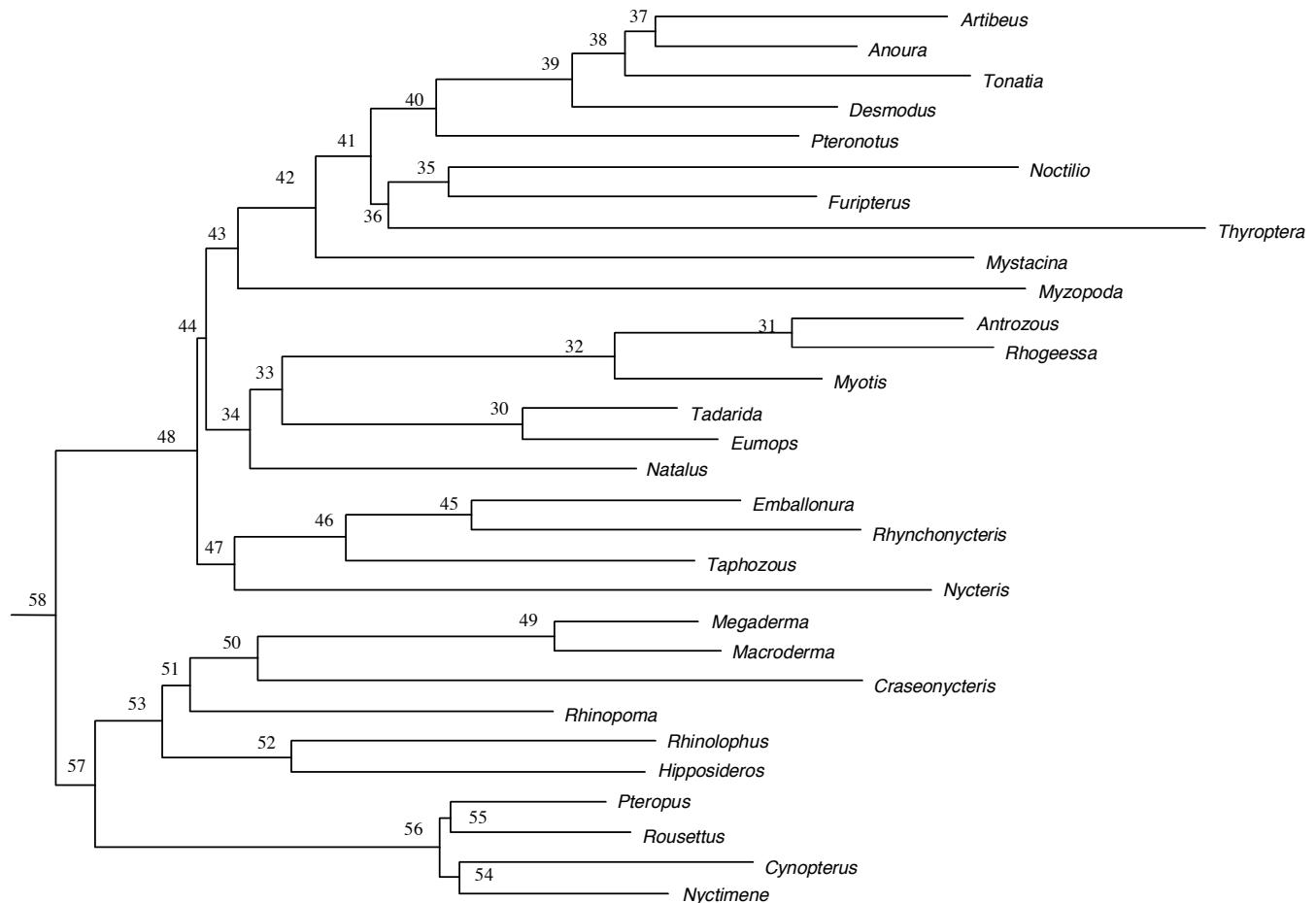
**Yangochiroptera**

### 15 base pair deletion *BRCA1*

### 7 base pair deletion *PLCB4* (3' UTR)

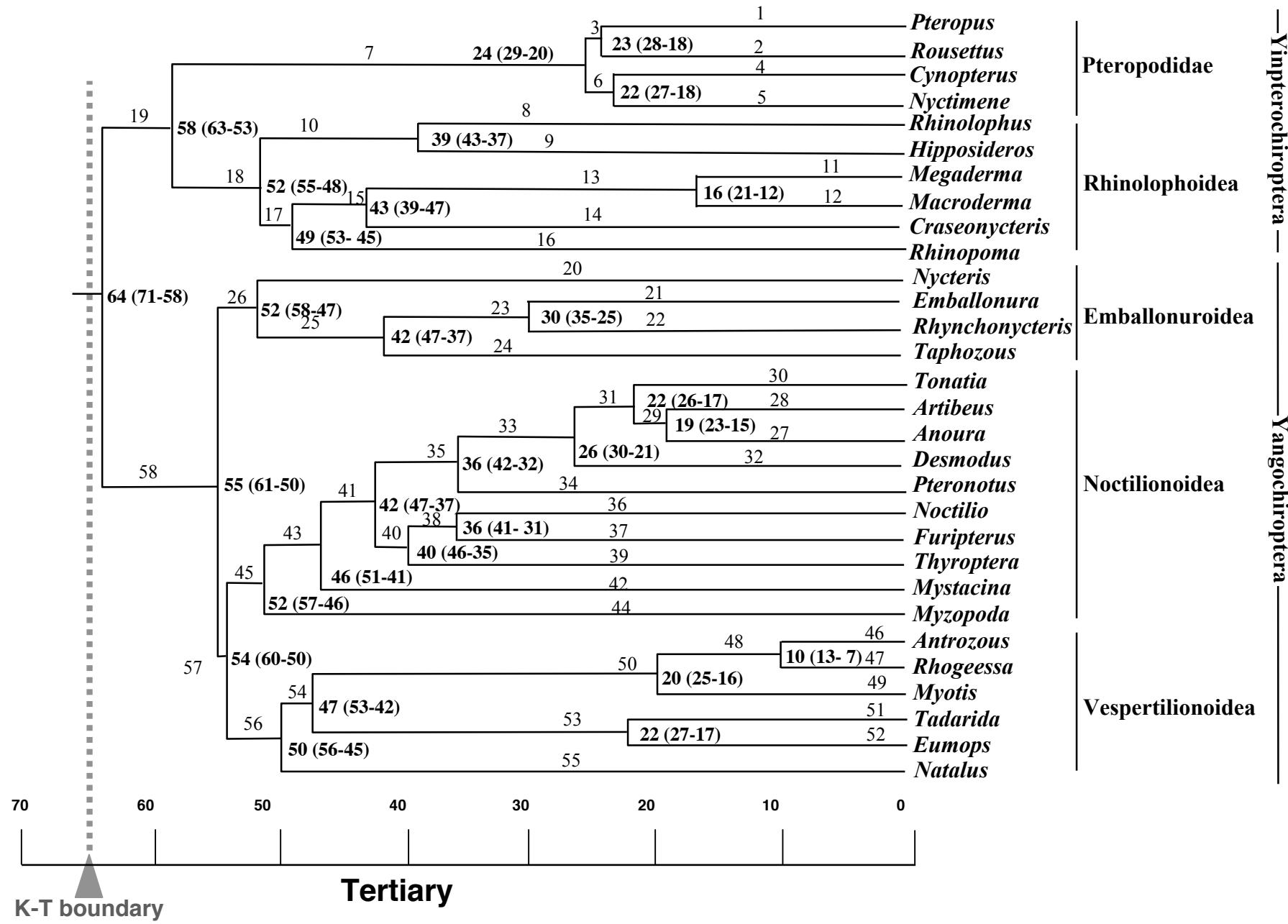
**Fig S1.** Depicts the two deletions found in all members of Yangochiroptera but are not found in any Yinpterochiropteran or outgroup taxa. These deletions were not included in the analyses, but instead are an independent source of support.

**Fig. S2.** ML tree incorporated in the DIVTIME and MULTIDIVTIME analyses. Numbers at the nodes indicate the nodes referred to in Table S3.



### Constraints incorporated in the Divtime and Multidivtime analyses

Nodes	Value in Mya
U39	34
L40	30
L33	37
L46	37
L52	37
U53	55



**Fig S3.** Molecular timescale for the order Chiroptera based on the *divtime* analyses, using the ML topology depicted in Fig. 1, six fossil constraints and a mean prior of 65 Mya for the base of the ingroup root. The values in bold face at the nodes are in millions of years, values in parentheses are the 95% credibility intervals. Branch numbers incorporated in table S5 are shown in normal face along each branch.

**Table S1.** Bootstrap values for the various clades. Bayesian posterior probabilities are shown as percentages. MLNNI, maximum likelihood with nearest neighbor branch swapping; MLTBR, maximum likelihood with tree bisection and reconnection branch swapping; MP, maximum parsimony; ME, minimum evolution; Bayes 1 & 2, Bayesian analyses with a single model of molecular evolution; Bayesian P1 & P2, Bayesian analyses with independent models of sequence evolution per gene partition.

(Table S1. continued)

Noctilionidae+Furipteridae	100	100	81	100	100	100	100	100
Noctilionidae+Furipteridae+Thyropteridae	76	77	18	36	95	96	88	89
Monophyly South American families	100	100	23	61	100	100	100	100
Basal position for Myzopodidae in Noctilionoidea	100	100	74	49	100	100	100	100
Noctilionoidea	78	77	28	50	100	100	100	99

**Table S2.** Comparison of phylogenetic hypotheses.

Phylogenetic hypotheses	Log likelihood score		$\Delta$ in $-\ln$ likelihood	<i>P</i> values for KH tests
	(a) Hutcheon	(b) Simmons		
1: (a) Yinpterochiroptera ( <i>S15</i> ) <b>versus</b> (b) Microbat monophyly ( <i>S5</i> )	92127.3772	92160.8491	33.47	0.006*
	(c) Pierson	(d) Kirsch		
2: (c) Noctilionidae + Mystacinidae ( <i>S16</i> ) <b>versus</b> (d) Mystacinidae basal to Noctilionidae + Phyllostomidae+Mormoopidae ( <i>S17</i> )	92127.3772	92187.8029	60.43	0.001*
	(e) Hoofer	(f) Simmons		
3: (e) Thyropteridae+Furipteridae with the exclusion of Natalidae and Myzopodidae ( <i>S18</i> ) <b>versus</b> (f) Nataloidea <i>sensu</i> Simmons and Geisler ( <i>S5</i> )	92127.3772	92577.4019	450	< 0.001*
	(g) Teeling	(h) Koopman		
4: (g) Rhinolophoidea <i>sensu</i> Teeling <i>et al.</i> ( <i>S1</i> ) <b>versus</b> (h) Rhinolophoidea <i>sensu</i> Koopman ( <i>S19</i> )	92127.3772	92556.3546	429	< 0.001*

\*Indicates hypotheses that were rejected at  $P < 0.01$  in pairwise comparisons

**Table S3.** Results from the Divtime and Multidivtime analyses. Nodes numbers are depicted in fig. S2.

DIVTIME	All six constraints		
Nodes	Point estimate (years $\times 10^6$ )	Standard deviation	95% credibility interval (years $\times 10^6$ )
30	<b>22.01425</b>	2.6303	(17.08512, 27.37454)
31	<b>9.57505</b>	1.42862	(7.05455, 12.62680)
32	<b>20.04225</b>	2.21498	(15.94317, 24.58184)
33	<b>47.2316</b>	2.76193	(41.98074, 52.76308)
34	<b>50.3167</b>	2.77173	(45.05088, 55.81135)
35	<b>35.6822</b>	2.63388	(30.68658, 40.99400)
36	<b>40.1843</b>	2.68585	(35.06333, 45.57177)
37	<b>19.17932</b>	2.02731	(15.36350, 23.28085)
38	<b>21.53562</b>	2.15676	(17.43630, 25.96897)
39	<b>25.54055</b>	2.31106	(21.20864, 30.24902)
40	<b>36.41302</b>	2.57925	(31.57647, 41.57388)
41	<b>41.57746</b>	2.66714	(36.57873, 46.88246)
42	<b>45.82662</b>	2.73156	(40.76129, 51.23790)
43	<b>51.6461</b>	2.81889	(46.37881, 57.30505)
44	<b>54.30112</b>	2.84889	(48.95887, 59.91360)
45	<b>29.84451</b>	2.72846	(24.85206, 35.48524)
46	<b>41.77661</b>	2.60572	(37.42366, 47.17763)
47	<b>51.87178</b>	2.79007	(46.75600, 57.51711)
48	<b>55.17114</b>	2.86814	(49.82219, 60.82810)
49	<b>15.93344</b>	2.18877	(11.96199, 20.51850)
50	<b>43.25103</b>	2.16147	(39.00689, 47.25939)
51	<b>49.40283</b>	1.99472	(45.28458, 52.69085)
52	<b>39.37232</b>	1.65184	(37.11268, 43.04464)
53	<b>52.02211</b>	1.98044	(47.88239, 54.86164)
54	<b>22.12157</b>	2.40312	(17.60806, 27.04482)
55	<b>22.70557</b>	2.34676	(18.25524, 27.55763)
56	<b>24.17959</b>	2.40802	(19.65148, 29.05913)
57	<b>58.3828</b>	2.59157	(53.18106, 63.02626)
58	<b>64.21475</b>	3.28638	(57.96385, 70.71400)

<b>DIVTIME</b>	<b>No maximum constraint at node 39</b>		
Nodes	Point estimate (years × 10 <sup>6</sup> )	Standard deviation	95% credibility interval (years × 10 <sup>6</sup> )
30	<b>22.05387</b>	2.68459	(17.02026, 27.50882)
31	<b>9.58986</b>	1.451	(7.03161, 12.68661)
32	<b>20.09802</b>	2.24885	(15.97420, 24.75936)
33	<b>47.22963</b>	2.78267	(42.00068, 52.87462)
34	<b>50.28611</b>	2.79476	(45.00059, 55.96544)
35	<b>35.64017</b>	2.63875	(30.66207, 40.99029)
36	<b>40.14065</b>	2.69879	(35.15759, 45.57933)
37	<b>19.14078</b>	2.06503	(15.29690, 23.28943)
38	<b>21.48856</b>	2.2023	(17.38339, 25.95847)
39	<b>25.49</b>	2.36304	(21.04831, 30.29247)
40	<b>36.35162</b>	2.61226	(31.45348, 41.59260)
41	<b>41.52902</b>	2.68391	(36.49644, 46.97722)
42	<b>45.76508</b>	2.73885	(40.61579, 51.27429)
43	<b>51.59512</b>	2.83767	(46.24887, 57.36281)
44	<b>54.25927</b>	2.858	(48.88471, 59.93840)
45	<b>29.8997</b>	2.74889	(24.82261, 35.51594)
46	<b>41.78458</b>	2.62838	(37.40777, 47.26224)
47	<b>51.85021</b>	2.8016	(46.73593, 57.45159)
48	<b>55.13365</b>	2.87842	(49.67935, 60.92174)
49	<b>15.95481</b>	2.18939	(11.97300, 20.53173)
50	<b>43.231</b>	2.17935	(38.80916, 47.19118)
51	<b>49.37014</b>	2.02857	(45.12063, 52.72436)
52	<b>39.37962</b>	1.64337	(37.10863, 43.11389)
53	<b>51.99631</b>	1.99435	(47.72762, 54.87382)
54	<b>22.11442</b>	2.3737	(17.68293, 26.93155)
55	<b>22.70781</b>	2.34987	(18.31995, 27.48823)
56	<b>24.16308</b>	2.38482	(19.71070, 28.96928)
57	<b>58.35715</b>	2.61397	(53.00823, 62.99476)
58	<b>64.15921</b>	3.32034	(57.74125, 70.66890)

<b>DIVTIME</b>	<b>No minimum constraint at node 40</b>		
Nodes	Point estimate (years $\times 10^6$ )	Standard deviation	95% credibility interval (years $\times 10^6$ )
30	<b>21.96768</b>	2.67121	(17.02820, 27.51227)
31	<b>9.56059</b>	1.42973	(7.00342, 12.60015)
32	<b>20.02047</b>	2.2193	(15.80762, 24.57238)
33	<b>47.20002</b>	2.74144	(42.02628, 52.73214)
34	<b>50.28794</b>	2.75161	(45.12864, 55.78763)
35	<b>35.61362</b>	2.6022	(30.58524, 40.90603)
36	<b>40.10992</b>	2.67135	(34.96761, 45.49021)
37	<b>19.15259</b>	2.0567	(15.32373, 23.36773)
38	<b>21.50061</b>	2.18343	(17.38660, 25.92077)
39	<b>25.49139</b>	2.33731	(21.09484, 30.16337)
40	<b>36.34151</b>	2.5689	(31.35613, 41.41163)
41	<b>41.50564</b>	2.64739	(36.43756, 46.78063)
42	<b>45.74839</b>	2.70605	(40.53972, 51.14237)
43	<b>51.59374</b>	2.79857	(46.33165, 57.18599)
44	<b>54.27235</b>	2.82602	(48.96274, 59.95378)
45	<b>29.89776</b>	2.72142	(24.88308, 35.37207)
46	<b>41.79987</b>	2.60051	(37.43305, 47.29352)
47	<b>51.87184</b>	2.7628	(46.78323, 57.41906)
48	<b>55.15021</b>	2.84737	(49.78835, 60.81107)
49	<b>15.89659</b>	2.18704	(11.92900, 20.45956)
50	<b>43.21599</b>	2.18565	(38.76634, 47.24439)
51	<b>49.37496</b>	2.01177	(45.18592, 52.76222)
52	<b>39.37009</b>	1.65095	(37.11242, 43.02544)
53	<b>52.01581</b>	1.97226	(47.78458, 54.87854)
54	<b>22.08963</b>	2.39218	(17.63437, 27.01381)
55	<b>22.67465</b>	2.33874	(18.30441, 27.50438)
56	<b>24.14352</b>	2.40055	(19.64078, 29.03648)
57	<b>58.3792</b>	2.58496	(53.03414, 62.97613)
58	<b>64.20364</b>	3.27871	(57.81929, 70.56990)

**DIVTIME**      **No minimum constraint at node 33**

Nodes	Point estimate (years $\times 10^6$ )	Standard deviation	95% credibility interval (years $\times 10^6$ )
30	<b>22.05953</b>	2.64686	(17.09330, 27.50099)
31	<b>9.58949</b>	1.43179	(7.01481, 12.63746)
32	<b>20.0949</b>	2.205	(15.99053, 24.57445)
33	<b>47.27561</b>	2.74116	(42.11734, 52.71460)
34	<b>50.348</b>	2.75856	(45.17492, 55.83958)
35	<b>35.69369</b>	2.6246	(30.81456, 41.03805)
36	<b>40.19085</b>	2.67831	(35.17772, 45.69075)
37	<b>19.17626</b>	2.06788	(15.37061, 23.46852)
38	<b>21.52786</b>	2.2002	(17.46500, 26.00521)
39	<b>25.54157</b>	2.34053	(21.19091, 30.26960)
40	<b>36.42262</b>	2.58116	(31.58427, 41.73129)
41	<b>41.59007</b>	2.64967	(36.67964, 47.01524)
42	<b>45.83048</b>	2.70298	(40.75674, 51.28142)
43	<b>51.66623</b>	2.793	(46.40025, 57.25894)
44	<b>54.33394</b>	2.82736	(49.05365, 59.91291)
45	<b>29.88566</b>	2.7229	(24.85255, 35.54656)
46	<b>41.81202</b>	2.61022	(37.44085, 47.29870)
47	<b>51.91428</b>	2.78092	(46.77293, 57.52692)
48	<b>55.21529</b>	2.84763	(49.87080, 60.82653)
49	<b>15.94205</b>	2.20251	(11.94084, 20.60110)
50	<b>43.27061</b>	2.20872	(38.78618, 47.35282)
51	<b>49.41393</b>	1.9854	(45.25785, 52.67294)
52	<b>39.41849</b>	1.68439	(37.09856, 43.25004)
53	<b>52.04785</b>	1.95423	(47.86885, 54.86462)
54	<b>22.13918</b>	2.39606	(17.62995, 27.03614)
55	<b>22.74054</b>	2.35102	(18.33962, 27.49763)
56	<b>24.20109</b>	2.40298	(19.75698, 29.04660)
57	<b>58.41379</b>	2.58208	(53.18114, 62.93713)
58	<b>64.24301</b>	3.27896	(57.89010, 70.74010)

<b>DIVTIME</b>	<b>No minimum constraint at node 46</b>		
Nodes	Point estimate (years × 10 <sup>6</sup> )	Standard deviation	95% credibility interval (years × 10 <sup>6</sup> )
30	<b>21.89526</b>	2.70914	(16.84624, 27.32230)
31	<b>9.52615</b>	1.46359	(6.90475, 12.64161)
32	<b>19.9437</b>	2.28153	(15.64157, 24.60319)
33	<b>46.95011</b>	2.9717	(41.25906, 52.71705)
34	<b>50.02168</b>	2.98091	(44.25967, 55.79341)
35	<b>35.46802</b>	2.73407	(30.31095, 40.88843)
36	<b>39.93492</b>	2.81536	(34.56757, 45.51297)
37	<b>19.05443</b>	2.06554	(15.33637, 23.34260)
38	<b>21.39672</b>	2.20179	(17.40666, 25.92270)
39	<b>25.37224</b>	2.36555	(21.04148, 30.20157)
40	<b>36.18969</b>	2.68617	(31.10991, 41.59280)
41	<b>41.32739</b>	2.80025	(35.99995, 46.87924)
42	<b>45.5418</b>	2.88925	(39.97069, 51.14157)
43	<b>51.33884</b>	3.01824	(45.41655, 57.12372)
44	<b>53.99842</b>	3.06825	(47.92449, 59.88447)
45	<b>29.59994</b>	2.97168	(23.86544, 35.59710)
46	<b>41.42011</b>	3.00028	(35.51467, 47.31390)
47	<b>51.57662</b>	3.03974	(45.56953, 57.40758)
48	<b>54.87521</b>	3.09411	(48.80173, 60.83938)
49	<b>15.92037</b>	2.18765	(11.92578, 20.52479)
50	<b>43.15362</b>	2.23681	(38.60790, 47.21777)
51	<b>49.26455</b>	2.08762	(44.89846, 52.67416)
52	<b>39.34013</b>	1.64064	(37.09397, 43.04125)
53	<b>51.87187</b>	2.0801	(47.41952, 54.85864)
54	<b>22.04246</b>	2.36713	(17.63286, 26.85218)
55	<b>22.62935</b>	2.31954	(18.33014, 27.30460)
56	<b>24.08553</b>	2.37749	(19.63565, 28.87939)
57	<b>58.15873</b>	2.73262	(52.46546, 62.90788)
58	<b>63.91615</b>	3.46616	(56.97773, 70.54494)

<b>DIVTIME</b>	<b>No minimum constraint at node 52</b>		
Nodes	Point estimate (years $\times 10^6$ )	Standard deviation	95% credibility interval (years $\times 10^6$ )
30	<b>21.34773</b>	2.62422	(16.57572, 26.91381)
31	<b>9.28355</b>	1.42667	(6.77623, 12.28983)
32	<b>19.47009</b>	2.23	(15.42916, 24.12170)
33	<b>45.88986</b>	3.02647	(40.49589, 52.03714)
34	<b>48.8658</b>	3.0921	(43.32832, 55.11682)
35	<b>34.64565</b>	2.7	(29.89437, 40.30714)
36	<b>39.02655</b>	2.81465	(34.11684, 44.89747)
37	<b>18.62183</b>	2.03545	(15.01355, 22.89212)
38	<b>20.90855</b>	2.17619	(17.03374, 25.45633)
39	<b>24.78217</b>	2.33768	(20.64410, 29.69183)
40	<b>35.36331</b>	2.64849	(30.73289, 40.82575)
41	<b>40.38715</b>	2.80871	(35.49667, 46.25210)
42	<b>44.50461</b>	2.94528	(39.32615, 50.51663)
43	<b>50.1492</b>	3.16308	(44.53010, 56.47155)
44	<b>52.73374</b>	3.24911	(46.90613, 59.14644)
45	<b>29.19569</b>	2.63059	(24.55965, 34.78426)
46	<b>40.77262</b>	2.58684	(37.17150, 46.48865)
47	<b>50.43056</b>	3.08857	(45.06616, 56.74647)
48	<b>53.5807</b>	3.28535	(47.65011, 60.10672)
49	<b>15.19289</b>	2.29085	(11.02194, 19.98611)
50	<b>41.49529</b>	2.99733	(35.20317, 46.76584)
51	<b>47.52084</b>	3.04977	(41.20802, 52.39643)
52	<b>37.12618</b>	2.97147	(31.12274, 42.62056)
53	<b>50.10261</b>	3.08002	(43.69569, 54.74975)
54	<b>21.13148</b>	2.5245	(16.31511, 26.33119)
55	<b>21.68906</b>	2.50937	(16.95288, 26.81186)
56	<b>23.09232</b>	2.58538	(18.16493, 28.38588)
57	<b>56.47714</b>	3.47449	(49.50979, 62.47537)
58	<b>62.31422</b>	3.97203	(54.86323, 69.91611)

<b>DIVTIME</b>	<b>No maximum constraint at node 53</b>		
Nodes	Point estimate (years $\times 10^6$ )	Standard deviation	95% credibility interval (years $\times 10^6$ )
30	<b>24.58874</b>	3.80255	(17.88082, 32.68125)
31	<b>10.70842</b>	1.9005	(7.45619, 14.85377)
32	<b>22.45833</b>	3.30125	(16.68241, 29.54966)
33	<b>52.94139</b>	5.94649	(43.28798, 65.77811)
34	<b>56.41059</b>	6.25991	(46.33369, 69.80977)
35	<b>39.77139</b>	4.57072	(31.92358, 49.19062)
36	<b>44.82122</b>	4.96776	(36.48208, 54.98004)
37	<b>21.13749</b>	2.60727	(16.21309, 25.96902)
38	<b>23.74269</b>	2.82513	(18.42858, 28.81535)
39	<b>28.19967</b>	3.16955	(22.19247, 33.63725)
40	<b>40.4895</b>	4.43321	(32.79652, 49.18699)
41	<b>46.39168</b>	5.08295	(37.83760, 56.68369)
42	<b>51.21968</b>	5.61047	(41.97662, 63.06672)
43	<b>57.87598</b>	6.36438	(47.61780, 71.60070)
44	<b>60.92267</b>	6.69946	(50.22354, 75.36590)
45	<b>33.35188</b>	4.4166	(26.00772, 42.96111)
46	<b>46.67718</b>	5.39452	(38.08998, 58.37587)
47	<b>58.17114</b>	6.4414	(47.87179, 72.05186)
48	<b>61.91836</b>	6.82066	(51.08204, 76.70061)
49	<b>18.01702</b>	3.16526	(12.72977, 25.08102)
50	<b>48.82393</b>	5.70779	(40.10657, 61.55083)
51	<b>55.79861</b>	6.26195	(46.28352, 69.73844)
52	<b>43.9951</b>	5.05288	(37.31893, 55.64816)
53	<b>58.77446</b>	6.52992	(48.99119, 73.29711)
54	<b>24.97485</b>	3.74898	(18.70666, 33.39214)
55	<b>25.64749</b>	3.75741	(19.45208, 33.88817)
56	<b>27.29468</b>	3.93265	(20.84595, 35.99214)
57	<b>65.85844</b>	7.32158	(54.53148, 82.22862)
58	<b>72.27564</b>	8.08191	(59.54893, 89.94825)

DIVTIME	56 Mya prior, all constraints		
Nodes	Point estimate (years × 10 <sup>6</sup> )	Standard deviation	95% credibility interval (years × 10 <sup>6</sup> )
30	<b>21.33793</b>	2.38352	(16.80436, 26.18619)
31	<b>8.97199</b>	1.30926	(6.64177, 11.76023)
32	<b>18.89551</b>	2.00702	(15.12632, 22.96247)
33	<b>45.27094</b>	2.26963	(40.99716, 49.83521)
34	<b>47.97888</b>	2.26165	(43.84005, 52.55103)
35	<b>34.11987</b>	2.21133	(30.02739, 38.68805)
36	<b>38.39314</b>	2.22401	(34.42466, 42.93658)
37	<b>18.27366</b>	1.80159	(14.89963, 21.99316)
38	<b>20.39328</b>	1.89044	(16.86486, 24.21081)
39	<b>24.18118</b>	1.99082	(20.52533, 28.23995)
40	<b>34.54925</b>	2.15141	(30.69293, 38.96936)
41	<b>39.65169</b>	2.19251	(35.77245, 44.19717)
42	<b>43.36807</b>	2.23178	(39.31622, 47.94655)
43	<b>48.79089</b>	2.28972	(44.55795, 53.46509)
44	<b>51.56538</b>	2.29131	(47.35061, 56.18495)
45	<b>28.83172</b>	2.31209	(24.66363, 33.64849)
46	<b>40.26807</b>	2.07337	(37.19502, 44.84675)
47	<b>49.61587</b>	2.25152	(45.59341, 54.33023)
48	<b>52.56092</b>	2.31496	(48.27777, 57.22868)
49	<b>16.20527</b>	2.18034	(12.27157, 20.83883)
50	<b>43.67076</b>	2.04639	(39.52830, 47.44108)
51	<b>49.85248</b>	1.84964	(45.96141, 52.86154)
52	<b>39.43535</b>	1.639	(37.10805, 43.04928)
53	<b>52.31517</b>	1.80433	(48.38404, 54.87443)
54	<b>22.7312</b>	2.36732	(18.30356, 27.62067)
55	<b>22.81523</b>	2.29371	(18.51443, 27.51406)
56	<b>24.65301</b>	2.3556	(20.26165, 29.53995)
57	<b>58.43236</b>	2.3532	(53.60806, 62.62887)
58	<b>62.63056</b>	2.81482	(57.11785, 68.17374)

MULTIDIVTIME	65 Mya prior, all constraints		
Nodes	Point estimate (years × 10 <sup>6</sup> )	Standard deviation	95% credibility interval (years × 10 <sup>6</sup> )
30	<b>20.82856</b>	1.98188	(17.17859, 24.93175)
31	<b>12.70627</b>	1.45693	(10.03162, 15.76665)
32	<b>22.08695</b>	1.92884	(18.47736, 26.11053)
33	<b>46.3575</b>	2.65601	(41.52793, 51.73516)
34	<b>47.67065</b>	2.67756	(42.82388, 53.16473)
35	<b>40.65657</b>	2.53756	(35.96456, 45.85757)
36	<b>42.26832</b>	2.55359	(37.64685, 47.49219)
37	<b>23.41805</b>	1.91708	(19.89542, 27.36130)
38	<b>25.11741</b>	1.97373	(21.53462, 29.17009)
39	<b>26.57768</b>	2.01355	(22.89254, 30.72336)
40	<b>37.57782</b>	2.38917	(33.23516, 42.44533)
41	<b>43.10977</b>	2.55345	(38.47039, 48.34105)
42	<b>45.40091</b>	2.63223	(40.60316, 50.75272)
43	<b>51.53168</b>	2.80495	(46.42331, 57.16523)
44	<b>52.17261</b>	2.80773	(47.11479, 57.89156)
45	<b>32.56694</b>	2.61272	(27.79477, 37.97400)
46	<b>42.54776</b>	2.59379	(37.83790, 47.93588)
47	<b>50.96774</b>	2.79501	(45.90431, 56.70255)
48	<b>52.68192</b>	2.81696	(47.61985, 58.40477)
49	<b>15.18104</b>	1.54169	(12.36128, 18.41456)
50	<b>46.54006</b>	2.26323	(42.33212, 51.08670)
51	<b>48.60186</b>	2.20142	(44.59773, 53.04033)
52	<b>38.5572</b>	1.34767	(37.04609, 41.96100)
53	<b>49.8588</b>	2.19226	(45.83383, 54.22694)
54	<b>21.30318</b>	1.78931	(18.04284, 24.95398)
55	<b>20.62245</b>	1.74171	(17.42808, 24.23068)
56	<b>22.0912</b>	1.80105	(18.77040, 25.82280)
57	<b>55.64543</b>	2.60594	(50.91364, 60.90003)
58	<b>60.44898</b>	2.91305	(55.21640, 66.31444)

MULTIDIVTIME	56 Mya prior, all constraints		
Nodes	Point estimate (years × 10 <sup>6</sup> )	Standard deviation	95% credibility interval (years × 10 <sup>6</sup> )
30	<b>20.57707</b>	1.94968	(16.94563, 24.54764)
31	<b>12.45105</b>	1.45591	(9.79628, 15.48864)
32	<b>21.68297</b>	1.9046	(18.19148, 25.62239)
33	<b>45.54137</b>	2.57698	(40.95932, 50.92988)
34	<b>46.81998</b>	2.58161	(42.21170, 52.22180)
35	<b>39.94301</b>	2.44261	(35.53363, 45.15050)
36	<b>41.52283</b>	2.4739	(37.08974, 46.76410)
37	<b>23.00935</b>	1.90231	(19.58066, 27.00308)
38	<b>24.67241</b>	1.96195	(21.11943, 28.77793)
39	<b>26.1062</b>	1.97865	(22.44924, 30.23892)
40	<b>36.93487</b>	2.33444	(32.72298, 41.86494)
41	<b>42.35226</b>	2.48569	(37.89967, 47.66580)
42	<b>44.58779</b>	2.57203	(40.05450, 50.04787)
43	<b>50.59176</b>	2.7044	(45.89938, 56.29430)
44	<b>51.2145</b>	2.71444	(46.51985, 56.97779)
45	<b>31.97307</b>	2.53973	(27.30625, 37.30240)
46	<b>41.77479</b>	2.47168	(37.58670, 47.05641)
47	<b>50.01694</b>	2.67861	(45.36714, 55.68119)
48	<b>51.70361</b>	2.7195	(46.99670, 57.42991)
49	<b>15.04461</b>	1.51617	(12.27389, 18.16130)
50	<b>45.90593</b>	2.16002	(41.99399, 50.43169)
51	<b>47.95238</b>	2.13354	(44.12568, 52.42482)
52	<b>38.33258</b>	1.21128	(37.03583, 41.49933)
53	<b>49.1896</b>	2.11897	(45.42244, 53.64849)
54	<b>21.14459</b>	1.75455	(17.87140, 24.72873)
55	<b>20.4743</b>	1.71465	(17.34764, 23.99715)
56	<b>21.92389</b>	1.76718	(18.62578, 25.51721)
57	<b>54.73889</b>	2.50785	(50.25747, 60.01673)
58	<b>59.30108</b>	2.82385	(54.33524, 65.30633)

**Table S4.** The ancestral reconstructions of geographic origin as mapped onto each of the six most parsimonious trees with both accelerated and delayed transformations for the nine-state and two-state analyses (Fig. 3).

Order	Trees					
	1	2	3	4	5	6
Chiroptera	N.America/ N.America/L	N.America/ N.America/L	N.America/ N.America/L	N.America/ N.America/L	N.America/ N.America/L	N.America/ N.America/L
Yinpterochiroptera	Asia/Asia/L	Europe/Europe/L	Europe/Europe/L	Asia/Asia/L	Europe/Europe/L	Asia/Asia/L
Rhinolophoidea	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L
Pteropodidae	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L	Asia/Asia/L
Yangochiroptera	Asia/Europe/L	Europe/Europe/L	Europe/Europe/L	Europe/Europe/L	Europe/Europe/L	Asia/Asia/L
Emballonuroidea	Asia/Europe/L	NK/Europe/L	NK/Europe/L	NK/Europe/L	NK/Europe/L	Asia/Asia/L
Vespertilioidea <sup>†</sup>	S.America/NK/L	S.America/NK/L	S.America/NK/L	S.America/NK/L	S.America/NK/L	S.America/ S.America/L
Noctilionoidea	S.America/NK/G	S.America/NK/G	S.America/NK/G	S.America/NK/G	S.America/NK/G	S.America/ S.America/G

\*Acctran/Deltran/G or L; G, Gondwanan; L, Laurasian; NK, not known <sup>†</sup>Discrepancy in the nine state versus two state analyses

**Table S5.** Estimation of the missing fossil record per lineage. Values are in millions of years unless specified otherwise.

Branch number	Taxon	Oldest fossil	Molecular age	Time interval of internal branches	Age of lineage	Total missing per lineage	Percent missing per lineage
<b>Pteropodidae*</b>							
1	<i>Pteropus</i>	0.89	23		23	22.11	96.13
2	<i>Rousettus</i>	0.13	23		23	22.87	99.43
3		0.89	24	24 – 23 = 1	1	1	100.00
4	<i>Cynopterus</i>	0.005	22		22	21.995	99.98
5	<i>Nyctimene</i>	0.005	22		22	21.995	99.98
6		0.005	24	24 – 22 = 2	2	2	100.00
7	Pteropodidae indet.	26.15	58	58 – 24 = 34	34	31.85	93.68
<b>Rhinolophoidea</b>							
8	<i>Rhinolophus</i>	43	39		39	0	0.00
9	<i>Hipposideros</i>	43	39		39	0	0.00
10	<i>Pseudorhinolophus</i>	45.15	52	52 – 39 = 13	13	6.85	52.69
11	<i>Megaderma</i>	28.5	16		16	0	0.00
12	<i>Macroderma</i>	13.8	16		16	2.2	13.75
13	<i>Necromantis</i>	43	43		43	0	0.00
14	<i>Craseonycteris</i>	0.005	43		43	42.995	99.99
15	<i>Necromantis</i>	43	49	49 – 43 = 6	6	6	100.00
16	<i>Rhinopoma</i>	0.005	49		49	48.995	99.99
17	<i>Necromantis</i>	43	52	52 – 49 = 3	3	3	100.00
18	<i>Pseudorhinolophus</i>	45.15	58	58 – 52 = 6	6	6	100.00
19	<i>Pseudorhinolophus</i>	45.15	64	64 – 58 = 6	6	6	100.00
<b>Emballonuroidea</b>							
20	<i>Nycteris</i>	26.15	52		52	25.85	49.71
21	<i>Emballonura</i>	0.89	30		30	29.11	97.03
22	<i>Diclidurus</i>	12.4	30		30	17.6	58.67
23	<i>Diclidurus</i>	12.4	30	42 – 30 = 12	12	12	100.00
24	<i>Vespertiliavus</i>	43.8	42		42	0	0.00

Table S5, continued

Branch number	Taxon	Oldest fossil	Molecular age	Time interval of internal branches	Age of lineage	Total missing per lineage	Percent missing per lineage
25	<i>Tachypteron</i> <sup>†</sup>	45.15	52	52 – 42 = 10	10	6.85	68.50
26	<i>Tachypteron</i>	45.15	55	55 – 52 = 3	3	3	100.00
<b>Noctilionoidea</b>							
27	<i>Anoura</i>	0.89	19		19	18.11	95.32
28	<i>Artibeus</i>	0.89	19		19	18.11	95.32
29		0.89	22	22 – 19 = 3	3	3	100.00
30	<i>Tonatia</i>	12.5	22		22	9.5	43.18
31	<i>Tonatia</i>	12.5	26	26 – 22 = 4	4	4	100.00
32	<i>Desmodus</i>	1.275	26		26	24.725	95.10
33	<i>Tonatia</i>	12.5	36	36 – 26 = 10	10	10	100.00
34	Mormoopidae indet.	31	36		36	5	13.89
35	Mormoopidae indet.	31	42	42 – 36 = 6	6	6	100.00
36	<i>Noctilio</i>	12.4	36		36	23.6	65.56
37	<i>Furipterus</i>	0.005	36		36	35.995	99.99
38	<i>Noctilio</i>	12.4	40	40 – 36 = 4	4	4	100.00
39	<i>Thyroptera</i>	12.4	40		40	27.6	69.00
40	<i>Noctilio</i>	12.4	42	42 – 40 = 2	2	2	100.00
41	Mormoopidae indet.	31	46	46 – 42 = 4	4	4	100.00
42	<i>Icarops</i>	20.1	46		46	25.9	56.30
43	Mormoopidae indet.	31	52	52 – 46 = 6	6	6	100.00
44	<i>Myzopoda</i>	1.275	52		52	50.725	97.55
45	Mormoopidae indet	31	54	54 – 52 = 2	2	2	100.00
<b>Vespertilioidea</b>							
46	<i>Antrozous</i>	7.5	10		10	2.5	25.00
47	<i>Rhogeessa</i>	0.005	10		10	9.995	99.95
48	<i>Antrozous</i>	7.5	20	20 – 10 = 10	10	10	100.00
49	<i>Myotis</i> <sup>‡</sup>	20	20		20	0	0.00
50	<i>Oligoryzomys</i>	31.1	47	47 – 20 = 27	27	15.9	58.89

Table S5, continued

Branch number	Taxon	Oldest fossil	Molecular age	Time interval of internal branches	Age of lineage	Total missing per lineage	Percent missing per lineage
51	<i>Tadarida</i>	35.5	22		22	0	0.00
52	<i>Eumops</i>	12.4	22		22	9.6	43.64
53	<i>Wallia</i>	43.25	47	47 – 22 = 25	25	3.75	15.00
54	<i>Wallia</i>	43.25	50	50 – 47 = 3	3	3	100.00
55	<i>Primonatalus</i> <sup>§</sup>	31	50		50	19	38.00
56	<i>Wallia</i>	43.25	54	54 – 50 = 4	4	4	100.00
57	<i>Wallia</i>	43.25	55	55 – 54 = 1	1	1	100.00
58	<i>Tachypteron</i>	45.15	64	64 – 55 = 9	9	9	100.00

\*Although it has been suggested that the oldest pteropodid is from the Late Eocene of Thailand (S38), this fossil consists of a single tooth that is considered derived among pteropodids. The validity and assignment of this fossil to crown group pteropodids has been recently questioned (S32); hence, it is considered an equivocal fossil and was not used in our analyses.

<sup>†</sup>*Eppsinycteris*, originally considered the oldest emballonurid, has recently been questioned as an emballonurid and even as a bat (S28); therefore, it was not used in our analyses.

<sup>‡</sup>Although it has been suggested that *Myotis* have been found from the Early Oligocene, these fossils have recently been identified as eptesicoid fossils rather than *Myotis* fossils (S39). The oldest unequivocal *Myotis* fossils are from about 20 Mya (S39).

<sup>§</sup>Other Tertiary genera have been considered natalids including the fossils *Ageina*, *Chadronycteris*, *Chamtwaria*, *Honrovotis*, and *Stehlinia*; however, these taxa have been questioned as belonging to the Natalidae (S31).

Table S6. GenBank accession numbers

Species	<i>ADRA2B</i>	<i>ADORA3</i>	<i>ADRB2</i>	<i>APP</i>	<i>ATP7A</i>	<i>BDNF</i>	<i>BMI</i>	<i>BRCA1</i>	<i>CREM</i>	<i>EDG1</i>	<i>PLCB4</i>	<i>PNC</i>	<i>RAG1</i>	<i>RAG2</i>	<i>TITIN2/3</i>	<i>TITIN6/7</i>	<i>TYR</i>	<i>VWF</i>	<i>ZFX</i>
1 <i>Pteropus rayneri</i>	AF337539							AF203751					AF203759	AF203769	AY834670	AY834703		AY012074	
<i>Pteropus giganteus</i>	AY011233	AY011293	AY011356	AY011420	AY011483	AY011541		AY011666	AY011727	AY011790	AY011847						AY012021		
<i>Pteropus hypomelanus</i>																		AF203777	
2 <i>Cynopterus sphinx</i>	AJ251181							AF203750					AF203758	AF203768				U31605	
<i>Cynopterus brachyotis</i>	AY834412	AY834436	AY834460	AY834484	AY834508	AY834532		AY057829		AY839080	AY834556	AY835940	AY834580	AF447512	AF447529				AY834625
<i>Rousettus amplexicaudatus</i>	AJ315937																	AY012022	
<i>Rousettus lanosus</i>	AY011234	AY011294	AY011357	AY011421	AY011484	AY011542		AY011667	AY011728	AY011791	AY011848							AF447547	
4 <i>Nyctimene albiventer</i>	AJ419805	AY834413	AY834437	AY834461	AY834485	AY834509	AY834533	AF447502	AY839081	AY834557	AY835941	AY834581	AF447514	AF447531	AY834673	AY834706	AY834606	AF447549	
5 <i>Rhinolophus creaghi</i>	AJ419806	AY834414	AY834438	AY834462	AY834486	AY834510	AY834534	AF447499	AY839082	AY834558	AY835942	AY834582	AF447511	AF447528	AY834674	AY834707	AY834607	AF447546	
6 <i>Hippopotideres commersoni</i>	AF337538	AY834415	AY834439	AY834463	AY834487	AY834511	AY834535	AF203752	AY059688	AY059692	AF203749	AY839083	AY059701	AY059708	AY059711	AF203757	AY834675	AY834608	
7 <i>Megaderma lyra</i>	AF337537	AY059670	AY059676	AY059680	AY059684	AY059688	AY059692	AF203749	AY839083	AY059701	AY059708	AY059711	AF203757	AF203767	AY834676	AY834709	AY059716	U31616	
8 <i>Macrotis gigas</i>	AY834404	AY834416	AY834440	AY834464	AY834488	AY834512	AY834536	AY834645	AY839084	AY834560	AY835944	AY834584	AY834654	AY834661	AY834677	AY834710	AY834609	AY834736	
<i>Nycterus grandis</i>	AJ419807							AF447484		AY834579	AY835945	AY834604	AF447506	AF447523	AY834678	AY834711	AY834610	AF447541	
<i>Nycterus thebaica</i>	AY011235	AY011295	AY011358	AY011422	AY011485	AY011543		AY011668										??????	
10 <i>Rhinopoma hardwickii</i>	AJ419809	AY834417	AY834441	AY834465	AY834489	AY834513	AY834537	AF447504	AY839085	AY834561	AY835946	AY834585	AF447535	AF447535	AY834679	AY834712	AY834611	AF447551	
11 <i>Emballonura atrata</i>	AJ419810	AY834418	AY834442	AY834466	AY834490	AY834514	AY834538	AF447505	AY839086	AY834562	AY835947	AY834586	AF447519	AF447536	AY834680	AY834713	AY834612	AF203776	
12 <i>Taphozous nudiventris</i>	AF337543	AY834419	AY834443	AY834467	AY834491	AY834515	AY834539	AF203748	AY839087	AY834563	AY835948	AY834587	AF203756	AF203766	AY834681	AY834714	AY834613	AF447540	
13 <i>Rhynchopteris naso</i>	AY834405	AY834420	AY834444	AY834468	AY834492	AY834516	AY834540	AY834646	AY839088	?????	AY835949	AY834588	?????	AY834662	AY834682	AY834715	AY834614	?????	
14 <i>Tonatia bidens</i>	AF337541							AF203745									U31622		
<i>Tonatia silvicola/saurophila</i>	AY834421	AY834445	AY834469	AY834493	AY834517	AY834541		AY839089	AY834564	AY835950	AY834589				AY834683	AY834716	AY834615	AY834632	
15 <i>Artibeus jamaicensis</i>	AY834406	AY011232	AY011292	AY011355	AY011419	AY011540	AY834646	AY011665	AY011726	AY011789	AY011846	AY834655	AY834663	AY834684	AY834717	AY012020	AY834737	AY012073	
16 <i>Desmodus rotundus</i>	AJ419811	AY834422	AY834446	AY834470	AY834494	AY834518	AY834542	AF447503	AY839090	AY834565	AY835951	AY834590	AF447517	AF447534	AY834685	AY834686	AY834616	AF447550	
17 <i>Annoura geoffroyi</i>	AY834407	AY834423	AY834447	AY834471	AY834495	AY834519	AY834543	AY834648	AY839091	AY834566	AY835952	AY834591	AY834656	AY834664	AY834686	AY834718	AY834617	AY834634	
18 <i>Noctilio albiventris</i>	AJ419812	AY834424	AY834448	AY834472	AY834496	AY834520	AY834544	AF447497	AY839092	AY834567	AY835953	AY834592	AF447509	AF447526	AY834687	AY834719	?????	AF447544	
19 <i>Antrorous pallidus</i>	AJ419813	AY834425	AY834449	AY834473	AY834497	AY834521	AY834545	AF447495	AY839093	AY834568	AY835954	AY834593	AF447509	AF447524	AY834688	AY834720	AY834618	AF447542	
20 <i>Rhogeessa tumida</i>	AJ419814	AY834426	AY834450	AY834474	AY834498	AY834522	AY834546	AF447496	AY839094	AY834569	AY835955	AY834594	AF447508	AF447525	AY834689	AY834721	AY834619	AF447543	
21 <i>Myotis daubentonii</i>	AF337540	AY834427	AY834451	AY834475	AY834499	AY834523	AY834547	AF203746	AY839095	AY834570	AY835956	AY834595	AF203754	AF203764	AY834690	AY834722	AY834620	AF203775	
<i>Myotis velifer</i>																	??????		
22 <i>Myzopoda aurita</i>	AY834408	AY834428	AY834452	AY834476	AY834500	AY834524	AY834548	AY834649	AY839096	AY834571	AY835957	AY834596	?????	AY834665	AY834690	AY834723	?????	AY834637	
23 <i>Pteronotus parnellii</i>	AY245422	AY834429	AY834453	AY834477	AY834501	AY834525	AY834549	AF245828	AY839097	AY834572	AY835958	AY834597	AY245418	AY245416	AY834692	AY834724	AY834621	AY245420	
24 <i>Thyroptera tricolor</i>	??????	AY834430	AY834454	AY834478	AY834502	AY834526	AY834550	AY834650	AY839098	AY834573	AY835959	AY834598	AY834657	AY834666	?????	AY834725	??????	AY834639	
25 <i>Mystacinia tuberculata</i>	AY245423	AY834431	AY834455	AY834479	AY834503	AY834527	AY834551	AY245829	AY839099	AY834574	AY835960	AY834599	AY245417	AY245417	AY834693	AY834726	AY834622	AY245421	
26 <i>Furipterus horrens</i>	AY834409	AY834432	AY834456	AY834480	AY834504	AY834528	AY834552	AY834651	AY839100	AY834575	AY835961	AY834600	AY834658	AY834667	AY834694	AY834727	??????	AY834740	
27 <i>Natalus stramineus</i>	AJ419815	AY834433	AY834453	AY834457	AY834481	AY834505	AY834529	AY834553	AF447498	AY839101	AY834576	AY835962	AY834601	AF447510	AF447527	AY834695	AY834728	AY834623	AF447545
28 <i>Tadarida brasiliensis</i>	AF337542	AY059669	AY059675	AY059679	AY059683	AY059687	AY059691	AF203747	AY059697	AY059700	AY059707	AY059710	AF203755	AF203765	AY834696	AY834729	AY059715	AF061061	
29 <i>Eumops auripendulus</i>	AY834410	AY834434	AY834458	AY834482	AY834506	AY834530	AY834554	AY834652	AY839102	AY834577	AY835963	AY834602	AY834659	AY834668	AY834697	AY834730	AY834624	AY834741	
30 <i>Craseonycteris thonglongyai</i>	AY834411	AY834435	AY834459	AY834483	AY834507	AY834531	AY834555	AY834653	AY839103	AY834578	AY835964	AY834603	AY834660	AY834669	AY834698	AY834731	??????	AY834742	
31 <i>Felis catus</i>	AJ251174	AY011246	AY011306	AY011369	AY011433	AY011496		AF284018	AY011679	AY011738	AY011802	AY011858	AF203761	AF203771	AY834699	AY834732	AY012029	AF061062	
<i>Panthera onca</i>																	AY012085		
32 <i>Tragelaphus eurycerus</i>		AY011240	AY011300	AY011363	AY011427	AY011490	AY011547		AY011673	AY011732	AY011796	AY011853			AY834700	AY834733	AY012026	AY012079	
<i>Bos taurus</i>	Y15944								AY077732						AF447520	AF447537			
33 <i>Condylostylus cristata</i>		AY011199	AY011260	AY011321	AY011385	AY011449	AY011508		AY011630	AY011693	AY01175	?????			AY834701	AY834734	AY011989	AY012041	
<i>Talpa europaea</i>	Y12520														AF447515	AF447532			
34 <i>Ceratotherium simum</i>		AY011244	AY011304	AY011367	AY011431	AY011494	AY011551	AF284007	AY057830	AY011677	AY011736	AY011800	AY011856		AY834702	AY834735	AY012028	AY011431	
<i>Equus caballus</i>	Y15945														AF447516	AF447533		AY012083	
<i>Equus asinus</i>																	U31610		

xxxxx=new sequence; ??????=missing

**Table S7.** Geographic characters and states used in the geographic ancestral reconstructions.

Characters Taxa	1	2
Pteropodidae	L	2&3&4&5
Rhinopomatidae	L	2&3
Megadermatidae	L	2&3&5
Rhinolophidae	L	1&2&3&4&5
Craseonycteridae	L	3
Emballonuridae	L	2&3&4&5&8
Nycteridae	L	2&3&4
Myzopodidae	G	2&4
Mystacinidae	G	5&6
Furipteridae	G	8
Thyropteridae	G	8
Noctilionidae	G	8&9
Mormoopidae	G	8&9
Phyllostomidae	G	8&9
Natalidae	G&L	8&9
Vespertilionidae	L	1&2&3&4&5&6&7&8&9
Molossidae	G&L	1&2&3&4&5&7&8&9
<i>Icaronycteris</i>	L	7
<i>Archaeonycteris</i>	L	1
<i>Hassianycteris</i>	L	1
<i>Paleochiropteryx</i>	L	1
Carnivora	L	1&2&3&4&5&7&8&9
Cetartiodactyla	L	1&2&3&4&5&6&7&8&9
Eulipotyphla	L	1&2&3&4&7&8
Perissodactyla	L	1&2&3&7&8
Pholidota	L	2&3&7&8

L, Laurasia; G, Gondwana; 1, Europe; 2, Africa; 3, Asia; 4, Madagascar; 5, Australian region; 6, New Zealand; 7, North America; 8, Central + South America; 9, West Indies

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