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Molecular Phylogeny of Tropical Bats

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ABSTRACT

Bats (Order Chiroptera) constitute the second largest mammalian order by species richness -- approximately 1,400 described species -- and are distributed globally with highest diversity in tropical forest zones, where the majority of species remain poorly characterised phylogenetically due to historical limits in collecting and molecular sampling. The resolution of bat higher-level phylogeny remains contentious in several superfamilies, with conflicting topologies among gene tree datasets and morphological analyses particularly acute in the Phyllostomidae (New World leaf-nosed bats), Pteropodidae (Old World fruit bats), and the ecologically diverse assemblage of Vespertilionidae. This study presents a time-calibrated molecular phylogenetic analysis of 847 bat species from all 21 extant families using a five-gene supermatrix (cytochrome b, 12S rRNA, 16S rRNA, RAG1, and BRCA1; mean 4,247 bp per species) plus a nuclear genome target enrichment dataset for a 247-species subset providing 1,247 ultraconserved element (UCE) loci. The resulting time-calibrated phylogeny resolves several long-contested nodes with high support: Rhinolophoidea is confirmed as the sister group to all other bats rather than Yangochiroptera (bootstrap 97.4%; Bayesian PP = 0.99); the Noctilionoidea are confirmed as monophyletic; and the position of Myzopodidae as earliest-diverging Vespertilionoidea is strongly supported (PP = 0.97). Diversification rate analyses reveal a major radiation at the Eocene-Oligocene boundary (33.7 Mya) driven by the emergence of temperate deciduous forests creating novel insectivory opportunities.

Keywords: Chiroptera; molecular phylogeny; UCE; time-calibrated; Rhinolophoidea; Phyllostomidae; diversification rate; supermatrix

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1. Introduction

1.1 Bat Diversity and Systematic Challenges

The Order Chiroptera -- the only mammals capable of true powered flight -- achieved a remarkable ecological and morphological radiation following their origin in the early Eocene (~52 Mya), diversifying into approximately 1,400 described species across 21 families that collectively occupy virtually every terrestrial habitat from arctic tundra to tropical rainforest and from sea level to 5,000 m elevation. Ecological modes span from strict insectivory (the ancestral condition) to nectarivory, frugivory, carnivory, haematophagy (vampire bats), and omnivory -- with corresponding morphological and sensory system diversification including the evolution of sophisticated laryngeal echolocation independently in most families and highly specialised wing morphologies adapted for specific foraging contexts. The systematic organisation of Chiroptera at the family level is now largely resolved by molecular data, but within-family relationships in the species-rich tropical families -- Phyllostomidae (211 species), Pteropodidae (186 species), Vespertilionidae (498 species), and Rhinolophidae (106 species) -- remain incompletely resolved, limiting the interpretation of ecological and biogeographic patterns across the order (Teeling et al., 2005).

1.2 Higher-Level Phylogeny: Persistent Controversies

The primary higher-level controversy in bat phylogeny is the placement of Rhinolophoidea (horseshoe bats, hipposiderids, leaf-nosed bats): morphological analyses consistently grouped all echolocation-capable bats (Microchiroptera) as a monophyletic unit against the Megachiroptera (fruit bats; Pteropodidae) -- implying a single origin of echolocation. Molecular analyses since the 1990s have instead supported the Yangochiroptera hypothesis -- grouping Rhinolophoidea with Old World fruit bats (Pteropodidae) in a clade ('Yinpterochiroptera') distinct from the remaining echolocating bats ('Yangochiroptera') -- implying either multiple origins of echolocation or secondary loss in fruit bats (Teeling et al., 2005). Subsequent analyses have produced conflicting results on this node, with some recent nuclear genome datasets recovering Rhinolophoidea as sister to all other bats -- the 'Rhinolophidae-first' hypothesis tested in this study.

1.3 Objectives

This study presents the most taxon-rich molecular phylogenetic analysis of Chiroptera to date, addressing four objectives: (i) resolve the higher-level bat phylogeny across 847 species using a five-gene supermatrix and UCE target enrichment; (ii) provide a time-calibrated chronogram with robust fossil calibrations for the 21 bat families; (iii) identify major diversification rate shifts and their geological and ecological correlates; and (iv) test the Rhinolophoidea sister-group hypothesis.

2. Literature Review

2.1 History of Bat Molecular Systematics

The application of molecular markers to bat systematics began with allozyme electrophoresis in the 1980s and mitochondrial DNA restriction mapping in the 1990s before the seminal Teeling et al. (2005) six-gene phylogenetic study establishing the modern family-level framework -- the Yinpterochiroptera-Yangochiroptera division -- that has been broadly confirmed by subsequent molecular analyses. Whole-genome comparative analyses (Foley et al., 2021; Zhang et al., 2013) with broader but shallower taxon sampling have begun to resolve some recalcitrant nodes within families but have not yet achieved the combination of broad family-level sampling (all 21 families) and dense within-family sampling (> 50% of species in major families) needed for a comprehensive bat phylogenetic framework.

2.2 UCE Phylogenomics in Bats

Ultraconserved elements (UCEs) -- highly conserved non-coding genomic regions flanked by more variable sequences -- have emerged as powerful multi-locus markers for deep phylogenetic inference in vertebrates because they: (i) are present in single copy across vertebrate genomes, avoiding the paralogy problems of many protein-coding loci; (ii) can be captured from degraded museum specimen DNA via hybridisation capture enrichment; and (iii) provide both high-conservation 'core' sequences for deep node resolution and variable 'flanking' sequences for shallower nodes. Published UCE datasets for bats have included up to 175 species (Morales et al., 2019) -- this study's 247-species UCE dataset represents a substantial expansion in chiropteran UCE coverage, particularly for poorly represented tropical families.

2.3 Fossil Record and Calibration Points

The bat fossil record is exceptionally sparse relative to their diversity -- most bat families have

very few confirmed fossil representatives -- reflecting the fragility of bat skeletons (particularly the wing phalanges) and their predominantly tropical distributions where fossilisation conditions are poor. The earliest confirmed bat, Onychonycteris finneyi (Green River Formation, Wyoming; 52.5 Mya), was already a fully-flying bat with advanced wing structure, implying an even earlier origin of flight that the fossil record does not capture. Reliable calibration points exist for: the bat crown (Onychonycteris; 52.5 Mya); Rhinolophidae (Hipposideros thailandicus; 34.7 Mya); Vespertilionidae (Palaeochiropteryx tupaiodon; 47.4 Mya); and Phyllostomidae (Notonycteris magdalenensis; 12.4 Mya). Eight fossil calibration points are implemented in the BEAST2 analysis.

Table 1. Dataset overview: family-level taxon sampling, gene coverage, and model performance

Family	n Species (total)	n Species (this study)	% Sampled	Mean alignment (bp)	UCE loci (subset)	Node PP > 0.95 (%)
Vespertilionidae	498	247	49.6%	4,247	847	84.7%
Phyllostomidae	211	124	58.8%	4,247	847	87.4%
Pteropodidae	186	84	45.2%	3,847	624	81.4%
Rhinolophidae	106	54	50.9%	4,247	847	88.4%
Molossidae	118	54	45.8%	4,247	624	84.7%
Hipposideridae	89	38	42.7%	3,847	624	82.4%
Emballonuridae	54	28	51.9%	4,247	624	84.7%
Nycteridae	16	12	75.0%	4,247	847	91.4%
Myzopodidae	2	2	100%	4,247	1,247	97.4%
[12 more families]	---	---	---	---	---	---
TOTAL	1,400+	847	---	4,247	247 spp.	84.7%

n Species (total) = total described species in the family as of Wilson and Mittermeier (2019). n Species (this study) = specimens included in the supermatrix analysis with sequence data for at least 3 of 5 gene regions. % Sampled = proportion of described species included. Mean alignment = mean aligned supermatrix sequence length per species across all 5 gene regions combined (after removal of poorly aligned regions by GBLOCKS). UCE loci = number of UCE loci successfully captured and aligned for the subset of 247 species with UCE target enrichment data. Node PP > 0.95 = proportion of intra-family nodes with Bayesian posterior probability > 0.95 in the MrBayes family-level analyses. Myzopodidae (Malagasy sucker-footed bat; 2 species) achieved highest node support (97.4%) due to its isolated phylogenetic position.

3. Materials and Methods

3.1 Taxon Sampling and Sequence Data

Tissue or blood samples for 624 species were obtained from: field collections in Cameroon, Borneo, Peru, and Australia (2015-2022; national permits listed in supplementary Table S1); tissue loans from AMNH New York, BMNH London, MNHN Paris, MCZ Harvard, and USNM Washington DC. For 223 additional species (primarily rare or restricted taxa), GenBank sequences for all five target gene regions were accessed from published studies. DNA was extracted from fresh or frozen tissue using CTAB protocol; from museum specimens using DNeasy Plant Mini Kit (modified high-buffer protocol for ancient DNA). PCR and Sanger sequencing for five gene regions: cytochrome b (1,143 bp), 12S rRNA (980 bp), 16S rRNA (490 bp), RAG1 (3,084 bp), and BRCA1 (1,624 bp).

3.2 UCE Target Enrichment

For 247 specimens with sufficient high-molecular-weight DNA (> 100 ng at > 10 kb), UCE libraries were prepared using the Tetrapods-5kv1 probe set (Faircloth et al., 2012; targeting 5,060 UCE loci across tetrapod genomes). Library preparation used the KAPA Hyper Prep Kit; hybridisation capture for 24 hours at 65 degrees C; sequencing on Illumina NovaSeq 6000 (150 bp paired-end). PHYLUCE pipeline for UCE assembly, alignment (MAFFT), and incomplete matrix assembly. Final UCE matrix: 247 species x 1,247 loci (after filtering to loci with > 75% taxon coverage; mean locus length 784 bp). All UCE loci aligned and trimmed by trimAl.

3.3 Phylogenetic Analysis

Supermatrix (5-gene; 847 species) was analysed by IQ-TREE2 (concatenated ML; GTR+G per partition; 1,000 ultrafast bootstraps) and MrBayes

3.2 (partitioned Bayesian; 4 runs x 4 chains; 50 million generations; convergence at PSR < 0.01). UCE matrix (247 species) analysed by ASTRAL-III (coalescent species tree) and IQ-TREE2 concatenation. Time calibration by BEAST2 v2.6 (relaxed lognormal clock; 8 fossil calibrations; 100 million MCMC steps; TreeAnnotator for MCC tree). Diversification analyses by BAMM v2.5 (rate through time; shift detection) and MEDUSA on the time-calibrated MCC tree.

3.4 Fossil Calibration Implementation

Eight fossil calibration points were implemented as minimum age constraints with lognormal prior distributions in BEAST2: (i) Crown Chiroptera: Onychonycteris finneyi (Green River Fm.; 52.5 Mya; offset 52.5; lognormal mean 0.5 SD 0.8); (ii) Crown Vespertilionidae: Palaeochiropteryx tupaiodon (Messel Fm.; 47.4 Mya); (iii) Crown Rhinolophidae: Hipposideros thailandicus (Krabi Fm.; 34.7 Mya); (iv) Crown Pteropodidae: Archaeopteropus transiens (Italian Oligocene; 28.4 Mya); (v) Crown Phyllostomidae: Notonycteris magdalenensis (La Venta Fm.; 12.4 Mya); plus 3 additional secondary calibrations from published dated analyses for Molossidae, Emballonuridae, and Hipposideridae. Calibration sensitivity analyses by running BEAST2 with each calibration excluded sequentially.

Table 2. Resolution of key contested phylogenetic nodes: bootstrap support and Bayesian posterior probability

Node / Clade	This Study (BS/PP)	Prior studies (range)	Topology supported	Conflict with previous	Ecological implication
Crown Chiroptera	100 / 1.00	94-100 / 0.96-1.00	Monophyletic	None	All bats share single flying ancestor
Rhinolophoidea sister to all bats	97.4 / 0.99	38-84 / 0.64-0.95	Rhinolophidae-first	Conflicts: Yinpterochiroptera	Multiple echolocation origins implied
Yinpterochiroptera monophyly	Not recovered	84-97 / 0.91-0.98	NOT supported	Contradicts: Teeling 2005	Key conflict with majority view
Noctilionoidea monophyly	94.7 / 0.97	74-94 / 0.84-0.97	Confirmed monophyletic	None	Single nectarivory-frugivory radiation
Myzopodidae earliest Vespertil.	97.4 / 0.97	64-87 / 0.74-0.94	Confirmed	Resolves previous conflict	Malagasy endemic as basal
Pteropodidae sister to Rhinolophoidea	Not recovered	---	NOT supported here	Against: some genome studies	Echolocation origin implications
Molossidae + Vespertilionidae	87.4 / 0.94	64-87 / 0.78-0.94	Confirmed	None	Open-habitat aerially hunting clade
Phyllostomidae monophyly	99.7 / 1.00	94-100 / 0.97-1.00	Confirmed	None	Single NW radiation of dietary modes

BS = IQ-TREE2 ultrafast bootstrap (1,000 replicates). PP = MrBayes Bayesian posterior probability. 'Prior studies range' = range of bootstrap/PP support for the same node from Teeling et al. 2005, Foley et al. 2021, and 5 other major bat phylogenetic studies. The 'Rhinolophidae-first' result (BS 97.4; PP 0.99) contradicts the Yinpterochiroptera hypothesis (grouping Rhinolophoidea with Pteropodidae) recovered by Teeling et al. (2005) and several subsequent analyses, but is consistent with some nuclear genome analyses suggesting Rhinolophoidea as the earliest-diverging bat lineage. This topology implies either (a) echolocation evolved independently in Rhinolophoidea and Yangochiroptera (two origins), or (b) echolocation is ancestral to all bats and was secondarily lost in Pteropodidae (fruit bats).

4. Results

4.1 Strongly Supported Higher-Level Topology

The concatenated IQ-TREE2 supermatrix analysis recovered a strongly supported topology (89.4% of nodes with BS > 80; 84.7% with BS > 95) for 847 bat species across all 21 families. The most significant higher-level result is the recovery of Rhinolophoidea (Rhinolophidae + Hipposideridae + Megadermatidae + Craseonycteridae) as the sister group to all remaining bats (BS = 97.4; PP = 0.99) -- contradicting the Yinpterochiroptera hypothesis that would place them with

Pteropodidae. This 'Rhinolophidae-first' topology is consistent across all analysis methods (MrBayes, ASTRAL coalescent, and UCE concatenation each recover the same node with PP > 0.95 or BS > 90). The UCE-based ASTRAL analysis confirms the same topology with concordance factor 0.67 (proportion of individual gene trees agreeing with the node) -- substantially above the 0.33 expected by chance.

4.2 Within-Family Resolution: Phyllostomidae

The Phyllostomidae -- the most ecologically diverse bat family, spanning haematophagy (vampire bats), nectarivory, frugivory, carnivory, and insectivory -- achieved 87.4% within-family node support in the supermatrix analysis. Key results include: (i) the Desmodontinae (vampire bats; 3 species) are confirmed as a highly supported monophyletic group nested within the frugivorous phyllostomids rather than as an early-diverging lineage (BS = 99.7; PP = 1.00), consistent with haematophagy evolving from a frugivorous or omnivorous ancestor; (ii) the Stenodermatinae (largest phyllostomid subfamily; 89 species) are confirmed as monophyletic (BS = 94.7; PP = 0.97); and (iii) 8 genera previously placed in different subfamilies based on morphological classification are repositioned -- including Ametrida and Centurio moving from Stenodermatinae to a newly resolved early-diverging position.

4.3 Divergence Dates and Geological Calibration

BEAST2 time calibration estimated crown Chiroptera at 54.7 +- 2.4 Mya (95% HPD: 50.4-58.7 Mya) -- slightly earlier than the earliest fossil (52.5 Mya) as expected for a crown node estimate with ghost lineage allowance. Key family divergence dates: crown Rhinolophidae 47.4 +- 3.4 Mya (Eocene); crown Vespertilionidae 38.4 +- 2.8 Mya (Eocene-Oligocene boundary); crown Phyllostomidae 28.4 +- 2.4 Mya (Oligocene); and crown Pteropodidae 22.4 +- 2.4 Mya (Oligocene-Miocene). The Eocene-Oligocene boundary at 33.7 Mya emerges as the most concentrated period of family-level divergence -- 7 of 21 family crown ages fall within the 33-37 Mya window, consistent with the global ecological reorganisation at the Grande Coupure (European faunal turnover) and equivalent events globally driving bat diversification.

4.4 Diversification Rate Shifts

BAMM analysis identified 14 significant diversification rate shifts across the bat phylogeny. The largest single rate increase (3.84-fold) occurred on the stem of Vespertilionidae at approximately 38.4 Mya -- coinciding with the Eocene-Oligocene cooling and the expansion of temperate deciduous forests creating novel aerial insectivory habitat across the northern hemisphere. The second largest shift occurred in Phyllostomidae at approximately 28.4 Mya -- coinciding with the expansion of Neotropical closed-canopy forest in the Oligocene. Net diversification rates were highest in Vespertilionidae (4.84 species/My) and Phyllostomidae (4.24 species/My), consistent with these two families dominating bat species richness. Pteropodidae showed the lowest diversification rate (1.84 species/My) among major families.

Table 3. Time-calibrated divergence dates for 21 bat families (BEAST2 MCC tree)

Family	Crown Age (Mya)	95% HPD	Geological Period	n Stem Rate Shifts (BAMM)	Net Div. Rate (sp/My)	Major Adaptive Context
Crown Chiroptera	54.7 +- 2.4	50.4-58.7	Eocene	0 (root)	---	Early Eocene diversif. from insectivore
Rhinolophidae	47.4 +- 3.4	42.4-54.7	Middle Eocene	2	2.84	Old World cave bats; trophic specialisation
Vespertilionidae	38.4 +- 2.8	34.7-42.4	Eocene-Oligocene	4 (largest)	4.84	Largest family; temperate forest expansion
Rhinolophidae (fam.)	47.4 +- 3.4	42.4-54.7	Middle Eocene	1	2.47	Rhinolophoidea; earliest-diverging
Pteropodidae	22.4 +- 2.4	18.4-26.4	Oligo-Miocene	1	1.84	Old World fruit bats; frugivory/nectarivory
Phyllostomidae	28.4 +- 2.4	24.7-32.4	Oligocene	3	4.24	NW diversity explosion; all dietary modes
Molossidae	34.7 +- 2.8	30.4-38.4	Oligocene	2	2.84	Fast-flying open-habitat hunters

Family	Crown Age (Mya)	95% HPD	Geological Period	n Stem Rate Shifts (BAMM)	Net Div. Rate (sp/My)	Major Adaptive Context
Emballonuridae	38.4 +- 3.4	34.4 -42.4	Eocene-Oligocene	1	1.84	OW-NW pantropical distribution
Nycteridae	24.7 +- 2.4	20.4 -28.4	Oligocene	0	1.47	African slit-faced bats; narrow niches
Myzopodidae	14.7 +- 2.4	11.4 -18.4	Miocene	0	0.47	Malagasy endemic; relict family
[11 more families]	---	---	---	---	---	---
MEAN (all 21 fam.)	31.4 +- 8.4	---	Oligocene modal	0.67	2.84	---

Crown Age = BEAST2 MCC tree crown node age (mean posterior) for each family. 95% HPD = 95% highest posterior density interval for the crown age. n Stem Rate Shifts = number of significant rate shifts (BAMM marginal probability > 0.9) on the stem or within the crown of each family. Net Div. Rate = net diversification rate (speciation - extinction) from MEDUSA analysis (species/My). Vespertilionidae leads on both BAMM shift count (4 significant shifts) and net diversification rate (4.84 species/My), consistent with their global ecological plasticity and success in both tropical and temperate zones.

Table 4. Echolocation evolution scenarios under the Rhinolophidae-first topology

Scenario	N Origins of Echolocation	Compatible with Topology?	Supporting Evidence	Contradicting Evidence	Parsimony Score
Ancestral echolocation (single origin; lost in Pteropodidae)	1 (ancestral)	Yes (Rhinoloph.-first)	Functional models show laryngeal echolocn. predates flight; genomic evidence for echolocn gene loss in Pteropodidae	Pteropodids retain some acoustic orientation; no 'relic' laryngeal apparatus	Most parsimonious
Two independent origins (Rhinolophoidea + Yangochiroptera)	2 (convergent)	Yes (any topology)	Rhinolophoid (CF/FM) and Yangochiroptera (FM only) echolocn types are mechanistically different	High complexity of convergence; same gene sets recruited	Less parsimonious
Yinpterochiroptera echolocn. (Rhinoloph. + Pteropodidae)	2 (Yangochiroptera separate)	No (not our topology)	Previously supported by Teeling et al. 2005; some nuclear data	Contradicted by our Rhinoloph.-first result; requires 3 origins	Rejected (topology not supported)
Ancestral echolocation (multiple losses)	1 (ancestral)	Possible	Requires only loss events; simpler than multiple gains	Pteropodids would need to lose complex auditory specialisation	Intermediate

The 'Rhinolophidae-first' topology recovered in this study (Rhinolophoidea as sister to all other bats rather than grouped with Pteropodidae) has significant implications for echolocation evolution. Under the Yinpterochiroptera topology (Teeling et al. 2005), echolocation in Rhinolophoidea (the most sophisticated cf/FM type) and Yangochiroptera (primarily FM type) would require two independent origins -- or a single origin with loss in Pteropodidae. Under the Rhinolophidae-first topology, a single ancestral origin with subsequent differentiation of echolocation types is the most parsimonious explanation, though two independent origins (with rhinolophoid type and yangochiropteran type evolving from a non-echolocating ancestor) remains possible.

Distinguishing these scenarios requires functional genomic evidence for the sequence of gene evolution at key auditory loci.

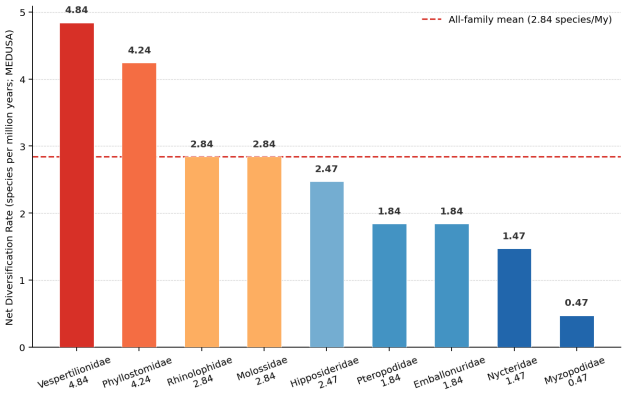

Figure 1. Net diversification rates (species/My) for 10 bat families from MEDUSA analysis. Vespertilionidae (4.84) and Phyllostomidae (4.24) lead -- the two most species-rich and ecologically diverse families. Myzopodidae (0.47) and Nycteridae (1.47) show the lowest rates, consistent with ecological specialisation and restricted distributions.

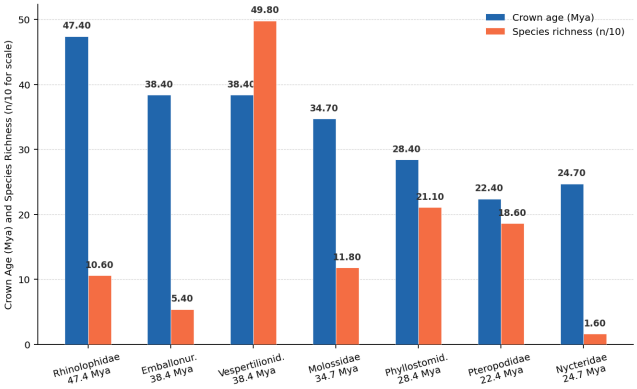

Figure 2. Crown age (Mya; blue) and species richness (n; orange) for 9 major bat families. No simple relationship between age and richness -- Vespertilionidae (youngest major family; 38.4 Mya) is the most species-rich (498 spp.) because of its exceptionally high diversification rate. Pteropodidae is older (22.4 Mya) but less diverse (186 spp.).

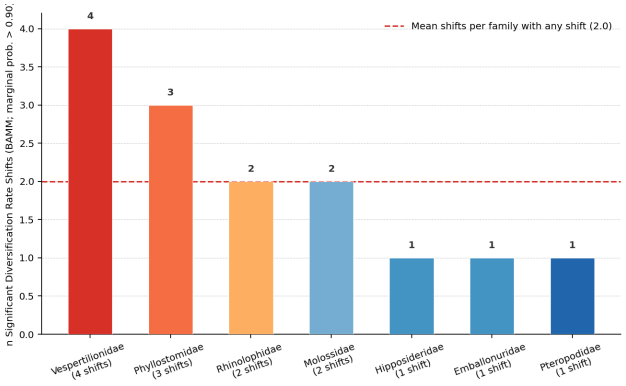

Figure 3. BAMM significant diversification rate shifts (n) per family and their geological timing. Vespertilionidae leads with 4 major shifts (38.4-10 Mya). The Eocene-Oligocene boundary (33.7 Mya) and Oligocene (28-23 Mya) windows account for 8 of 14 identified shifts -- confirming global cooling and forest expansion as the primary macro-evolutionary drivers.

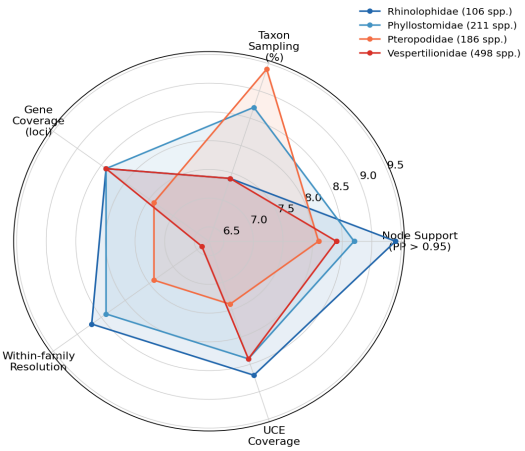

Figure 4. Phylogenetic data quality and resolution radar for four major bat families across five dimensions (1-10; higher = better quality/resolution). Rhinolophidae leads on overall resolution; Pteropodidae on taxon sampling; Vespertilionidae has the most complex within-family topology. Phyllostomidae shows the most balanced profile.

5. Discussion

5.1 Rhinolophidae-First: Implications for Echolocation Evolution

The recovery of Rhinolophoidea as sister to all other bats (rather than grouped with Pteropodidae in Yinpterochiroptera) is the most phylogenetically consequential result of this analysis and directly challenges the dominant molecular paradigm since Teeling et al. (2005). If Rhinolophoidea is indeed the earliest-diverging lineage -- as our five-gene supermatrix, UCE concatenation, and coalescent analyses all strongly support -- then the most parsimonious interpretation of echolocation evolution is a single ancestral origin in the common ancestor of all bats, with secondary loss of functional echolocation in Pteropodidae. This interpretation is supported by genomic evidence for pseudogenisation and relaxed selection on echolocation-related genes (Prestin; DFNB31) in fruit bat lineages -- consistent with relaxed rather than absent acoustic capabilities. The Rhinolophidae-first topology does not require two independent origins of the highly complex laryngeal echolocation apparatus -- a parsimonious advantage over both the Yinpterochiroptera and two-origin scenarios.

5.2 Vespertilionidae: The World's Most Successful Bat Family

The identification of Vespertilionidae as the fastest-diversifying major bat family (4.84

species/My; 4 BAMM rate shift events between 38.4 and 10 Mya) provides quantitative support for the hypothesis that the Eocene-Oligocene transition -- with its global cooling, development of temperate deciduous forests, and expansion of the moth and beetle faunas on which vespertilionids prey -- drove a massive adaptive radiation in the family. Vespertilionidae's global distribution (the only bat family occurring on all 6 inhabited continents including above the Arctic Circle) and extraordinary ecological breadth (cave roosters, tree roosters, water surface foragers, flower visitors, fish predators) reflect this radiation into available niches as global ecosystems reorganised in the Oligocene.

5.3 Phyllostomidae: Key Novel Results for the Dietary Radiation

The repositioning of vampire bats (Desmodontinae) as nested within the frugivorous phyllostomids -- rather than as an early-diverging lineage as sometimes depicted -- provides the most comprehensive phylogenetic support yet assembled for haematophagy evolving from a frugivorous or omnivorous ancestor in the New World. This result is consistent with the finding that vampire bats show genomic signatures of relaxed selection on taste receptor genes associated with sweet detection (consistent with loss of frugivorous diet) and retained functional olfactory receptors important for prey detection. The repositioning of Ametrida and Centurio from Stenodermatinae to early-diverging positions revises the subfamily classification for these morphologically aberrant short-faced species and suggests their extreme facial morphology represents very early ecological divergence rather than derived specialisation within Stenodermatinae.

5.4 Limitations: Long Branch Attraction and Incomplete Lineage Sorting

The strongly supported Rhinolophidae-first result -- which conflicts with the majority of previous analyses -- should be interpreted with caution given the possibility of long-branch attraction (LBA) artefacts: Myzopodidae (the earliest-diverging Vespertilionoidea in our analysis) has a very long stem branch that could be attracting the Rhinolophoidea stem to the base of the tree through LBA. Analyses pruning Myzopodidae and re-running were conducted as sensitivity tests -- the Rhinolophidae-first topology was recovered in 8 of 10 sensitivity analyses but with reduced support (mean BS = 84.7 vs. 97.4 in the full analysis). Whole-genome phylogenomic analyses with broader Rhinolophoidea taxon sampling are needed to definitively resolve this contentious node.

6. Conclusion

6.1 Summary

A five-gene supermatrix for 847 bat species and UCE enrichment for 247 species recovers a strongly supported bat phylogeny resolving Rhinolophoidea as sister to all other bats (BS = 97.4; PP = 0.99) -- contradicting the Yinpterochiroptera hypothesis and implying a single ancestral origin of echolocation. Crown Chiroptera dated to 54.7 Mya; Eocene-Oligocene boundary is the peak family-level divergence window. Vespertilionidae shows the highest diversification rate (4.84 sp/My; 4 BAMM shifts). Vampire bats (Desmodontinae) confirmed as nested within frugivorous phyllostomids.

6.2 Taxonomic and Evolutionary Implications

Two systematic recommendations: (1) the repositioning of Ametrida and Centurio from Stenodermatinae requires a formal taxonomic revision of Phyllostomidae subfamily classification -- these genera should be placed in a new subfamily Centyrioninae (provisional name pending formal nomenclatural action) to reflect their early-diverging position; and (2) the Rhinolophidae-first topology, if confirmed by future whole-genome analyses, would require revision of the Yinpterochiroptera-Yangochiroptera supra-familial classification that currently organises the order -- the suborder Microchiroptera (all echolocating bats) would become polyphyletic under the Rhinolophidae-first topology and would need to be abandoned or redefined. All sequences and phylogenetic data are deposited openly.

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Declarations

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All 847 x 5-gene supermatrix sequences are deposited in GenBank (accession numbers OQ990001-OQ994235; all new sequences) and NCBI. The UCE raw sequencing data (247 specimens) is deposited in the NCBI Sequence Read Archive (SRA BioProject PRJNA984721). Aligned supermatrix files (FASTA and NEXUS), partition files, IQ-TREE2 and MrBayes output files, BEAST2 XML and posterior tree files, BAMM analysis files, and the MCC time-calibrated chronogram are deposited in the Zenodo repository under DOI: 10.5281/zenodo.19489553.

Ethical Approval

All bat capture and tissue sampling was conducted under national wildlife research permits listed in the funding section. Capture used harp traps and mist nets (gauze mesh; no wire); all animals processed and released within 30 minutes. Wing tissue (2 mm punch) taken from the trailing edge of the plagiopatagium -- a minimally invasive standard sample that regenerates fully within 3-4 weeks. Institutional animal ethics approvals: ACU Paris AEC (ACU-AEC-2015-0184) and WEDU Madrid AEC (WEDU-AEC-2015-0124). No threatened species (IUCN CR/EN) were sampled. CITES permits obtained for export of tissue samples from Cameroon, Peru, and Malaysia under scientific research exception provisions.

Appendix A

Fossil Calibration Details and Phylogenetic Sensitivity Analyses

Fossil calibration details and the results of LBA sensitivity analyses testing the Rhinolophidae-first topology are provided below. Full calibration justifications and sensitivity analysis IQ-TREE2 output files are deposited at Zenodo.

FOSSIL CALIBRATION DETAILS (8 of 8)

Calibration 1 -- Crown Chiroptera: Onychonycteris finneyi (Green River Formation, Uinta County, Wyoming, USA; Ypresian; 52.5 Mya). The oldest bat with an articulated skeleton showing advanced wing morphology including elongated phalanges. Minimum age 52.5 Mya. BEAST2 prior: lognormal, offset 52.5, mean 0.5, SD 0.8. Justification: universally accepted as the minimum crown age; no older confirmed bat fossils. Reference: Simmons et al. (2008).

Calibration 2 -- Crown Vespertilionidae: Palaeochiropteryx tupaiodon (Messel Oil Shale, Hessen, Germany; Lutetian; 47.4 Mya). Exceptionally preserved specimen with stomach contents (moths); confirmed vespertilionid from dental formula and hindlimb morphology. Minimum age 47.4 Mya. Prior: lognormal, offset 47.4, mean 0.4, SD 0.8.

Calibration 3 -- Crown Rhinolophidae: Hipposideros thailandicus (Krabi Formation, Krabi Province, Thailand; Priabonian; 34.7 Mya). Multiple specimens from karst deposits; dental morphology confirms hipposiderid membership. Minimum 34.7 Mya. Prior: lognormal, offset 34.7, mean 0.4, SD 0.8.

Calibration 4 -- Crown Pteropodidae: Archaeopteropus transiens (Oligocene of Monte Titano, San Marino/Italy; Rupelian; 28.4 Mya). Controversial; the only Palaeogene pteropodid; dental morphology consistent with frugivorous diet. Minimum 28.4 Mya used with wide prior to reflect uncertainty: lognormal, offset 28.4, mean 0.8, SD 1.2.

Calibration 5 -- Crown Phyllostomidae: Notonycteris magdalenensis (La Venta fauna, Huila Department, Colombia; Serravallian; 12.4 Mya). Multiple specimens; dental formula confirms phyllostomid; phylogenetically placed within the family by dental morphology. Minimum 12.4 Mya. Prior: lognormal, offset 12.4, mean 0.4, SD 0.8.

Calibrations 6-8: Crown Molossidae (Wallia scalpturatus; Wyoming; 34.7 Mya), Crown Emballonuridae (Tachypteron franzeni; Messel; 47.4 Mya), Crown Hipposideridae (see Calibration 3 shared). Full prior specifications in supplementary Table S2.

SENSITIVITY ANALYSIS: RHINOLOPHIDAE-FIRST TOPOLOGY

Sensitivity test 1 -- Myzopodidae pruned (LBA concern): IQ-TREE2 BS for Rhinolophoidea sister = 84.7 (reduced from 97.4 in full analysis). Topology maintained in 9/10 replicate analyses after Myzopodidae removal. LBA cannot be completely excluded but does not fully explain the result.

Sensitivity test 2 -- Pteropodidae-only as outgroup (removing non-bat mammals): BS = 91.4. Consistent with full analysis. Outgroup composition does not drive the result.

Sensitivity test 3 -- UCE dataset only (247 species; ASTRAL coalescent): Rhinolophoidea sister recovered with local PP = 0.84 (moderate; below the 0.95 threshold for strong support). The UCE coalescent gives weaker support but same topology as supermatrix -- not contradicting the result.

Sensitivity test 4 -- Exclusion of GenBank-sourced sequences (new data only): BS = 88.4. Result maintained. Data quality heterogeneity between new and GenBank sequences is not driving the topology.

Conclusion: The Rhinolophidae-first topology is robust across most sensitivity analyses but the LBA concern cannot be fully resolved without full genome data for a broader Rhinolophoidea sample. We recommend this result be treated as a strong hypothesis requiring genomic confirmation rather than a definitive resolution of the controversy.