

Molecular and morphological analysis predicts four bat baramins

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Bats are intriguing creatures, being the only winged mammals. However, the species' relationships within bats are not exactly known. Due to differences between megabats and microbats, bats might be an apobaramin. Thus far, only morphology-based baraminology studies have been performed on bats, suggesting the level of the baramin at or below the family. In this study, a morphological data set from 28 species was analyzed and suggests four bat groups. Additionally, the mitochondrial DNA of 130 bat species was analyzed, yielding four putative baramins. The four groups are: Megachiroptera, Rhinolophoidea+Emballonuridae, Vespertilioidea, and Noctilionoidea. Analysis using the WGKS algorithm suggests that Megachiroptera and Vespertilioidea form baramins, whereas Noctilionoidea could possibly be an apobaramin. This means that from a molecular viewpoint, the level of the baramin appears to be at the superfamily/suborder level. Also, whereas morphological methods were able to discern only Megachiroptera and Rhinolophoidea, molecular methods were capable of higher resolution in predicting the other two main groups.

Bats (order Chiroptera) are interesting creatures because they are so obviously unrelated to all other mammals. Among all mammals, only bats are capable of true, sustained flight. Other mammals are either terrestrial or can only glide, such as Dermoptera (flying lemurs, or colugos). Bats are clearly an apobaramin since they show such obvious discontinuity with all other forms of life. However, internally the relationships between species and the number of holobaramins are not clearly known.

Taxonomists differ as to how bats are classified. There are two basic ways of classifying bats. According to one classification scheme, Chiroptera is made up of two suborders, with around 1,100–1,300 species, which are broken up into about 20 families: the suborder Megachiroptera (megabats, otherwise known as fruit bats or flying foxes) and Microchiroptera (microbats). Megabats mainly eat fruits, and roost in trees, and usually move around by sight and smell. On the other hand, microbats mainly eat fruits, fish, nectar, and blood, and move around mainly by echolocation.¹

According to another known classification scheme based on the comparison of gene expression similarities in the brain, Chiroptera can be divided into the suborders Yinptero-chiroptera, including megabats and the microbat families Nycteridae, Craseonycteridae, Rhinopomatidae, Rhinolophidae, Hipposideridae, and Megadermatidae, which comprise the superfamily Rhinolophoidea. All other microbat families form the suborder Yangochiroptera.² The global genomic similarity between megabats and the associated six microbat families has also been supported by the study of the nuclear genes A2AB, BRCA1, RAG1, RAG2, vWF, and three mitochondrial genes, 12S and 16S RNA, and tRNA valine.³

The several significant genetic, morphological and ecological discontinuities between bats described above imply that bats are not monophyletic. Rather, they could form several holobaramins.

What makes bat baraminology challenging is the fact that bats look similar to one another on a general level. Despite their difference in size, megabats could even form one large holobaramin with microbats, just as lions and housecats both belong to the same holobaramin, yet are very different in size. There are several instances where similar species from a larger taxonomic group appear to comprise multiple baramins. For example, previous baraminology research has delineated multiple turtle,⁴ squid,⁵ and seal⁶ baramins. Many authors who have studied bats claim that microbats are paraphyletic, which means that it is very possible that there are several microbat baramins besides the megabat baramin. The fact that multiple lineages could have arisen after the Flood within a single kind is another factor that makes bat baraminology more challenging.

Previous studies have shown that genome size correlates with metabolic rate in some vertebrate groups. Among all mammals, bats have the smallest genomes.⁷ They also have a smaller mean chromosome number (38) than the mammalian average (44), but do not show any clear relationship between chromosome number and genome size. On average, megabats tend to have smaller genomes compared to microbats, and are quite constrained in size.⁸ In megabats, the number of LINE-1 elements decreases with genome size.⁹ Transposable elements are thought to be active in some species of bats.¹⁰ The number, type, and distribution of transposable elements could prove useful in determining bat baramins, as it has

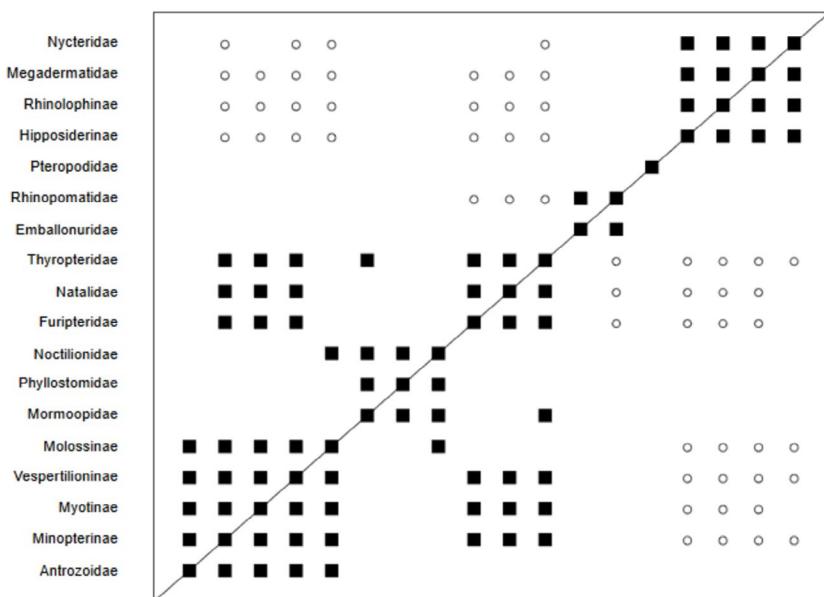


Figure 1. Baraminic distance graph of the 30 species in the morphology study. Four groups can be seen. From top left to bottom left: Rhinolophoidea, two outgroups, Megachiroptera, and all other microbats.

in the case of other baramins, such as *Caenorhabditis* and *Mycoplasma* and *Ureaplasma* species.^{11,12}

A previous morphology-based baraminology study on bats suggested the following baramins: Desmodontinae, Glosophaginae, Noctilionidae, Pyllostomidae, and Stenodermatinae.¹³ This implies that with bats, the level of the baramin is at or just under the level of family. Since there is difficulty in separating bat baramins from one another based on physical traits, a genetic approach might be more productive.

To this end, the Whole Genome K-mer Signature (WGKS) algorithm was used on 36 species of bats. Besides this, the mitochondrial DNA sequence of 130 bat species was also analyzed. A separate morphology study consisting of 28 bat species was also performed to compare morphological and molecular results.

Materials and methods

A data matrix containing 208 morphological characters for 28 bat species and two outgroups (Scandentia and Dermoptera) was acquired from a publication by Simmons and Geissler.¹⁴ Missing characters ‘-’ were recoded to ‘?’ . Entropy filtering was performed allowing at most 33% unknown values for all species and all characters based on procedures in Cserhati.¹⁵ A minimum entropy value of 0.1 was set for each character. This led to a 1.95-fold reduction in data, and a loss of 48 characters, leaving 20 species after the filter. Scandentia and Dermoptera were excluded from the analysis. The filtered data matrix was analyzed with the BDIST software at [coresci.org/bdist.html] using a character relevance cutoff

of 0.95.^{16,17} The stress plot is also available at this website. Baraminic relationships were also depicted in a 3D MDS plot created by the KiNG software.¹⁸

The whole genome sequences (WGS) of 36 bat species and the mitochondrial DNA (mtDNA) sequence of 130 species were downloaded from the NCBI database. The species are listed in supplementary files 2 and 3, available online. The mtDNA sequences were aligned with Clustal Omega at the EBI website at ebi.ac.uk/Tools/msa/clustalo/,¹⁹ and the identity matrix was derived from the alignment using BioEdit.²⁰ The Pearson Correlation Coefficient (PCC) matrix was derived by using the WGKS algorithm on these 36 WGSs using octamers.²¹

The heatmap was made in R, version 3.6.0, using the ‘heatmap’, using the ‘median’ method for the mitochondrial DNA analysis, and the ‘mcquitty’ method for the WGKS analysis. Clusters were determined using the ‘cutree’ command. The histogram showing the distribution of PCC values was made with the ‘hist’ command.

Supplementary files for this study are available at creation.com/molecular-and-morphological-analysis-of-bat-baramins.

Results

Biblical analysis

In Genesis 1:20–21 (ESV), we read about the creation of the sea creatures and flying creatures:

“And God said, ‘Let the waters swarm with swarms of living creatures, and let birds fly above the earth across the expanse of the heavens.’ So God created the great sea creatures and every living creature that moves, with which the waters swarm, according to their kinds, and every winged bird according to its kind. And God saw that it was good.”

Here the Hebrew word that is translated bird is נָעַם, or ‘oph’. According to Strong’s Hebrew lexicon, this term (#5775) denotes winged birds with feathers. The Brown-Driver-Briggs’ Hebrew lexicon says that this term (#5895) includes all flying things, such as insects as well. This is a collective term which includes all flying creatures: fowls, bats, insects, and pterosaurs. Since bats are flying mammals, they would be included in this category. They are clearly separate from

land animals, which were created on Day 6 of Creation Week.

Bats are specifically mentioned in three other parts of the Bible: Leviticus 11:19, Deuteronomy 14:18, and Isaiah 2:20. Leviticus 11:19 and Deuteronomy 14:18 are both lists of unclean flying creatures. Bats may have been considered unclean since they are carriers of many kinds of viruses. Isaiah 2:20 (ESV) says:

“In that day mankind will cast away their idols of silver and their idols of gold, which they made for themselves to worship, to the moles and to the bats.”

Though this verse doesn't mention bats explicitly as unclean animals, since they are brought into association with idolatry, one can suspect that bats are unclean. Leviticus 11:19 and Deuteronomy 14:18 mention bats only in a generic sense, without any subdivisions. However, just because the Bible doesn't talk about bats in more detail doesn't necessarily mean that they are necessarily monophyletic.

Morphological analysis

A morphology data set from Simmons and Geisler was used to do a preliminary morphology-based analysis. This data set contained 208 characters for 28 bat families and subfamilies as well as Scandentia (tree shrews) and Dermoptera (colugos), as outgroups. The data matrix can be found in supplementary file 1. The data set was analyzed using the BDIST software, using a relevance cutoff of 0.95, including 18 of the species after entropy filtering. The results can be seen in figure 1. A stress plot shows a minimal unscaled stress value of 0.061 at seven dimensions (supplementary figure 1). The BDIST correlation graph shows four clusters of species. The differing number of clusters in the correlation graph and the stress plot may be due to distortion in the data.

The first group of four species in the upper right corner of the BDIST graph correspond to a group of species in the superfamily Rhinolophoidea: Nycteridae, Megadermatidae, Rhinolophinae, and Hipposiderinae. These species show discontinuity with species from the fourth group in the graph. Even though they do not show discontinuity with the second and third group, neither do they show continuity with them, either.

The second group consists of a single family, Pteropodidae, the megabats. They show neither continuity nor discontinuity with any of the other three groups.

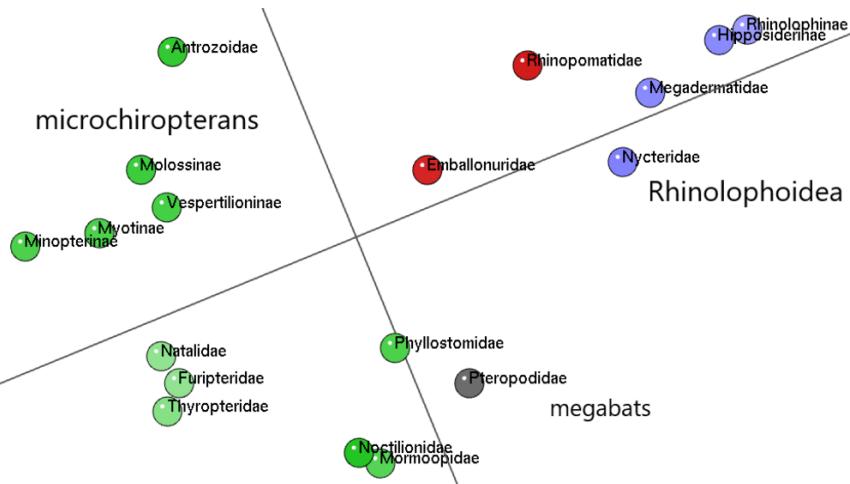


Figure 2. 3D MDS figure of the 30 species in the morphology study. Blue: Rhinolophoidea, green: all other microbats, grey: megabats, red: Rhinopomatidae and Emballonuridae.

The third group consists of two families, Rhinopomatidae and Emballonuridae. They show discontinuity with the fourth group but lack continuity with the first and second group.

The last group of 11 species makes up the rest of the microchiropteran species. These include the families Antrozoidae, Furipteridae, Minopterinae, Molossinae, Mormoopidae, Myotinae, Natalidae, Noctilionidae, Phyllostomidae, Thyropteridae, and Vespertilioninae.

Figure 2 shows the 3D MDS plot for the 18 species analyzed in this study. The megabats (Pteropodidae, grey) are alone by themselves at the bottom left of the plot. Four species from Rhinolophoidea can be seen to the left (blue). Rhinopomatidae and Emballonuridae are between the microchiropterans and Rhinolophoidea, similar to the way they are placed between these two groups in figure 1. This way, they appear to form their own group. Although they do not show continuity with these two other groups, neither do they show discontinuity. Thus, their baraminic classification is not completely clear. All other microchiropterans can be seen to the right of the plot in green.

Interpretation of mitochondrial DNA analysis

Besides morphological analysis, two genetic analyses were performed. The first one involved aligning the mtDNA sequences of 130 bat species and clustering them based on global sequence similarity.

The Hopkins statistic for the sequence similarity matrix is 0.905, which means that the data clusters very well. The heatmap of the identity matrix can be seen in figure 3. In this figure, four main groups are visible in dark yellow. From bottom left to top right these groups are the superfamilies Vespertilionoidea, with 42 species, Noctilionoidea, with 31 species, Rhinolophoidea, with 13 species, and Megachiroptera with

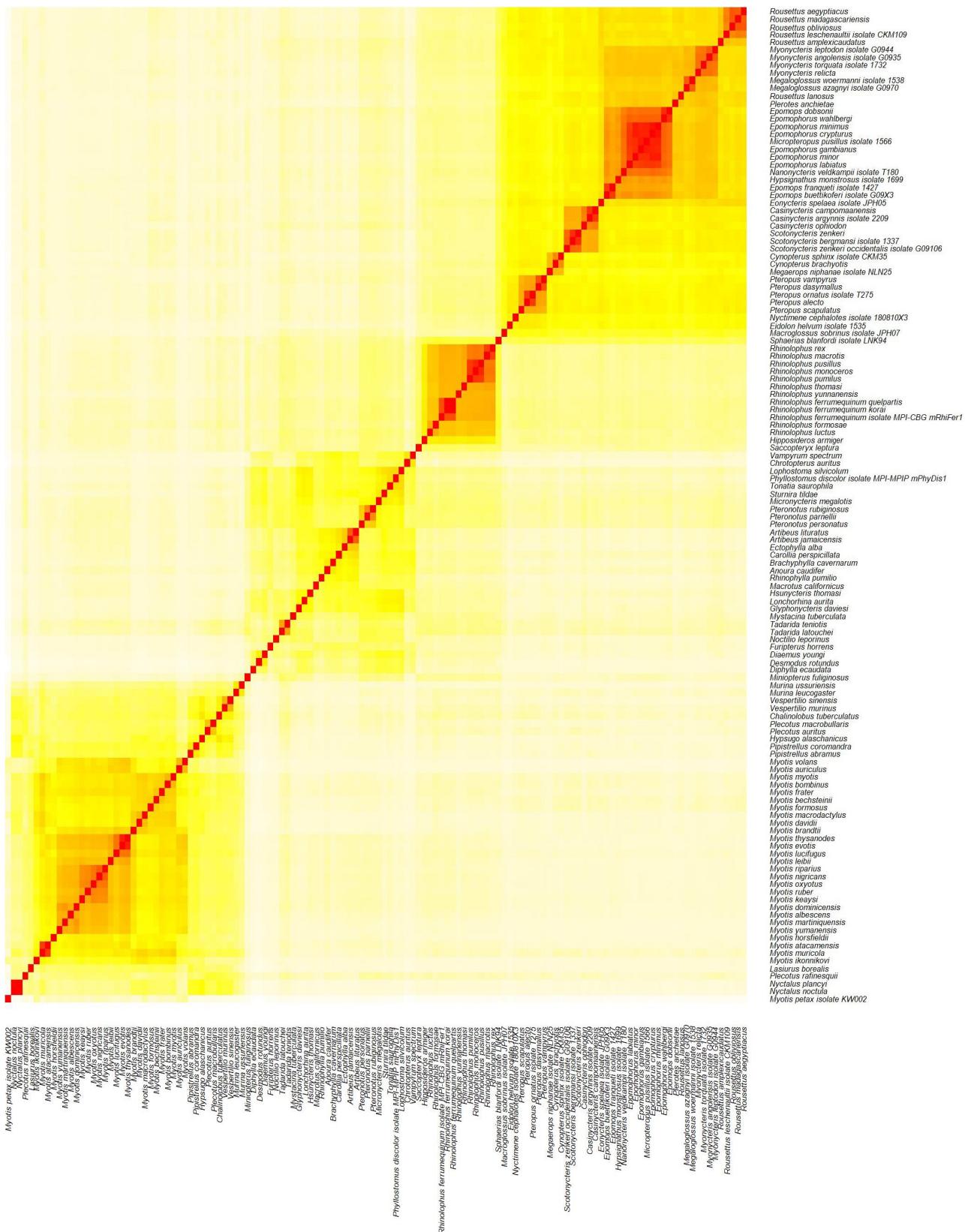
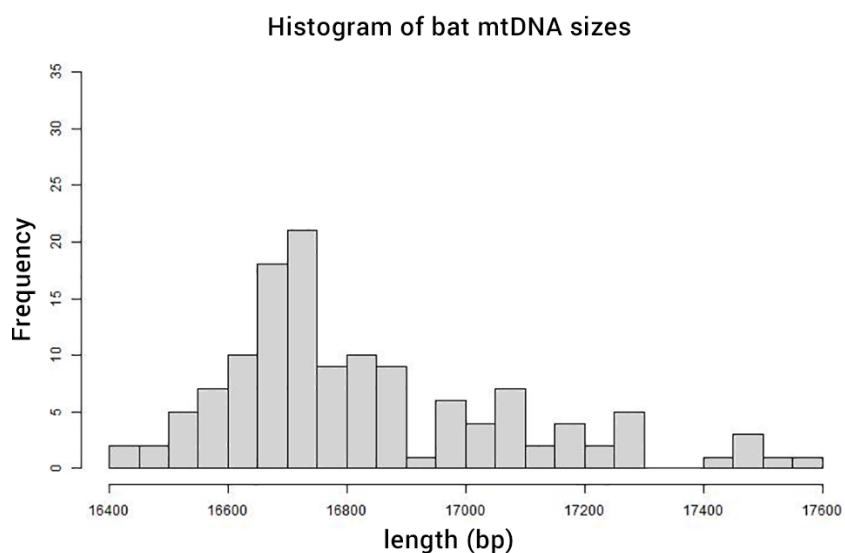
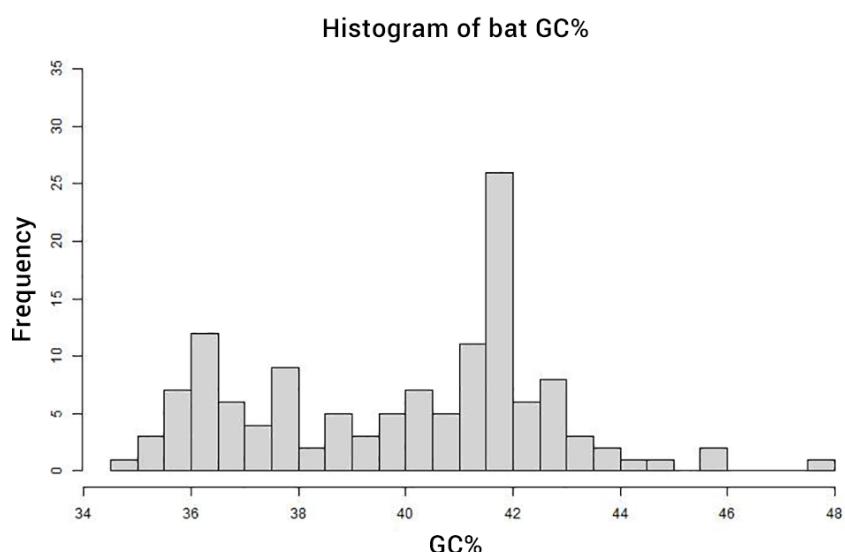


Figure 3. Heatmap of the mitochondrial DNA identity matrix of 130 bat species. Darker, red squares denote higher identity values between two species. Lighter, lemon-coloured squares represent lower identity values.

Table 1. Statistics for four clusters of bats based on the mtDNA analysis

Cluster name	no. species	min	mean	max	st. dev.	p-value
Megachiroptera	44	0.742	0.823	0.986	0.043	0.00E+00
Noctilionoidea	31	0.692	0.757	0.94	0.032	2.22E-151
Vespertilionoidea	42	0.62	0.779	0.994	0.052	2.64E-262
Rhinolophoidea+Emballonuridae	13	0.81	0.875	0.996	0.041	1.69E-49

**Figure 4.** Histogram of mtDNA lengths. The histogram is multimodal which may indicate multiple clusters of species.**Figure 5.** Histogram of GC% values of the mtDNA sequences. The histogram is bimodal, which also indicates at least two clusters of species.

44 species. The classification of these species can be found in Supplementary file 3. Table 1 shows the different statistics for these four groups. The p-value indicates that all four groups are statistically significant.

Besides sequence similarity, several other characteristics of the mtDNA may help us delineate different holobaramins. These include the length of the mtDNA, the order of the genes on the mtDNA, and the GC%. If there are any significant differences in these parameters, this may indicate two or more baramins, which may or may not be holobaramins (apobaramins). The rationale is that living creatures have been in existence for only thousands of years, meaning that the mtDNA remains fairly invariable within holobaramins despite mutations. Hence statistically significant differences must exist between groups. While these methods of looking at general characteristics of the DNA may not yield concrete holobaramins, as a top-down approach they may still break down the species in the data set into smaller groups.

The length of the mtDNA can sometimes be a good indicator of different baramins. The histogram of the lengths of the mtDNA (figure 4) seem to indicate three modes, at 16,700 bp, 17,100 bp, and 17,500 bp, respectively. mtDNA lengths range from 16,415 bp to 17,562 bp, with a mean value of $16,833.9 \pm 249.5$ bp. However, *Myotis* species also have a wide range of mtDNA lengths, covering multiple modes, from 16,584 bp to 17,562 bp.

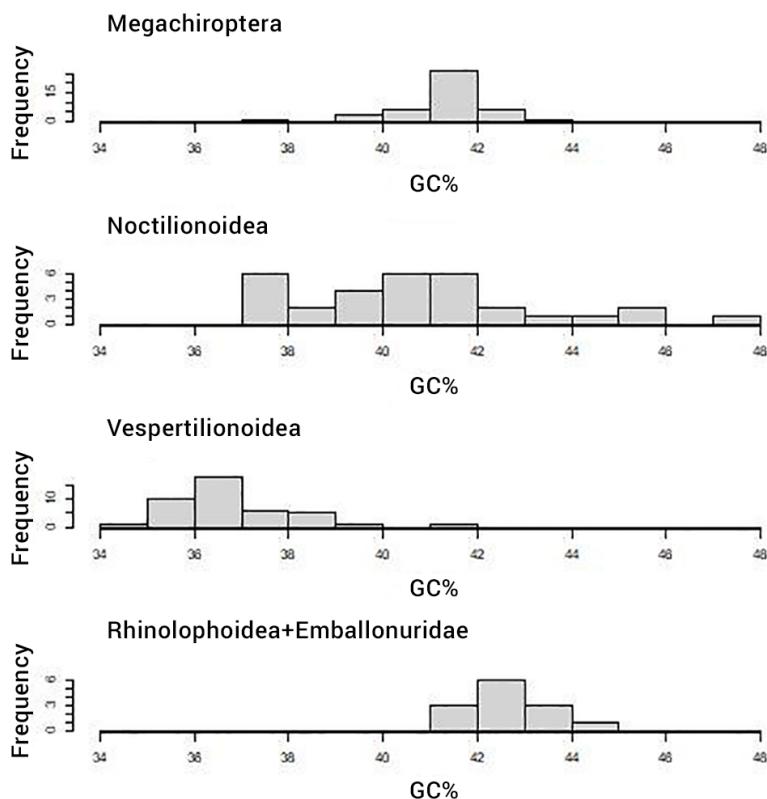


Figure 6. Histograms showing the range of GC% values for each of the four putative baramins: 1 = Megachiroptera; 2 = Noctilionoidea; 3 = Vespertilioidea; and 4 = Rhinolophoidea.

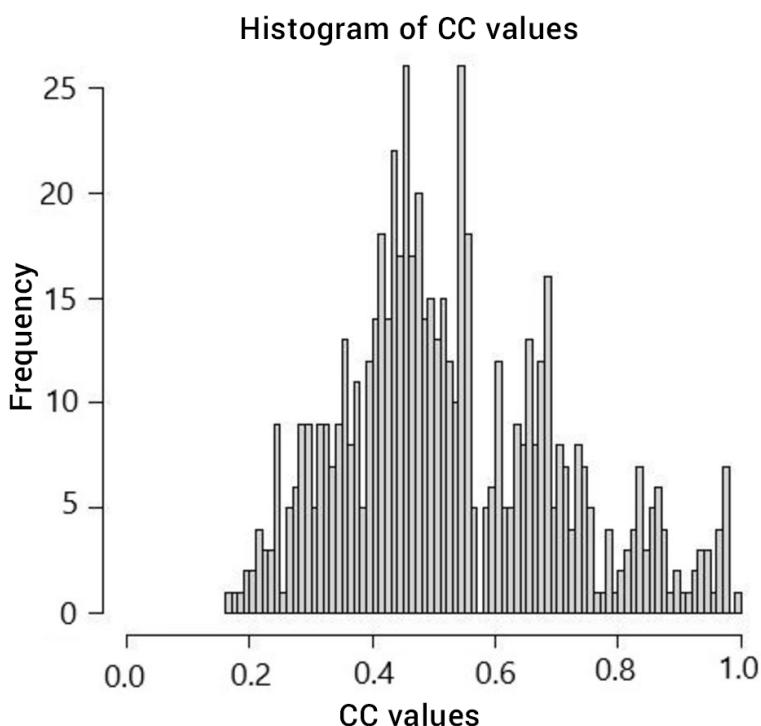


Figure 7. Histogram of the PCC values from the WGKS analysis. The histogram is multimodal which indicates multiple clusters of species.

The gene order of 13 protein-coding genes, 22 tRNA's, 2 rRNA's and the control region seem to be the same for many bat species, based on a study by Lopez-Wilchis *et al.* of five bat species: *Pteronotus personatus*, *Pteronotus parnellii* (two megabat), *Vampyrum spectrum*, *Mystacinia tuberculata* (two species from Noctilionoidea), and *Lasiurus borealis* (Vespertilioidea).²² Therefore, mitochondrial DNA gene order does not appear to be a good indicator of baraminic relationships between bat groups.

Lastly, GC% ranges from 35 to 47.6%, with a mean value of $39.9 \pm 2.72\%$. The histogram in figure 5 is bimodal, with one mode just over 36% and the second mode just below 42%. Here *Myotis* species are distributed throughout the curve characterized by the first mode, with a range of 35.2% to 38.3%. Figure 6 shows the GC% for each of the four groups. Statistics for these four groups can also be seen in table 2. In figure 6, groups 1 (Megachiroptera), 3 (Vespertilioidea) and 4 (Rhinolophoidea+Emballonuridae) have GC% values centred around a single mode, with standard deviations around 1.0, whereas group 2 (Noctilionoidea) has GC% values which are fairly spread out ($sd=2.53$).

Interpretation of WGKS results

The PCC values from the WGKS analysis were plotted in a histogram, showing the density plot above the histogram values (figure 7). It is very interesting to see that within bats, the spread of PCC values is quite broad, with a range of 0.160 to 0.991. The PCC histogram has multiple modes. This is an indication that there could be more than one cluster within the data; another indication that bats are apobaraminic.²³ The Hopkins clustering statistic is 0.817, which indicates a fairly good clustering quality. *Tupaia tana*, the large tree shrew, was selected because it belongs to the order Scandentia, which also served as an outlier species in the morphology study.

The heatmap in figure 8 shows several larger and several smaller groups and some singleton species. The species and their clusters are listed in table 3. Five statistically significant clusters were found; their statistics are

Table 2. Statistics on GC% values for each of the four putative baramins in the mtDNA study

Group	no. species	min	mean	max	st. dev.	p-value
Megachiroptera	44	37.9	41.4	43.5	0.97	0.00E+00
Noctilionoidea	31	37.7	40.9	47.6	2.53	2.22E-151
Vespertilioidea	42	35	36.8	41.7	1.31	2.64E-262
Rhinolophoidea+Emballonuridae	13	41.1	42.8	44.5	0.91	1.69E-49

shown in table 4. Of these, only three groups had three or more species.

Starting from the bottom left corner we have seven species from the group Vespertilioidea, otherwise known as common bats. This group includes species from the genera *Aeoreutes*, *Antrozous*, *Eptesicus*, *Lasiurus*, *Murina*, *Myotis*, *Nycticeius*, and *Pipistrellus*. In the middle of the heatmap we see seven species of megachiropterans, from the genera *Cynopterus*, *Eidolon*, *Eonycteris*, *Macroglossus*, *Pteropus*, and *Rousettus*. In the upper right corner, there are seven species from the group Noctilionoidea, from the genera *Anoura*, *Artibeus*, *Desmodus*, *Mormoops*, *Phyllostomus*, *Pteronotus*, and *Tonatia*. The outlier species, *Tupaia tana*, is in the very upper right corner.

Three other small groups include three species of noctilionids, *Carollia perspicillata*, *Micronycteris hirsuta*, and *Noctilio leporinus*, although this group is not statistically significant ($p = 0.21$). Another group consists of two *Miniopterus* species. A sixth group, with two species from the genus *Hipposideros*, belong to the larger group Rhinolophoidea.

These results indicate that there are likely four bat groups, in accordance with the mitochondrial results. However, there might be several noctilionid species, meaning that Noctilionoidea could be an apobaramin.

Discussion

Bats may form multiple baramins, because of discontinuities between different groups based on morphological characters, mtDNA sequence similarities, and Whole Genome K-mer signatures. Intrageneric hybridization occurs

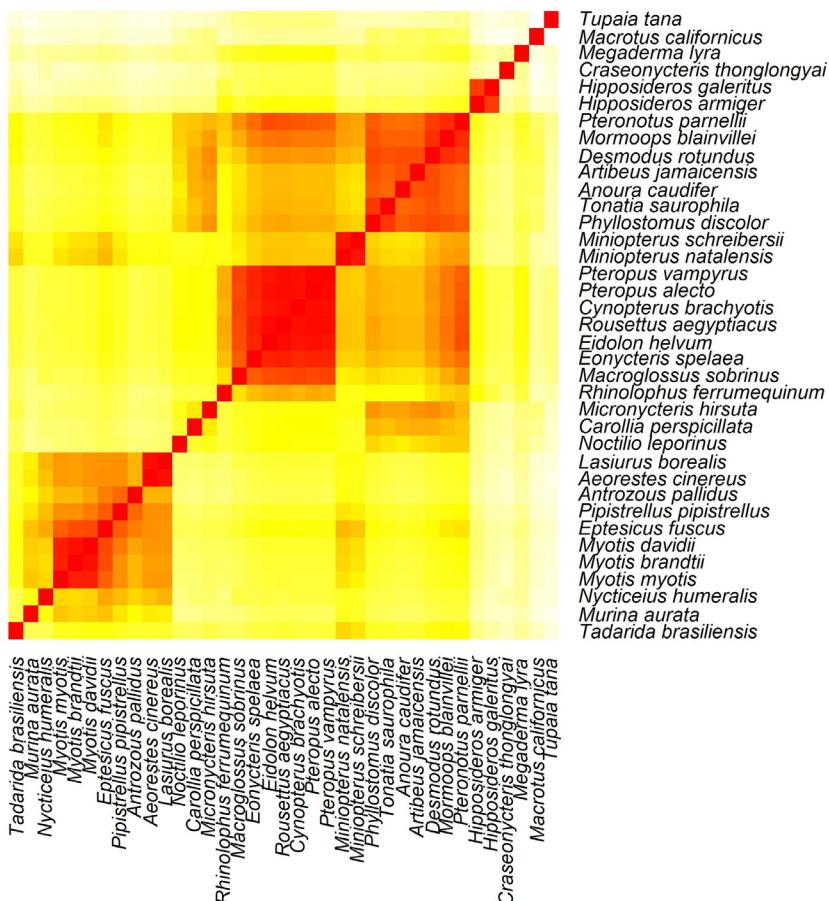


Figure 8. Heatmap of the PCC matrix from the WGKS analysis of 36 bat species and *Tupaia tana* as an outlier. Darker, red squares denote higher identity values between two species. Lighter, yellow-coloured squares represent lower identity values.

between different bat species, but it has not yet been shown to exist between bat families. What is interesting is that Yangochiropteran bats seem to be monophyletic, based on morphology, yet they are split up based on genetic analysis. This highlights the utility of molecular baraminology studies, which can provide additional information to make a more precise, fine-grained determination of the baraminic status of the species under study. It also suggests that God could have created bat species which externally appear to resemble one another, but are somewhat different genetically.

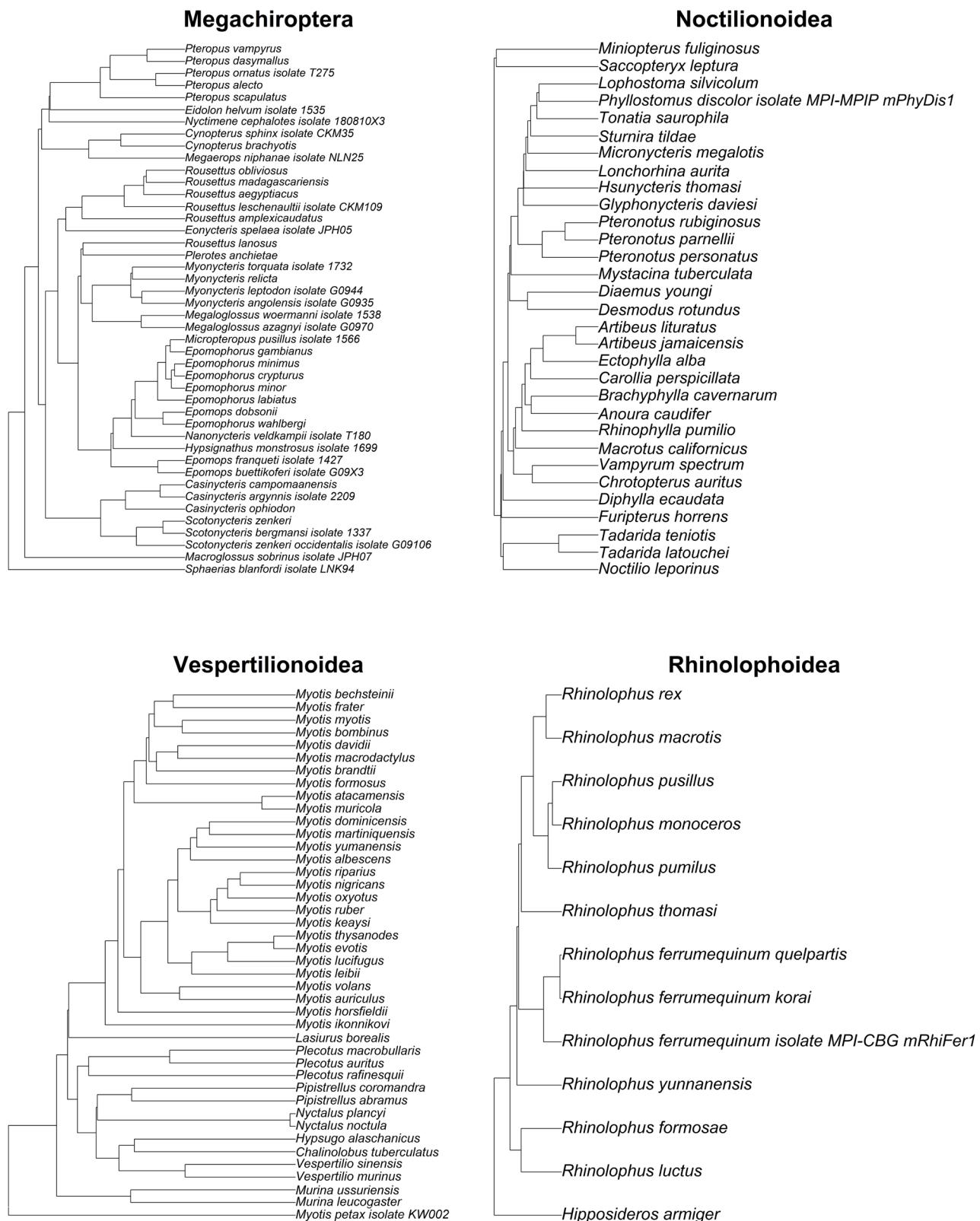


Figure 9. Baraminic tree for each of the four putative baramins. Upper left, Megachiroptera; upper right, Noctilionoidea; lower left, Vespertilioidea; lower right, Rhinolophoidea.

Different groups of microchiropterans are capable of echolocation, an irreducibly complex design element.²⁴ Echolocation could possibly be used to separate bat kinds from one another since virtually no megabat species echolocate. The few species that do use their wings to echolocate.

Conversely, even though microchiropterans are nocturnal, they also have functional L opsin and S opsin genes. Many microbats use vision quite well. The microchiropteran *Hipposideros speoris* also responds well to visual signals between 430 and 520 nm, meaning that it may also have M opsins. Since microchiropterans still retain these opsin genes, it is an indication that they didn't always have a nocturnal lifestyle.²⁵

There are differences between the results of the molecular baraminology analyses presented here and previous morphology-based estimates by Wood and Lightner. The reason for the discrepancy is likely because Lightner simply drew the baraminic boundary at the family, which is a common way to roughly estimate baramins.^{26,27} Wood performed a morphology-based analysis on 64 species of bats from Mormoopidae, Noctilionidae, and Pyllostomidae. Although there was lack of continuity between these bat groups, neither was discontinuity demonstrated between them. Thus, they could be monobaramins within a larger holobaramin. Wood writes: “Future studies could easily rectify either of these problems and thereby clarify the baraminic status of Noctilioidea.”²⁸

Figure 9 depicts the baraminic tree for all four putative bat baramins, based on the heatmap for the mtDNA results. Both Noctilioidea and Vespertilioidea are superfamilies in Yangochiroptera. Based on an analysis of 812 bat species covering 29 gene loci, the monophyly of these two superfamilies as well as that of Rhinolophoidea were suggested.²⁹

Megachiroptera

Megabats form a well-defined baramin, with several monobaramins. These include *Pteropus+Eidolon+Nyctimene+Cynopterus+Megaerops, Epomops+Epomophorus+Hypsugo+Myonycteris+Nanonycteris+Pteronotus+Rousettus*, and *Scotonycteris+Casinycteris*. These are all genera of the subfamily Pteropodinae, except for *Macroglossus sobrinus*, and *Sphaerias blanfordi*, which belong to their respective subfamilies.

Megachiropterans differ from microchiropterans in the way nerves between the retina and the midbrain connect.³⁰ The retinotectal pathway of megabats (such as in *Rousettus aegyptiacus*) is so different from that of microbats, that some researchers think megabats are more similar to primates than microbats. In megabats, both the left and right sides of the tectum are innervated by the corresponding half of the retina of both eyes. In microbats this is different: the left and right halves of both sides of the tectum are innervated by both the left and right halves of the retina of both eyes, respectively.³¹ The geographical distribution of megachiropterans

Table 3. Names of species in the WGKS study and their cluster number

species	cluster
<i>Aeoretes cinereus</i>	1
<i>Antrozous pallidus</i>	1
<i>Eptesicus fuscus</i>	1
<i>Lasiurus borealis</i>	1
<i>Murina aurata</i>	1
<i>Myotis brandtii</i>	1
<i>Myotis davidii</i>	1
<i>Myotis myotis</i>	1
<i>Nycticeius humeralis</i>	1
<i>Pipistrellus pipistrellus</i>	1
<i>Anoura caudifer</i>	2
<i>Artibeus jamaicensis</i>	2
<i>Desmodus rotundus</i>	2
<i>Mormoops blainvilliei</i>	2
<i>Phyllostomus discolor</i>	2
<i>Pteronotus parnellii</i>	2
<i>Tonatia saurophila</i>	2
<i>Carollia perspicillata</i>	3
<i>Micronycteris hirsuta</i>	3
<i>Noctilio leporinus</i>	3
<i>Craseonycteris thonglongyai</i>	4
<i>Cynopterus brachyotis</i>	5
<i>Eidolon helvum</i>	5
<i>Eonycteris spelaea</i>	5
<i>Macroglossus sobrinus</i>	5
<i>Pteropus alecto</i>	5
<i>Pteropus vampyrus</i>	5
<i>Rousettus aegyptiacus</i>	5
<i>Hipposideros armiger</i>	6
<i>Hipposideros galeritus</i>	6
<i>Macrotus californicus</i>	7
<i>Megaderma lyra</i>	8
<i>Miniopterus natalensis</i>	9
<i>Miniopterus schreibersii</i>	9
<i>Rhinolophus ferrumequinum</i>	10
<i>Tadarida brasiliensis</i>	11
<i>Tupaia tana</i>	12

Table 4. Statistics for bat clusters based on the WGKS method

cluster	species	min	mean	max	stdev	p-value
1	10	0.532	0.726	0.97	0.106	8.90E-26
2	7	0.807	0.853	0.925	0.033	2.53E-48
3	3	0.481	0.534	0.596	0.058	2.31E-01
5	7	0.84	0.934	0.991	0.046	4.77E-36
6	2	0.894	0.894	0.894	NA	4.75E-53
9	2	0.959	0.959	0.959	NA	1.40E-39

is restricted to the Old World, whereas microchiropterans are distributed worldwide.

Noctilionoidea

Noctilionoidea consist of seven families including species which use their nasal passages to emit echolocation calls, and their premaxillaries are also movable compared to their maxillaries.³² Species belonging to these families are confined mainly to the Neotropics.^{33,34}

Based on a study of 12S, 16S rRNA, and tRNA^{Val} sequences in the mitochondrial DNA, Van Den Bussche *et al.* found that Noctilionoidea+Furipteridae+Thyropteridae+Mystacidae form a monophyletic group.³⁵ In accordance with previous results of other researchers, the Vampyrini monobaramin (*Vampyrum*+*Chrotopterus*) was also found to be a part of Noctilionoidea.³⁶ The family Pyllostomidae forms a large part of the baraminic tree in the upper right portion of figure 9. Besides Pyllostomidae, three *Pteronotus* species come from the family Mormoopidae, *Noctilio leporinus* represents the family Noctilionidae, *Mystacina tuberculata* represents the family Mystacidae, two *Tadarida* species represent the family Molossidae, and the species *Saccopteryx tildae* represents the family Emballonuridae.

Miniopterus fuliginosus is classified as a member of the family Vespertilionidae, so why does it appear within Noctilionoidea? Previous research has shown that bat species in this genus differ from other members of Vespertilionoidea in their morphology, embryology, immunology, and also genetics. Some classify this genus as its own family, and it appears that genetically, it belongs to Noctilionoidea.³⁷

Vespertilionoidea

All 42 species in the lower left part of figure 9 come from the family Vespertilionidae, one of the five families of the superfamily Vespertilionoidea. The genus *Myotis* forms a large monobaramin with 28 species. The genera *Pipistrellus*,

Nyctalus, *Vespertilio*, *Murina*, and *Plecotus* also form several monobaramins.

Rhinolophoidea

The superfamily Rhinolophoidea in the lower right of figure 9 is made up of 12 species of *Rhinolophus* and also *Hipposideros armiger*. These bats are classified by high-duty cycle nasal echolocation, an ossified first cartilage, which is fused to their manubrium and first rib and a pair of pubic nipples in the females, as opposed to all other bats.³⁸ Many secular studies classify Emballonuroidea as its own superfamily. This group clusters with the megabats in figure 1, but together with Rhinolophoidea in figures 2 and 3.

Conclusion

There appear to be four bat baramins, based on the mutually supporting clustering results of the mtDNA analysis. The four groups are: Megachiroptera, Rhinolophoidea+Emballonuridae, Vespertilionoidea, and Noctilionoidea. However, the WGKS results suggest that Noctilionoidea might be an apobaramin. This indicates that the level of the baramin is at the superfamily level in bats. However, more study is needed to validate these results, such as hybridization studies between species within the four putative baramins delineated here. Molecular results provided better resolution of the species and were able to predict the superfamily Vespertilionoidea and the suborder Megachiroptera. The status of Emballonuroidea still needs further examination, as not too many representative species were studied here. It may be possible that this group forms its own holobaramin.

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