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Molecular Baraminology of Primates

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Abstract

Twenty-six species of primates were examined by the Gene Content method, and 93 mitochondrial genome sequences were downloaded from the NCBI database, aligned, and analyzed. In total, 96 species were studied, about half of all primates. The Gene Content method predicts at least six putative primate holobaramins: cercopithecines, colobines, New World monkeys, lemurs, great apes, and humans. The mitochondrial DNA analysis predicts sixteen groups, which are likely monobaraminic lineages within the groups predicted by the Gene Content method. This study predicted a larger number of groups compared to a previous study of primates and other vertebrates. Interestingly, examination of the newly sequenced mitochondrial genome of *Homo heidelbergensis* places it within the human holobaramin, in agreement with previous studies that included it based on morphological data alone. The human holobaramin also clearly separates from all other primate species, even its alleged closest relatives, the great apes. Hylobatidae is possibly a seventh primate group, and *Pongo* (orangutan) is a possible eighth group.

Key Words: primate, Homo, molecular baraminology, mitochondrial DNA, Old World monkey, New World monkey, great ape, colobine, cercopithecine, Gene Content method

Introduction

There are 194 species of living primates (Stringer and Andrews, 2011), which range in size from the tiny, palm-sized Moholi bush baby (*Galago moholi*) (around 6 oz. in weight) to the large gorilla (*Gorilla gorilla*) (up to 227 kg in males) and are (excluding humans) found on all continents except Australia and Antarctica. Primates are classified into two suborders (see Figure 1)—Strepsirrhini, such as lemurs, lorises, and galagos; and Haplorhini: tarsiers and anthropoids. Anthropoids can be classified as either flat-nosed monkeys (Platyrrhini), otherwise known as New World monkeys, and Catarrhini, or Old World monkeys with downward-looking nostrils. Platyrrhini is subdivided into five families: Callitrichidae (marmosets and tamarins), Cebidae (capuchins and squirrel monkeys), Aotidae (owl monkeys), Pitheciidae (titis, sakis, and uakaris), and Atelidae (howler monkeys and spider monkeys). Catarrhini is subdivided into two superfamilies, Cercopithecoidea, which has one family, Cercopithecidae (including langurs, macaques and baboons), and Hominoids. Hominoids are further classified into two

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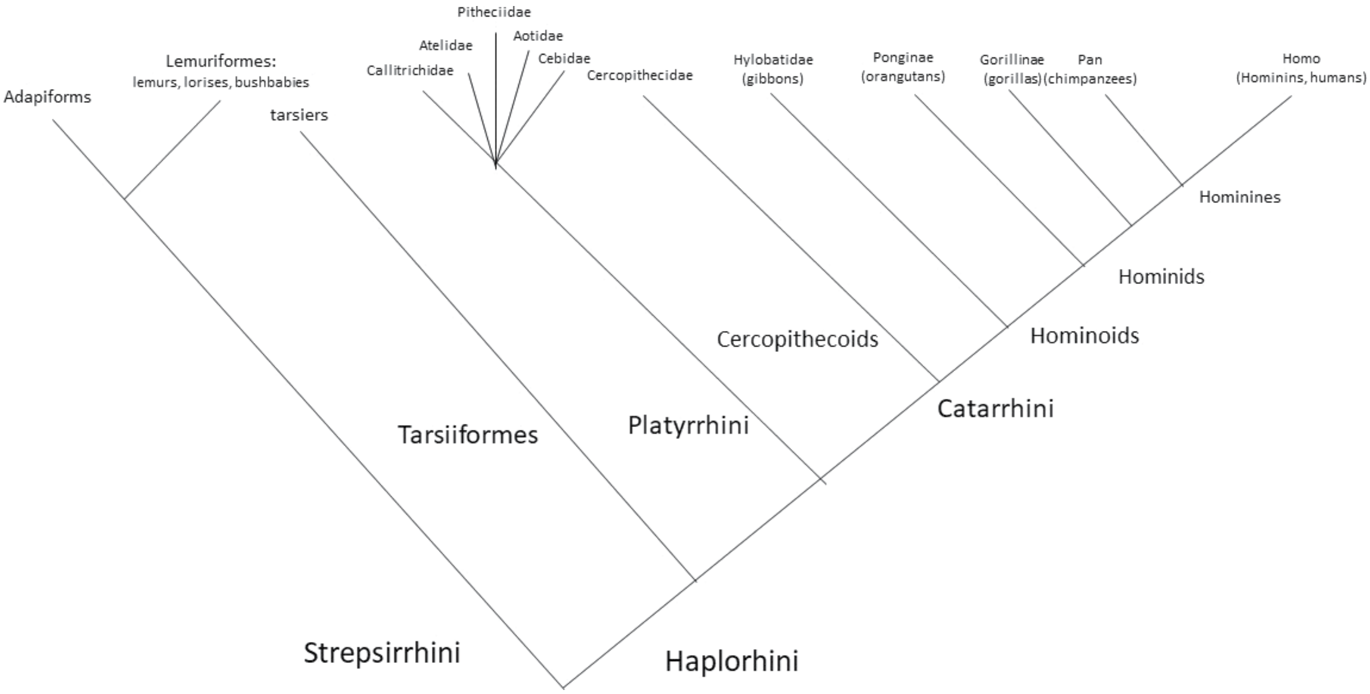

Figure 1. Taxonomic tree of primates. Primates are divided into Strepsirrhini and Haplorhini, which are classified as Tarsiiformes (tarsiers), Platyrrhini (New World monkeys) and Catarrhini (Old World monkeys). Catarrhini is made up of cercopithecoids and hominoids, which themselves are made up of Hylobatidae (gibbons) and Hominids. This latter group is made up of gorillas and orangutans and hominins (chimpanzees and humans). The human species *(Homo sapiens)* is unique among all primates.

families Hylobatidae (gibbons), and Hominidae. Hominidae is subdivided into the subfamilies Ponginae (orangutans), Gorillinae (gorillas), and Homininae. Hominines are made up of the tribes Panini (chimpanzees, genus *Pan*) and Hominini, otherwise known as hominins, made up of the genus *Homo* (humans) (Park, 2009; Stringer and Andrews, 2011; Gebo, 2014).

The human species (*Homo sapiens*) is unique among all extant primates, even all extant animals, in that we alone were created in God's image (Genesis 1:26–27) (Lightner and Cserhati, 2019). Archaic humans, such as Neanderthals (*Homo sapiens neanderthalensis*), Denisovans (*Homo sapiens denisova*), and other possible groups are also members of humankind (Lubenow, 2004; Savanne, 2014; Rupe and Sanford, 2017; O'Micks, 2018a, 2018b). Based on their morphological characteristics, several other fossil species have also been considered to be members of the human holobaramin. These include *Homo erectus*, *Homo heidelbergensis*, *Homo naledi*, and *Homo rudolfensis* (Wood, 2010; Line, 2013; O'Micks, 2016; Wood, 2016; Rupe and Sanford, 2017. Some of these placements are controversial, so molecular data provide another line of evidence that can be useful in classifying these taxa. With the mitochondrial genome sequenced for *Homo heidelbergensis* (Meyer et al., 2014) we now have the opportunity of placing this fossil taxon into its proper category with even more confidence.

Almost all primates are characterized by grasping feet with a divergent hallux, presence of nails on most digits, an elongated calcaneus, hind limb locomotor dominance, grasping hands with opposable thumbs, forward rotation of the closely set eyes, large brain size, relatively long gestation compared to body weight, slow fetal growth, prolonged life history, and a loss of an incisor and premolar from the tooth row (Stringer and Andrews, 2011).

Until now, the creationist literature has dealt mainly with the analysis of groups or individual hominins or australopithecines. For example, the families Cercopithecidae (Hartwig-Scherer, 1993), Galagonidae (Wood, 2008), and the subfamilies Gorillinae (Hartwig-Scherer, 1998) and Ponginae (Hartwig-Scherer, 1998) have also been studied individually. A more comprehensive study of the order Primates has been lacking. With proteomic data available

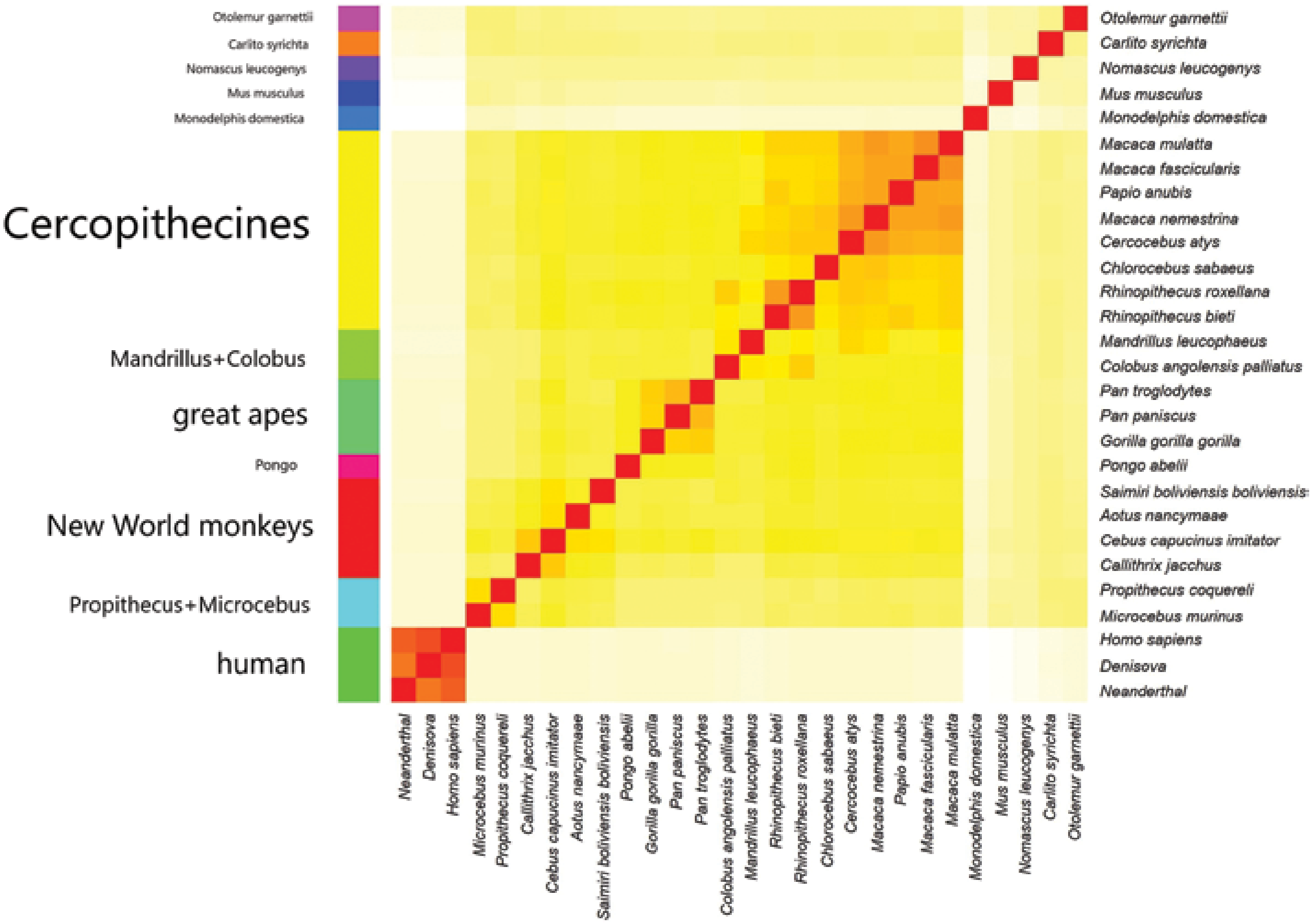

Figure 2. Heatmap showing putative baraminic relationships between primates based on analysis by the Gene Content method. Darker, redder colors correspond to higher Jaccard Coefficient values, corresponding to species showing significant continuity with one another. Lighter, yellower colors correspond to lower Jaccard coefficient values denoting species from two separate baramins.

from the NCBI database, a comprehensive molecular baraminology study can be done using the Gene Content (GC) method of O'Micks (2017).

Materials and Methods

Gene Content Method Analysis

The whole proteomes of 26 primate species were downloaded from the NCBI database. *Monodelphis domestica* and *Mus musculus* were used as outlier species. Protein predictions were made for Neanderthal and Denisova using the Augustus gene prediction software. Proteins were assigned to OrthoMCL clusters following the GCM protocol (Stanke and Morgenstern, 2005; Fischer et al., 2011; O'Micks, 2017). The number of proteins and the number of proteins mapped to orthologs for each species were noted and can be seen in the 'species' tab of Supplementary File #1, which also contains the results for this analysis. It is available on Zenodo at https://zenodo.org/record/6635435#.YqWDGOzMLrc.

Mitochondrial DNA Analysis

The mitochondrial genome sequences of 92 primate species were downloaded from the NCBI Organelle database at ncbi.nlm.nih.gov/genome/browse#!/organelles. *Monodelphis domestica* was used as an outlier. One sequence each for *Gorilla gorilla gorilla* and for *Pan paniscus* was excluded because it was a duplicate. The alignment of

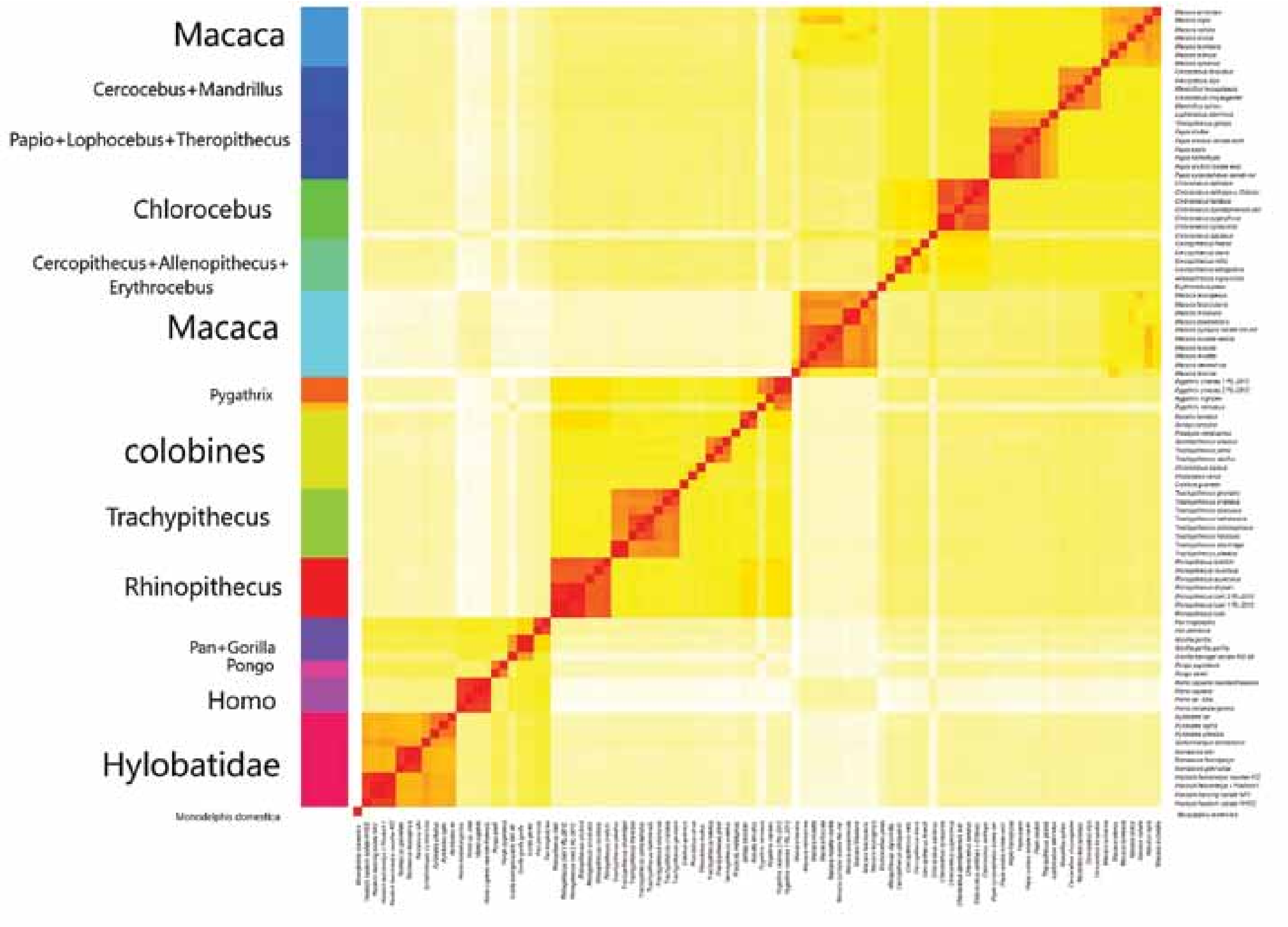

Figure 3. Heatmap showing putative baraminic relationships between primates based on analysis of the mitochondrial genome. Darker, redder colors correspond to higher sequence similarity values, corresponding to species within the same baramin. Lighter, yellower colors correspond to lower sequence similarity values suggesting species from two separate baramins.

these mtDNA sequences was done using the CLUSTALW2 alignment tool (Thompson et al., 2002). The results of the mtDNA analysis are available in Supplementary File #2.

Results and Discussion

Results of the Gene Content Method

The JCV matrix (see Supplementary File #1) has a Hopkins measurement value of 0.793, which is good quality for clustering. An elbow plot (Supplementary Figure 1) generated from the matrix shows an optimal number of clusters at k=12. The Gene Content method was run to search for twelve baramins according to protocol (O'Micks, 2017). The species classification and the statistics for the predicted baramins can be found under the tabs 'species classification' and 'stats' in Supplementary File #1. It is available on Zenodo [https://zenodo.org/record/6635435#.YqWDGOzMLrc].

On the heat map in Figure 2, several clusters of species can be seen. Group 1 (red) includes four species of New World Monkeys: *Aotus nancymaae*, *Callithrix jacchus*, *Cebus capucinus imitator*, and *Saimiri boliviensis boliviensis*. The last three belong to the family Cebidae (marmosets, tamarins, capuchin monkeys, and squirrel monkeys). *Aotus nancymaae* (Ma's Night Monkey) belongs to the family Aotidae (owl monkeys). However, according to newer primate taxonomies based on the phylogeny of the epsilon globin and interphotoreceptor binding protein (IRBP) intron 1 (Schneider and Sampaio, 2015) the genus *Aotus* is now included in the family Cebidae as well, meaning that all species used in this study can be considered to represent a single family. This group has a p-value of 1.4E-10, and a mean JCV of 0.947. Hybrids have been reported between species from the genus *Callithrix* and *Callicebus*, which belong to the families Cebidae and Pitheciidae (Neusser et al., 2005; Fantini et al., 2011).

Group 2 (yellow) makes up the largest group, in the middle, with eight species of cercopithecine Old World monkeys, with a p-value of 3.84E-28, and a mean JCV of 0.96. These include species from the genera *Cercocebus*, *Chlorocebus*, *Macaca*, *Papio*, and *Rhinopithecus*.

Group 3 (olive green) is made up of another group of Old World monkeys, the colobines, with two species, *Colobus angolensis palliates* (Angolan black-and-white colobus monkey), and *Mandrillus leucophaeus* (the mandrill). Interestingly, there have been two recorded hybridizations between members of the genus *Mandrillus* and *Cercocebus* from the previous group (Van Gelder, 1977).

Humans (group 4, light green) form a very compact cluster, visibly discontinuous with all other primate species, with a p-value of 1.8E-07, and a mean JCV of 0.979. This is in accordance with results from a previous molecular baraminology study performed on mammals by Lightner and Cserhati (2019). The present study compared 26 primates with one another, as opposed to 21 in the previous analysis. Both studies underscore the uniqueness of humans compared to all other animals, even other primates, which are those animals to which humans are most similar. Whereas humans have a JCV of 0.979±0.005, this drops down to 0.8±0.001 when the JCV are compared between humans and the great apes. This means that if we add the great apes to the human baramin, we get meaningless results since the mean JCV is greatly reduced. If we calculate a z-score on the two JCV distributions, we get a value of 16.01, which means that the two distributions are very highly dissimilar.

Group 5 (bluish green) is made up of the great ape species: the gorilla (*Gorilla gorilla*), and two chimpanzee species (*Pan paniscus* and *Pan troglodytes*). These three species show discontinuity with all other species, with a p-value of 2.4E-12 and a mean JCV of 0.956.

Two species of lemurs, the Gray Mouse Lemur (*Microcebus murinus*) and Coquerel's sifaka (*Propithecus coquereli*) make up cluster 6 (cyan), with a mean JCV of 0.951.

Several individual species could not be assigned to any cluster. These include *Carlito syrichta* (Philippine tarsier, orange) and *Otolemur garnettii* (Small-Eared Galago, purple), *Nomascus leucogenys*, the White-Cheeked Gibbon (dark purple), from the family Hylobatidae, as well as *Pongo abelii* (the Sumatran orangutan, magenta). *Monodelphis domestica* (blue) and *Mus musculus* (dark blue), the two outlier species also cluster separately from all of the other species.

Mitochondrial DNA Analysis

Putative clusters were predicted based on aligning the mtDNA sequences of 92 primate species and an outlier, *Monodelphis domestica*. The silhouette plot in Supplementary Figure 2 shows an optimum value at k=16. Thirteen of the sixteen clusters were comprised of at least 3 members and were statistically significant with a p-value less than 5%. The mtDNA sequence similarity cluster had a Hopkins clustering value of 0.897, which indicates very good clustering. The list of species used in this study, the mtDNA identity matrix, the putative clusters, and the statistics for the predicted baramins can be found in see Supplementary File #2.

The sixteen clusters are as follows: the first cluster (in red in Figure 2) consists of five species from the genus *Rhinopithecus* (snub-nosed monkeys): three *Rhinopithecus bieti* individuals, *Rh. strykeri*, *Rh. roxellana*, *Rh. brelichi*, and *Rh. avunculus*. This is a genus of Old World colobine monkeys.

The second and third clusters (orange and dark orange) are made up of three species from the genus *Pygathrix* (douc langurs): *Pygathrix cenerea*, *Pygathrix nigripes*, and *Pygathrix nemaeus*. This is a genus of Old World colobine monkeys.

Nine species make up cluster 4 (yellow): *Simias concolor* (the pig-tailed langur), *Nasalis larvatus* (the proboscis monkey), *Presbytis melalophos* (black-crested Sumatran langur), *Trachypithecus johnii*, *Trachypithecus vetulus*, *Procolobus verus* (olive colobus monkey, or Van Beneden's colobus), *Semnopithecus entellus* (the northern plains gray langur), *Piliocolobus badius* (western red colobus), and *Colobus guereza* (mantled guereza). Some researchers prefer to put the first two species into the same genus (Groves, 1970). Whittaker et al. (2006) also came to the same conclusion based on similarities between cytochrome b and several RNA genes. These nine species in cluster 4 are all colobine monkeys. An analysis of 54 genes covering 35 Kb of nuclear DNA (Perelman et al., 2011) places *T. vetulus* within the genus *Semnopithecus*, thus explaining the incongruency of why this species is seemingly misplaced from the next group, *Trachypithecus*. This might be true of *T. johnii*, another misplaced *Trachypithecus* species. Indeed, based on an analysis of a 573 bp segment of the mitochondrial cytochrome b gene, *T. johnii* forms a clade with the South Indian form of *Semnopithecus entellus* (Osterholz et al., 2008).

Group 5 (yellow-green) consists of eight species of Old World colobine monkeys, namely lutungs (leaf monkeys) within the genus *Trachypithecus*

(*T. cristatus*, *T. francoisi*, *T. germaini*, *T. hatinhensis*, *T. obscurus*, *T. pileatus*, *T. poliocephalus*, and *T. shortridgei*).

Group 6 (light green) consists of six Chlorocebus species: *Chlorocebus aethiops*, *Ch. cynosuros*, *Ch. djamdjamensis*, *Ch. pygerythrus*, *Ch. tantalus*, and *Ch. aethiops x Ch. pygerythrus*. Here the letter 'x' between two species names denotes a new hybrid species which is a result of the cross between the two species that make up the hybrid's name. These are all cercopithecine monkeys. A seventh group consists of only *Ch. sabaeus*. The reason that this one species of *Chlorocebus* separates from all the rest may be due to mtDNA heteroplasmy, which is known to occur in this genus (Rensch et al., 2016).

Group 8 (light cyan) includes: *Cercopithecus diana*, *C. albogularis*, *C. mitis*, *C. lhoesti*, *Erythrocebus patas*, and *Allenopithecus nigroviridis*. These are all species of cercopithecine monkeys.

The ninth and tenth cluster of primate species (cyan and blue) includes seventeen species of macaques (genus *Macaca*). Macaques are cercopithecine monkeys and have a widespread geographic distribution, and live in places such as deserts to rainforests, from sea level to mountains, from North Africa to Southeast Asia. This genus might be split up into two groups because they might represent two monobaramins. Members of the first group are: *Macaca arctoides*, *M. nigra*, *M. radiata*, *M. silenus*, *M. sinica*, *M. sylvanus*, and *M. tonkeana*, whereas members of the second group are: *Macaca assamensis*, *M. cyclopis*, *M. fascicularis*, *M. fuscata*, *M. leonina*, *M. leucogenys*, *M. mulatta*, *M. mulatta vestita*, *M. nemestrina*, and *M. thibetana*.

Previous studies have usually discovered four groups of macaques (the *fascicularis*, *silenus*, *sinica*, and *sylvanus* groups). The membership of these groups varies as to which region of the mtDNA is under study, be it NADH4 and several tRNAs, the 12S and 16S ribosomal regions, the 3.1 kb TSPY and the SRY regions, the 2322 bp region of 12S, tRNA^{Glu}, COI, COII and COIII, or the *Alu* elements of 358 loci, or the region around the COII gene (Rahim et al., 2014). However, since these studies did not examine the entire mtDNA, they remain inconclusive.

Group 11 (darker blue) is made up of five species from two cercopithecid genera: *Cercocebus* (mangabeys) and *Mandrillus* (forest baboons or mandrills). These are: *Cercocebus atys*, *C. chrysogaster*, *C. torquatus*, *Mandrillus leucophaeus*, and *M. sphinx*. Liedigk et al. (2014) created a multiple alignment of the mitochondrial genomes of papionins (*Macaca*, *Mandrillus*, *Cercocebus*, *Lophocebus*, *Theropithecus*, *Rungwecebus*, and *Papio*). They found that *Cercocebus* + *Mandrillus* forms a clade amongst papionins. The present results reinforce previous molecular results which show that drills are more closely related to mangabeys from the genus *Cercocebus* as opposed to a previously held view grouping them together with baboons (Disotell, 1994; Fleagle, 1999). In fact, Fleagle and McGraw (1989) suggest that similar jaw, fore- and hindlimb morphology between *Mandrillus* and *Cercocebus* allow for a foraging lifestyle on the forest floor. The previously mentioned study be Perelman et al. (2011) also found the *Cercocebus*-*Mandrillus* clade to be monophyletic (Devreese and Gilbert, 2015).

Six species from the genus *Papio* make up group 12 (darkest blue), as well as two species from two other genera. These are *Papio anubis*, *P. cynocephalus*, *P. hamadryas*, *P. kindae*, *P. papio*, and *P. ursinus*. These monkeys are also cercopithecids. The two other species are *Lophocebus aterrimus* (black-crested mangabey) and *Theropithecus gelada* (gelada). These papionid monkeys form a clade together based on the molecular analysis of 57 loci (Pugh and Gilbert, 2018). According to these same authors, the genera *Cercocebus* and *Mandrillus* form another clade. This clade was also found by Liedigk et al. (2014) in a multiple mitochondrial genome alignment. A phylogenetic tree based on the testis-specific Y-encoded protein (TSPY) shows that *M. leucophaeus* and *M. sphinx* intermingle with three species of *Cercocebus*, strongly suggesting that the species in these two genera belong to the same holobaramin (Kim and Takenaka, 1996; Harris, 2000).

Group 13 (dark purple) consists of three gorillas (*Gorilla gorilla gorilla*, *G. gorilla* and *G. beringei*) and two chimpanzee species: *Pan paniscus* and *P. troglodytes*.

The next group (14, purple) is made up of four individuals belonging to the genus *Homo*: *Homo heidelbergensis*, *H. sapiens*, *H. sapiens neanderthalensis*, and *H. sp. Altai* (Denisovan). This is a very interesting result, in that there is now molecular evidence to support the human status of *H. heidelbergensis*, which until now was classified as human based only on morphological traits (Line, 2013). It also reinforces the results that Neanderthals and Denisovans are both human.

Two species of orangutan make up group 15 (light purple), *Pongo abelii* and *P. pygmaeus*. These two species have been known to hybridize (*P. abelii x pygmaeus*) (Botting and Bastian, 2019).

Group 16 (magenta) is made up of eleven species from four genera: *Hoolock* (hoolock gibbons), *Hylobates* (gibbons), *Nomascus* (gibbons), and *Symphalangus* (siamangs): *Hoolock hoolock*, *H. leuconedys*, *H. leuconedys x H. tianxing*, *H. tianxing*, *Hylobates agilis*, *H. lar*, *H. pileatus*, *Symphalangus syndactylus*, *Nomascus gabriellae*, *N. leucogenys*, and *N. siki*. All these species belong to the family Hylobatidae.

The outlier, *Monodelphis domestica* (white), shows discontinuity with all other species.

Summary and Conclusion

Table 1 summarizes the overlap in the results between the Gene Content

Table 1. Comparison of results between the Gene Content method and the mtDNA analysis. Names in parentheses are present in one analysis but are missing from the other.

Gene Content method	mtDNA analysis
Colobines	*Pygathrix* (2 groups)
	Rhinopithecus
	Simias+Nasalis+Trachypithecus+ Semnopithecus+Colobus+Piliocolobus+ Presbytis+Procolobus
	Trachypithecus
Colobus+Mandrillus	*Cercocebus+Mandrillus*
Cercopithecines	*Macaca* (2 groups)
	Papio+Lophocebus+Theropithecus
	Chlorocebus (2 groups)
	Allenopithecus+Cercopithecus+Erythrocebus
Great apes	*Gorilla+Pan*
Orangutan	*Pongo*
Human	*Homo sapiens, Neanderthal, Denisovan, Homo heidelbergensis*
(Gibbons)	*Hoolock+Hylobates+Symphalangus+ Nomascus*
New World monkeys, family Cebidae: *Aotus*, *Callithrix*, *Cebus*, *Saimiri*	(New World Monkeys)

method and the mtDNA analysis. As we can see, the Gene Content method found four groups (colobines, cercopithecines, great apes, human) also found by the mtDNA analysis. Each of these four groups, however, match several groups found by the mtDNA analysis. One group found by the mtDNA analysis (Hylobatidae) was not found by the Gene Content method. New World monkeys and lemurs were found by the Gene Content method but not by the mtDNA analysis.

What do these results mean? They most likely indicate that primate molecular baraminology has discovered seven simiform primate kinds: colobines, cercopithecines, great apes, New World monkeys (Cebidae), great apes, gibbons, and humans. The previous study by Lightner and Cserhati (2019) predicted only four primate groups: humans, great apes, Old World monkeys, and New World monkeys. This previous study only examined 21 species, among other vertebrate groups, whereas the present study examined 98 species.

The fact that the mtDNA analysis uncovered a larger number of groups could be because these groups are monobaraminic lineages within the corresponding larger group found by the Gene Content method. Indeed, the genera *Pygathrix*, *Macaca*, and *Chlorocebus* were each split into two groups. Further, two species of *Trachypithecus* cluster with *Semnopithecus*, in separate group from other *Trachypithecus*. Mitochondrial DNA analysis can only go so far in providing congruent results. Factors, such as heteroplasmy or higher mutation rates may obscure baraminic relationships based on mtDNA analysis.

The Gene Content method provides a more holistic view of baraminic relationships as opposed to the mtDNA study which examined only a small fraction of the genome. Interestingly, both the Gene Content method and the mtDNA analysis placed gorillas and chimpanzees into the same baramin. This may seem unusual due to the size disparity between these two species, however, such large size differences exist in other kinds, such as the cat kind (lion and house cat). Humans clearly separate from all other primates in this study, including their alleged closest relatives, the great apes, based on both the Gene Content method as well as mitochondrial DNA analysis. This highlights their unique position within creation. Lastly, a very interesting result shows that based on molecular data, *Homo heidelbergensis* is likely a member of the human holobaramin (Cserhati, 2022), confirming what many creationists had suspected, based on morphological analysis. With the isolation of genetic material from even more fossil hominins our picture of baraminic relationships within primates is becoming more complete.

Acknowledgments

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