Sorting out the phylogeny of the Order of Primates - Chuck Kennedy (*Futurum ignotum*)

Phylogenetic trees are prepared with the purpose of showing evolutionary relationships among species being studied. The species that are closer together in the process of divergence into different species should have similar characteristics compared to species that diverged from the lineage at a much earlier or later time. Additionally, divergence of organisms within the lineage is marked by the occurrence of particular traits within the divergent group that makes them different from other species in the ancestral lineage. These derived traits will continue to occur through mutation, natural selection and possibly other microevolution processes while maintaining many earlier shared traits. This exemplifies the definition of evolution as 'descent with modification'. The phylogenetic tree will consist of 'nodes' that indicate a common ancestor at the time of a divergence between groups of organisms. In Fig. 1, node 1 represents ancestral divergence between species 'A' and the other three species, node 2 represents ancestral divergence between species 'B' and the two species 'C' and 'D', and node 3 represents common ancestry at the time of divergence between species 'C' and 'D'.

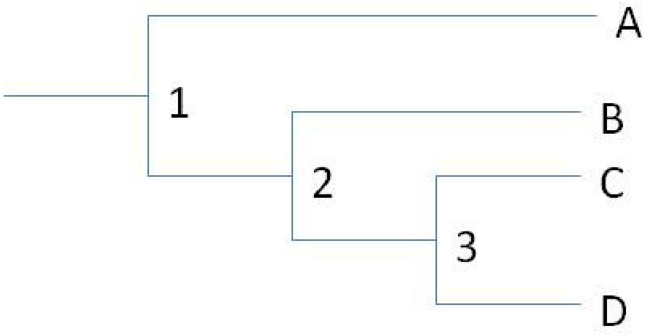
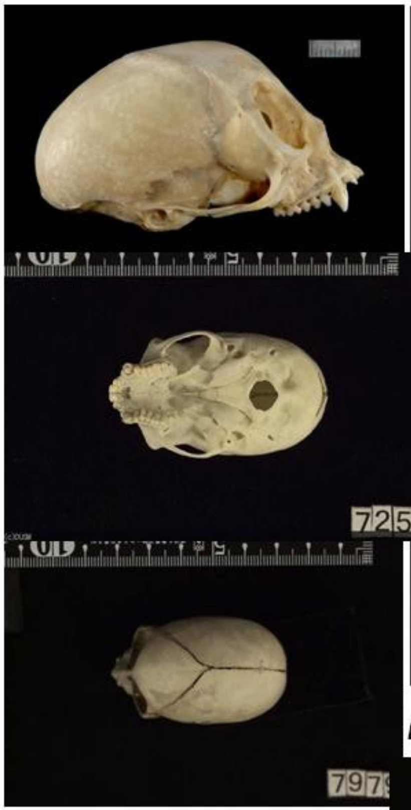


Figure 1. Example of a hypothetical phylogeny tree.

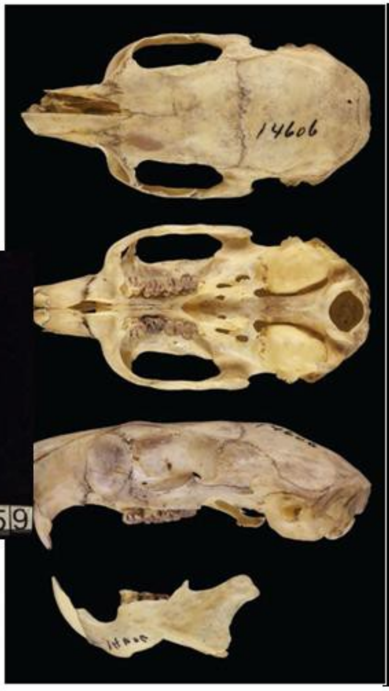
According to the concept of phylogeny trees, species 'A' would have the fewest shared, derived traits with the other species and the sister taxons 'C' and 'D' would have the most shared traits. Sister taxons (or sister groups) are those which share the most traits within a phylogeny tree (also called a cladogram) relative to other groups or species within the tree. 'Relative' is the key word here since cladograms normally do not include all possible species in the evolution of lineages represented. So some sister taxons could be very close in evolutionary divergence and while others may actually be more distant than the tree may suggest.

Traits that could be used to identify similarities include morphological, anatomical, physiological, and biochemical characteristics.

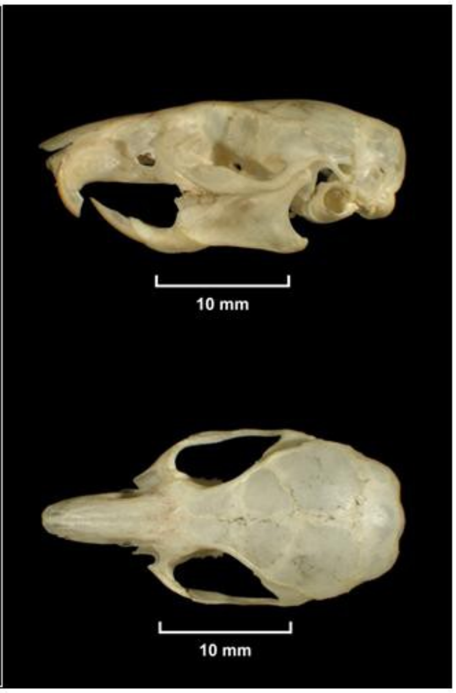
Your first objective here is to construct a phylogenetic tree of several primate species along with two outlier non-primate species using skull anatomy and body morphology. The order of primates includes our species. Primates show an evolutionary trend towards prehensile hands/feet, shortened snout, different types of teeth, forward-facing eye orbits, differentiation between forelimbs and hind limbs, skulls with a larger cranium and a trend of the foramen magnum moving towards the center of the skull base. The foramen magnum is the opening allowing the spinal cord to enter into the skull.



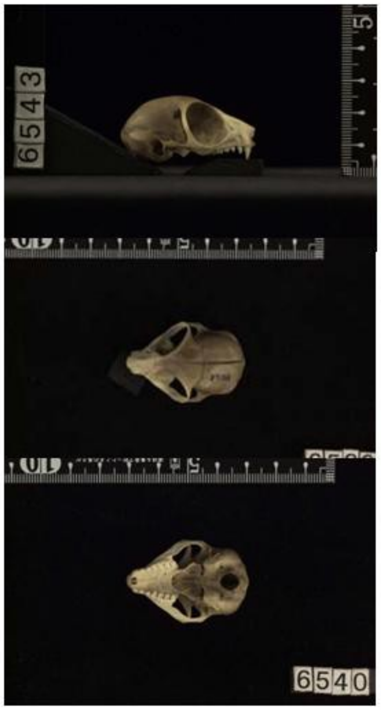
Saimiri sciureus



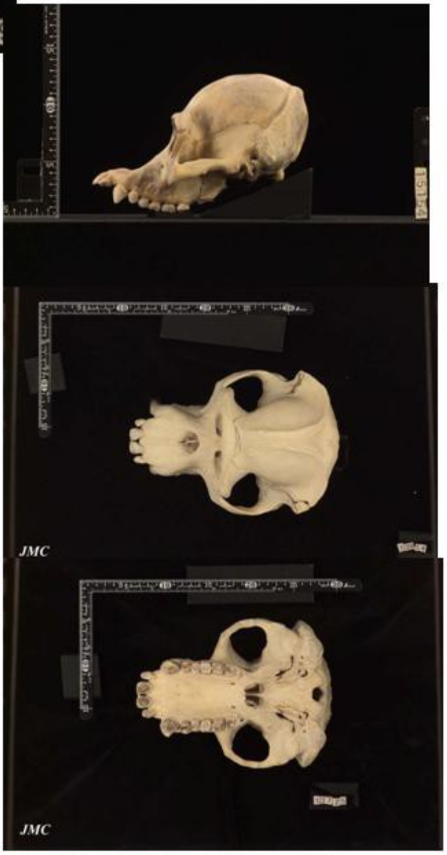
Dactylomys boliviensis



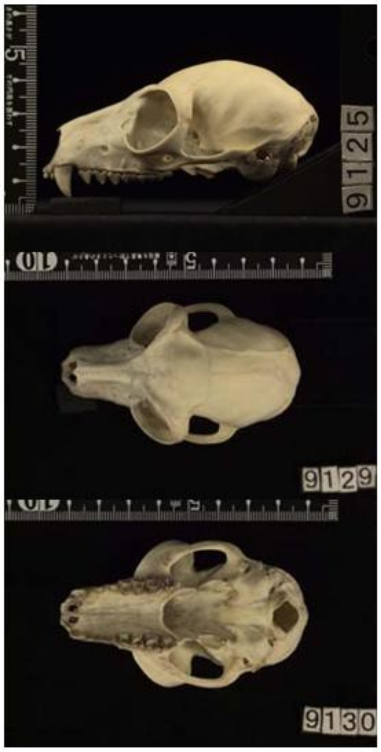
Acomys spinosissimus



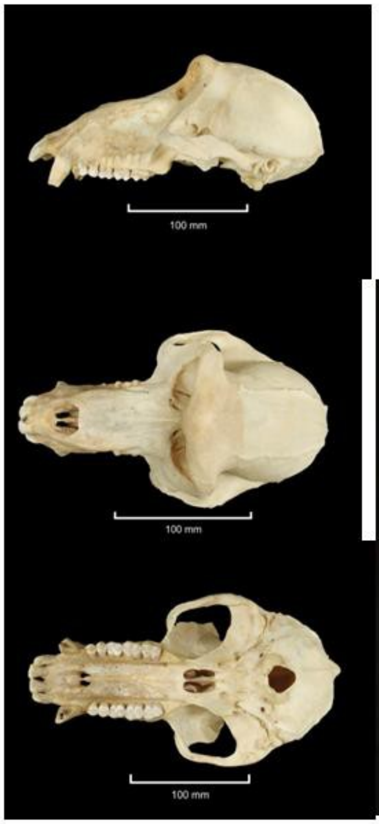
Galago crassicaudatus



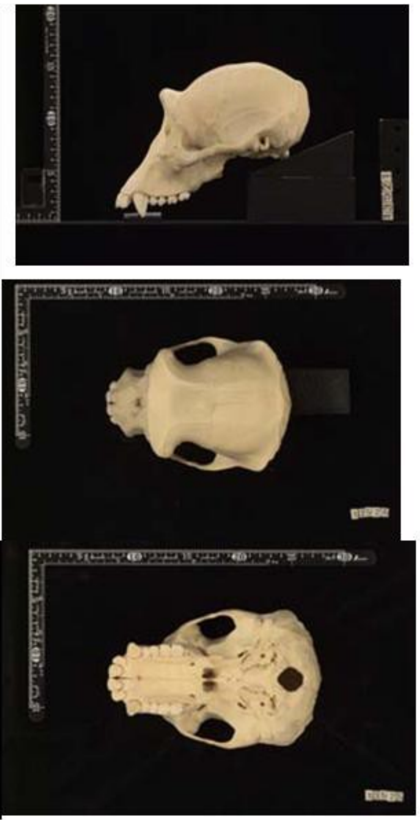
Pongo pygmaeus



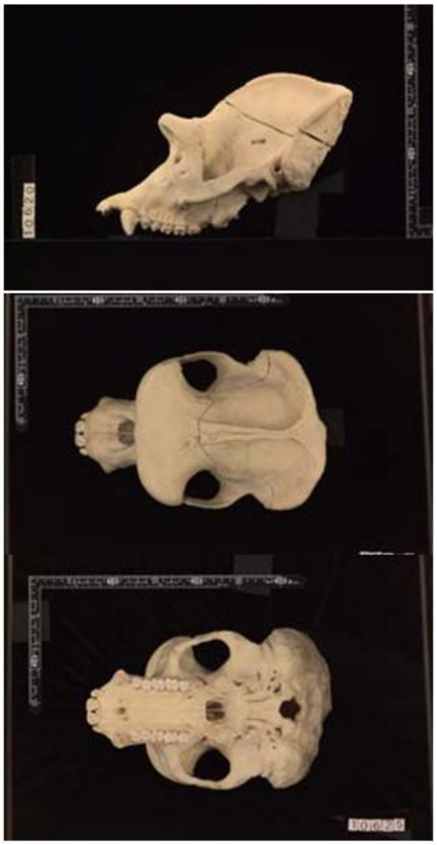
Lemur catta



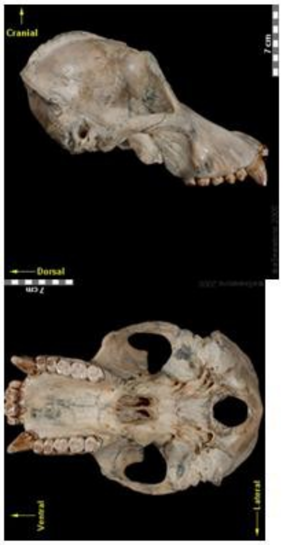
Papio cynocephalus



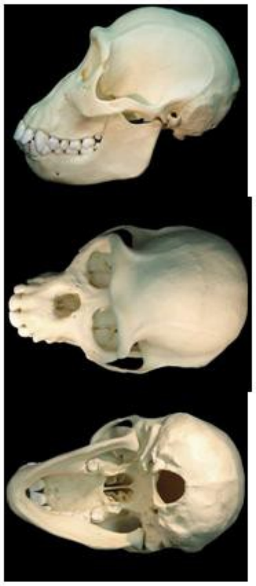
Pan troglodytes



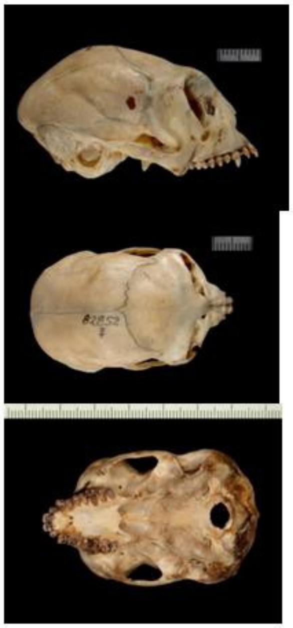
Gorilla gorilla



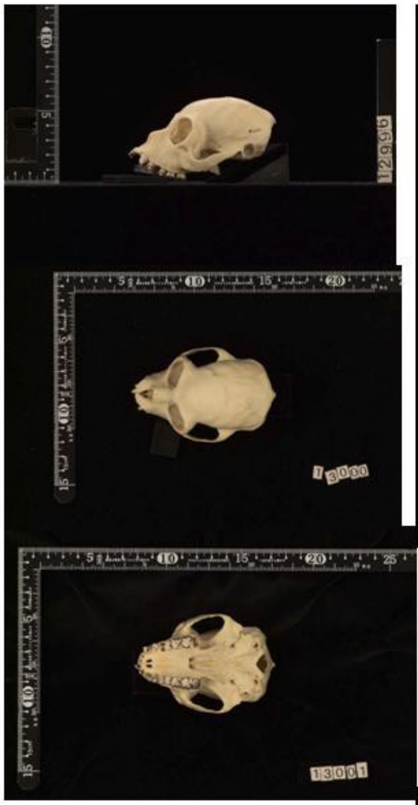
Pongo abelii



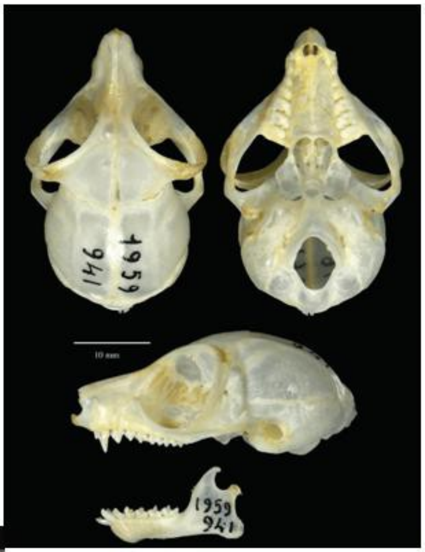
Pan paniscus



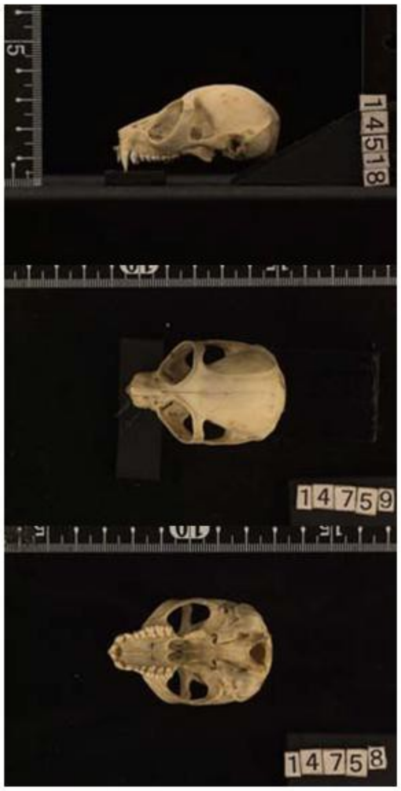
Callicebus donacophilus



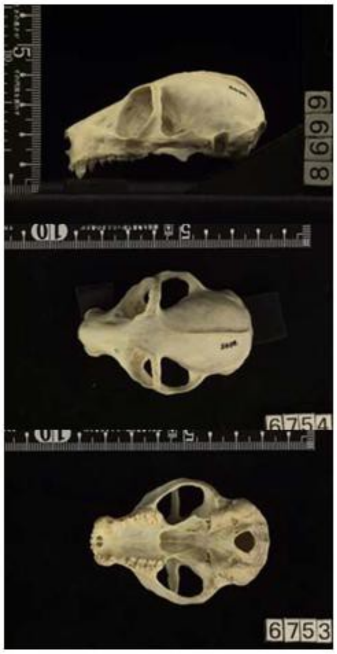
Alouatta caraya



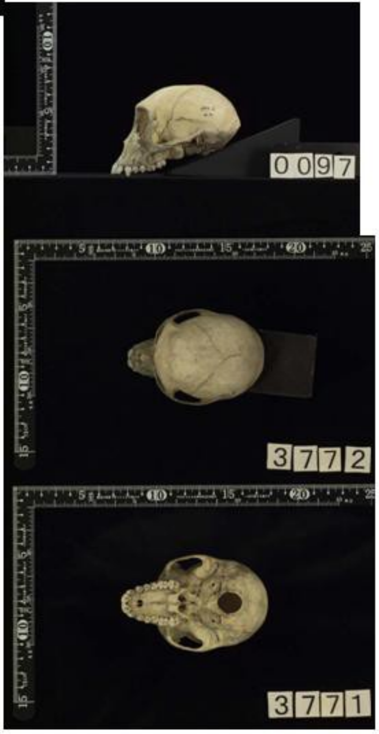
Galagoides demidoff



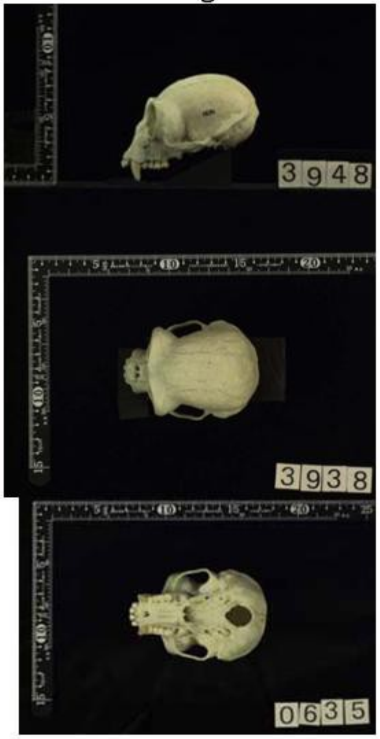
Loris tardigradus



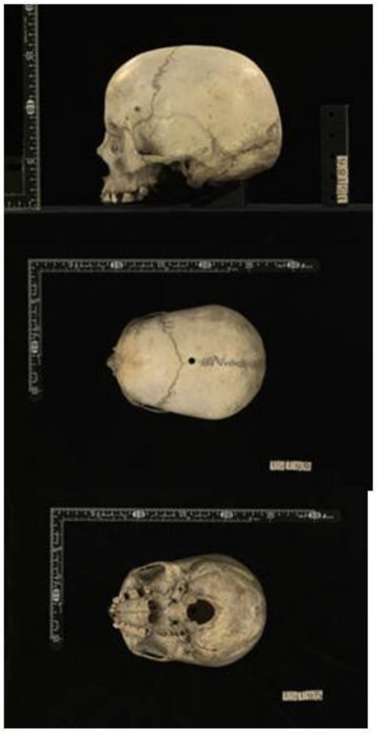
Otolemur garnettii



Macaca nemestrina



Hylobates gabriellae



Homo sapiens

Acomys spinosissimus

Saimiri sciureus

Dactylomys boliviensis

Galago crassicaudatus

Pongo pygmaeus

Lemur catta

Papio cynocephalus

Pan troglodytes

Gorilla gorilla

Pongo abelii

Hylobates gabriellae

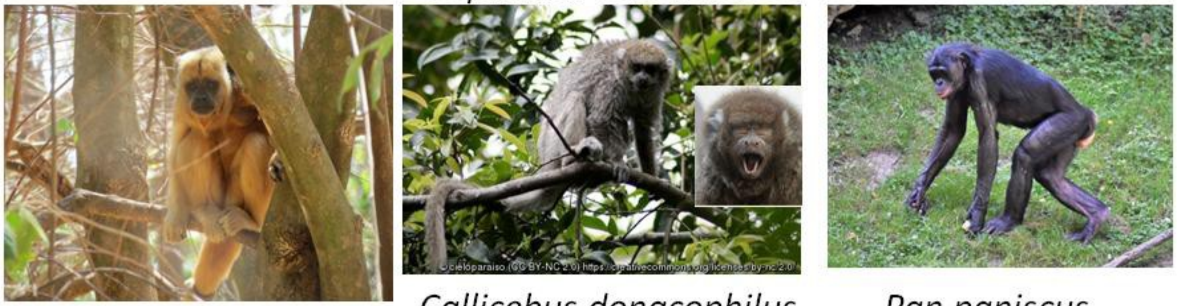
Galagoides demidoff

Otolemur garnettii

Loris tardigradus

Lepilemur edwardsi

Macaca nemestrina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Alouatta caraya

Callicebus donacophilus

Pan paniscus

Homo sapiens

Figure 2. Skull anatomy and body morphology of primate and other species

Table 1. Additional characteristics to assist in determining clade position							
Species	Fingernails not claws	Prehensile tail	Opposable thumb	Wet nose, big olfactory	Dry nose, less olfactory	Nares down	Nares fwd or to side	Rel. Brain Size
Acomys spinosissimus	N	N	N	Y	N	N	Y	S
Pongo abelii	Y	N	Y	N	Y	Y	N	L
Hylobates gabriellae	Y	N	Y	N	Y	Y	N	L
Galagoides demidoff	P	N	Y	Y	N	N	Y	M
Otolemur garnettii	P	N	Y	Y	N	N	Y	M
Loris tardigradus	P	N	Y	Y	N	N	Y	M
Lepilemur edwardsi	P	N	Y	Y	N	N	Y	M
Macaca nemestrina	Y	N	Y	N	Y	Y	N	ML
Alouatta caraya	Y	Y	Y	N	Y	N	Y	ML
Gorilla gorilla	Y	N	Y	N	Y	Y	N	L
Callicebus donacophilus	Y	N	Y	N	Y	N	Y	ML
Pan paniscus	Y	N	Y	N	Y	Y	N	L
Saimiri sciureus	Y	N	Y	N	Y	N	Y	ML
Dactylomys boliviensis	N	N	N	Y	N	N	Y	S
Galago crassicaudatus	P	N	Y	Y	N	N	Y	M
Homo sapiens	Y	N	Y	N	Y	Y	N	V
Pongo pygmaeus	Y	N	Y	N	Y	Y	N	L
Lemur catta	P	N	Y	Y	N	N	Y	M
Papio cynocephalu	Y	N	Y	N	Y	Y	N	ML
Pan troglodytes	Y	N	Y	N	Y	Y	N	L
Presence	Y = yes N = no P = partially							
Relative Brain size	S = small M=Moderate ML= moderately large L= large V= very large							

Table 2. Species in the Order Primates, plus two outlier species.

Acomys spinosissimus

Saimiri sciureus

Dactylomys boliviensis

Galago crassicaudatus

Homo sapiens

Pongo pygmaeus

Lemur catta

Papio cynocephalus

Pan troglodytes

Gorilla gorilla

Pongo abelii

Hylobates gabriellae

Galagoides demidoff

Otolemur garnettii

Loris tardigradus

Lepilemur edwardsi

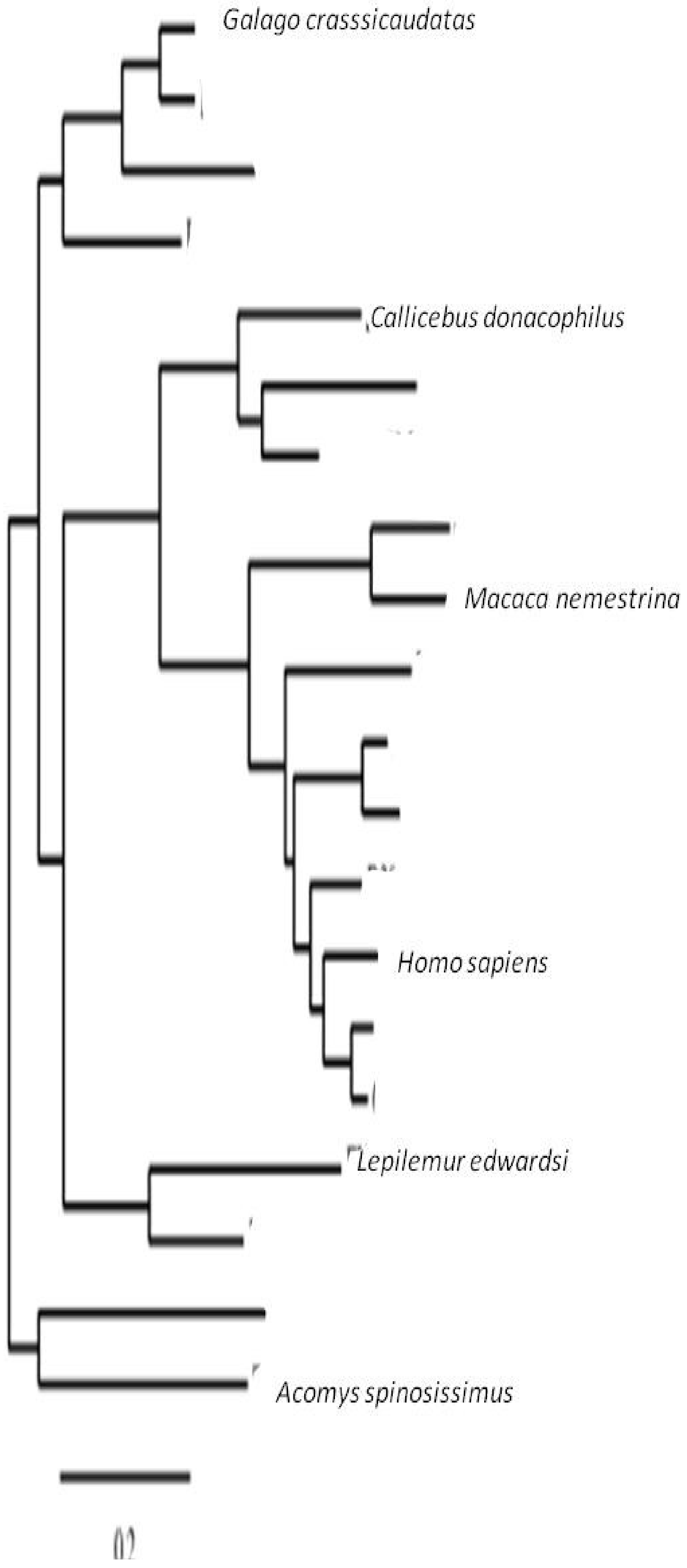
Macaca nemestrina

Alouatta caraya

Callicebus donacophilus

Pan paniscus

Part 1. Using the above pictures in Fig. 2 and characteristics in Table 1, try to develop a phylogenetic tree of the species listed in Table 2. Use the following tree scheme to fill in the species according to characteristics that you think are similar. To help you get started, a bracketing guideline for placement of the rest of the species is included. This consists of one outlier species , our species (*Homo sapiens*) , and four other species dispersed within the tree. Hints: taxonomy often reflects phylogeny. Do this with your background knowledge and the information at hand and do not resort to accessing additional information from the internet. You won't learn as much if you take shortcuts at this time.


Galago crasssicaudatas
Callicebus donacophilus
Macaca nemestrina
Homo sapiens
Lepilemur edwardsi
Acomys spinosissimus
0.2

Determining phylogeny on the basis of nucleotide sequence in genes.

With the increased ease of DNA sequence analysis, more and more data are being accumulated for genes of many species and are used to determine phylogeny. A change in the nucleotide sequence is termed a mutation and these will accumulate in a species over time. The greater amount of time that has passed since organisms diverged from a common ancestry the greater number of mutations will occur that will make differences in DNA sequences. For some parts of an organism's genome, the change has been so great that it would be difficult to determine phylogeny beyond the Genus or possibly family level. There are other genes that are highly conserved; genes associated with basic cellular metabolism common to most life are such examples. Genes that produce proteins involved in the electron transfer chain in mitochondria cannot change so much that it would result in lethality. Therefore the mutation rate is slow and can be used to separate organisms into larger phylogeny groups.

Part 2. We will be using the nucleotide sequence of the gene for Cytochrome B, a protein in the electron transport chain of mitochondria.

Step 1. Log into the website: https://www.ncbi.nlm.nih.gov/

2. In the drop down box where it says 'All Databases', click on the drop down arrow and scroll down until you find ' Nucleotide' and select it by clicking on it. It should then appear in the drop down box.

3. In the adjacent Search box copy and paste each of the following GenBank numbers individually and then click on 'Search'. A page should come up with the scientific name of the animal. If you did Part 1, the name may look familiar. Below and to the left of the name will be the term 'FASTA'.

4. Click on that and a page will appear with the GenBank number, Scientific name, gene description, followed by a N-base sequence for the gene. Highlight all of this including the GenBank number and scientific name, and copy and paste into a word file. Repeat steps 3 and 4 for each GenBank number except for the last four numbers in the list.

-------------------- GenBank number --------------------

Z96068.1

L23339.1

U53582.1

U53579.1

U09500.1

X97717.1

U53575.1

GQ148710.1

U38274.1

AY374378.2

KP410669.1

KP410663.1

KP410640.1

EU200437.1

EU204975.1
FJ785423.1:14147-15286
D38114.1:14169-15309
Y13307.1
GU189671.1:14171-15311
JF727211.2:14168-15308

5. For the last four accessions beginning with ‘F’ , ‘G’, or ‘J’ : after you copy and paste the accession as you did previously, a different page will appear. Click on the FASTA link within the boxed area and the correct sequence will appear. Copy and paste the sequence for each.

Getting the sequence for D38114.1:14169-15309 is a bit more difficult. First copy and paste only D38114.1 and not the rest (:14169-15309). Those numbers are the N base location for the gene on the chromosome. You will use that to find the gene on the ‘Graphic’ of the GenBank page. 1) click on the Graphic link. 2) locate the number sequence (ranging from 1 to 16,364)below the tool bar. 3) find the gene located below the range of 14168 to 15309. It’s a rough estimate, but you will find it easily enough; there is only the one gene there. 4) run your cursor over the gene and a popup window will appear. 5) find the FASTA Record (it will include the range you have used) and click on it. The page with the cytochrome b gene sequence will open. 6) Copy and paste as you did with the other accessions.

Note: Each of your copied FASTA results should look like this (Figure 3.):

The GeneBank number occurs first followed by the scientific name of the species and the description of the gene. The lines of genetic code will follow that. Make sure you copy the '>' at the beginning of the information

```
>AB185152.1 Euroscaptor malayana mitochondrial cytb gene for cytochrome b, complete cds, isolate:
SIK0557
ATGACAAACATCCGAAAAACACACCCTCTAATAAAAATCATCAACAACTCATTCATTGATCTACCAGCAC
CATCCAACATTTCATCCTGGTGAAACTTCGGATCACTACTTGGAATTTGCCTAACTCTGCAAATCCTCAC
AGGATTATTCCTAGCAATACACTATACCTCAGACACAACAACAGCCTTCTCATCCGTGACCCACATCTGC
CGAGATGTAAACTATGGCTGACTTATTCGATATCTACACGCAAACGGAGCATCAATATTCTTTATCTGTT
TATTCCTGCATGTAGGACGAGGCCTATACTACGGATCTTACATATTTATAGAGACCTGAAACATCGGAGT
TATCCTACTTTTCACCGTAATAGCCACTGCATTCATAGGGTATGTTTTACCGTGGGGTCAAATATCCTTT
TGAGGGGCCACAGTAATTACAAATTTACTGTCAGCCATTCCATACATCGGAACAGACCTAGTAGAATGAA
TCTGAGGGGGTTTCTCAGTAGATAAAGCAACACTCACACGATTCTTCGCCTTTCACTTCATCCTACCATT
TATCATTGCAGCCCTAGCAGGAGTACACCTGTTATTCTTACACGAAACAGGCTCCAACAATCCATCAGGA
CTCTCATCAGACACAGACAAAATCCCATTCCACCCATACTACACTATCAAAGACATCCTAGGAGCACTAG
TAATGATCCTAGCCCTATCAATTCTAGTACTATTTTCACCCGACCTCCTAGGAGACCCAGACAACTATAT
CCCAGCCAACCCACTGAACACACCACCCCATATTAAGCCAGAATGATATTTCTTATTTGCATATGCCATC
CTACGATCCATCCCTAACAAACTAGGAGGTGTGCTAGCACTAGTATTCTCAATCCTTATCCTAGCACTCA
TGCCACTCCTACATACCTCAAAACAACGAAGCATAATATTCCGACCCATCAGTCAGTGTTTATTCTGACT
ACTAGTAGCCGACTTACTCACCCTAACATGAATTGGAGGACAGCCAGTAGAGCACCCATTCATCATCATT
GGCCAACTAGCATCAATTCTCTACTTCGCACTTATCCTAATCCTTATGCCACTAGCAAGCCTAATAGAAA
ACAACCTCCTTAAATGAAGA
```

Figure 3. Example of copied FASTA result for a species' cytochrome b gene.

At this time you should have a word document file or equivalent that has the nucleotide sequence of the cytochrome b gene for 20 organisms. If you wish, you can save the file to complete the project later.

Step 6. Access the web site http://www.phylogeny.fr/index.cgi

7. Scroll down until you find 'Phylogeny analysis'

8. Click on the ' "One Click" ' . The page where you will enter your data set will appear.

9. Open your word file and copy the entire file. You can use 'select all' to speed up the process prior to selecting 'copy'.

10.Paste the file in the indicated box.

11. Click on 'Submit'

12. The phylogeny tree will be generated in a few minutes

13. In our case we want to reroot the outgroup . To do this locate the 6th action that can be selected. It will indicate 'reroot' (outgroup).

14. Click on that action and then go to the tree and find the internal branch with the sister taxons of *Acomys spinosissimus* and *Dactylomis boliviensis.* The hand cursor (pointer) should appear and you should click with it on the line just to the left of the ancestral node of the two species.

15. The phylogeny tree should reset and the tree branch of these two species will be placed as an outgroup.

16. **Your phylogeny tree of primate species based on the cytochrome B gene nucleotide sequence differences among species is complete! You can download the tree as selected from several file types or take a screenshot of it.** FYI: The short bar located below the tree with a value of '0.2' is the scale bar for the amount of genetic change that has occurred in each lineage. The number represents the number of mutations (excluding deletions) per nucleotide for the gene. If a branch length is longer than the scale bar then that species had more mutations than that since it diverged from its ancestor; if the length is less, then mutations would be less than '0.2'. The greater the length is, the higher chance for more divergence and additional species that may not be in the tree.

--------------------------------------Alternative Tree Generating Procedure---

IF (and only IF) you are having incompatibility problems with the software on the Phylogeny.fr website, you can do the nucleotide sequence analysis using the software on the Clustal Omega website.

Before starting this, here is a helpful hint: the accession number is at the front of the sequence. You can put an easier species identifier right after the ' > ' that will show up when you run the Clustal Omega program. Example: replace ' > Y13307.1 Hylobates gabriellae mitochondrial DNA for cytb gene ' with ' > Hylobates_gabriellae (space) Y13307.1 (space) (pertinent descriptors) ' . By doing this, only 'Hylobates_gabriellae' will appear in the results for this accession instead of the accession number. Be sure to include the underscore symbol (_) between the Genus and species name (all continuous, no spaces) to avoid problems.

Step 6a. Access the website https://www.ebi.ac.uk/Tools/msa/clustalo/ .

7a. Select 'DNA' as the set you will paste into the box.

8a. Copy your word file (you can use 'select all') and paste it into the box in the website.

9a. Leave all the default settings as is.

10a. 'Submit'

11a. It will take a while for the program to run; be patient.

12a. Upon completion, the Results page will appear. Move the cursor to the 'Phylogenetic Tree' tab and click on it. This will reveal the phylogenetic tree. This result is fairly representative, but with a bit more tweaking you can produce one that would be even closer to the one generated by the Phylogeny.fr website software.

13a. When the the Job Results page appears for your initial submission, click on the 'Results Viewers' tab. Scroll to the bottom of the page and find 'Send to Simple Phylogeny' and click on it. The 'Simple Phylogeny' page appears.

14a. Step1 of that page should show your original Clustal job number has already been entered.

15a In Step 2, the options you need to change are the 'Distance Correction' [on]; 'Exclude Gaps' [on] , and change 'P.I.M.' to 'on'. The 'Clustering Method' should remain 'Neighbor joining' and 'Tree Format' should remain as 'default'. (P.I.M.=percent identity matrix)

16a. Click on 'submit'. The phylogeny cladogram and the PIM should arrive soon. Take a screen shot of the cladogram.

17a. FYI the number after the species name is the Distance Value. This is the number of nucleotide substitutions (mutations) as a proportion of the alignment (gene), excluding gaps, that has occurred to the species since it diverged from its ancestor. The larger the number is, the higher chance for more divergence and additional species that may not be in the tree.

Part 3.Results of the two phylogenetic trees.

1. Compare the tree based on morphology and skull anatomy you filled in to the tree based on the cytochrome B gene constructed with software. Were you able to correctly identify both outlier species? What order of mammals do you suspect they are in? Which area of the trees was most different between the two trees and which areas were most similar? Why do you think that occurred?

We will now focus on the phylogenetic tree based on nucleotide sequences.

2. Based on taxonomy, the phylogentic tree, and the Percent Identity Matrix (last page) which nodes represent the strongest sister taxons? Do you think these sister taxons would have more nucleotide differences or fewer nucleotide differences compared to the differences between sister taxons that are not as closely related.

3. Why do the other node pairings like the branch at *Macaca nemestrina* and *Papio cynocephalus* suggest other species not in the tree may have closer ancestry to either of these species? Use the percent identity matrix located on the last page of the exercise to confirm your conclusion.(Hint: Is this phylogenetic tree of primates complete? (Consider the Distance Value)).

4. What species are the nearest relatives to humans? Is the nearest ancestor of these species the same nearest ancestor to humans? Explain.

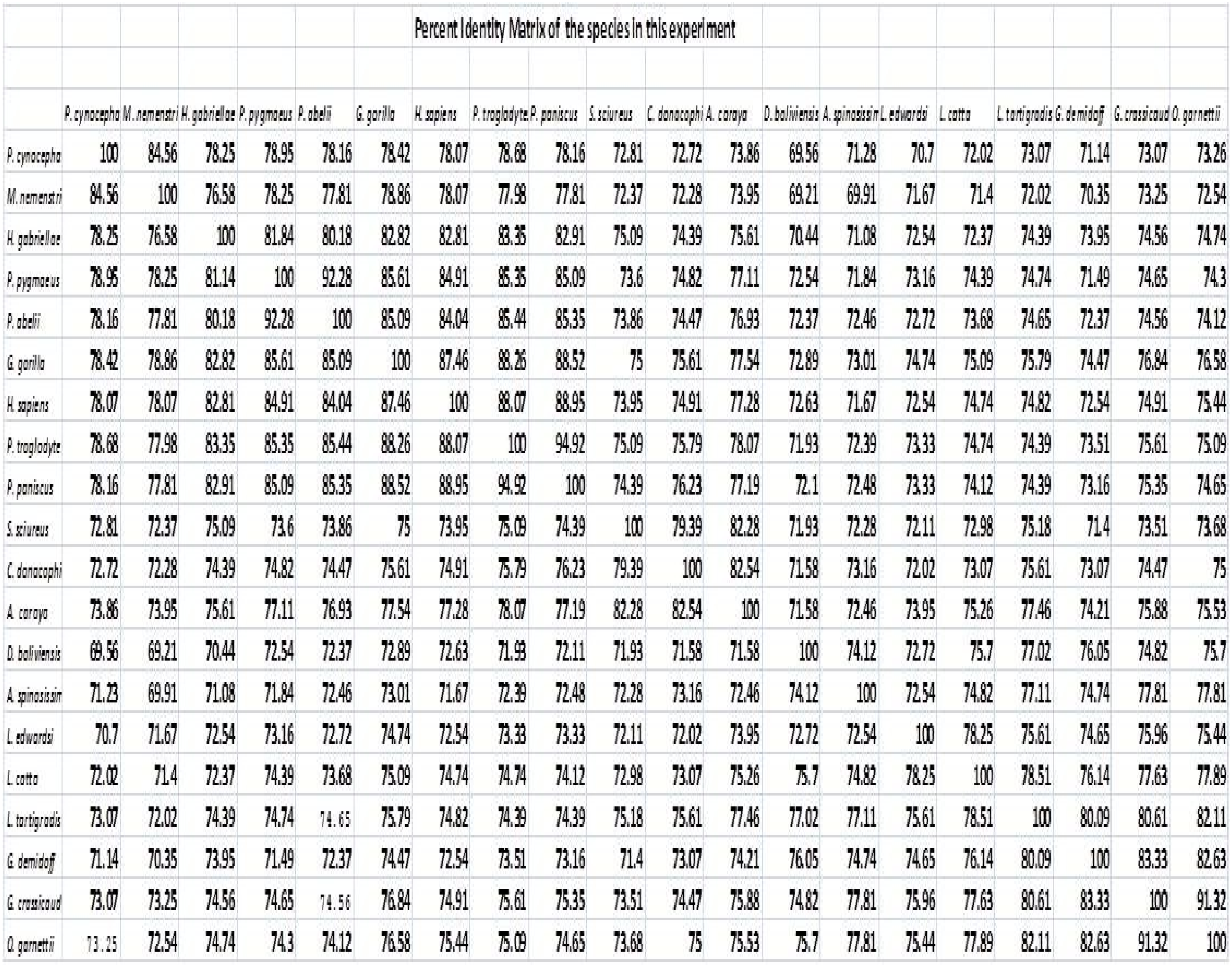
5. Which species in this tree belong to the superfamily Hominoideae, the family of humans and apes?

6. In that group, which species was the earliest to diverge from the lineage leading to humans?

7. This lineage diverged from what group of monkeys; old world or new world? Hint: Which species in the tree are in the lineage of 'old world monkeys' and which are in the lineage of 'new world monkeys' and which group is closer to Hominoideae in this phylogenetic tree?

8. Cytochrome b is a transmembrane protein in the inner membrane of mitochondria. It consists of approximately 400 amino acids. The average similarity in the nucleotide sequence in our study was about 75% . With this amount of mutation in this gene, how can it still be functional for all these organisms? (Hint: think about makeup of the universal genetic code , the *types* of mutations that can occur, and amino acid function according to classifications.)

9. Now that the study is completed, what do you think could additionally be done to confirm the phylogeny of these species?



Percent Identity Matrix of the species in this experiment

	P. cynocepha	M. nemenstri	H. gabriellae	P. pygmaeus	P. abelii	G. gorilla	H. sapiens	P. tragladyte	P. paniscus	S. sciureus	C. donacophi	A. caraya	D. boliviensis	A. spinosissin	L. edwardsi	L. catta	L. tartigradis	G. demidoff	G. crassicaud	O. garnettii
P. cynocepha	100	84.56	78.25	78.95	78.16	78.42	78.07	78.68	78.16	72.81	72.72	73.86	69.56	71.28	70.7	72.02	73.07	71.14	73.07	73.26
M. nemenstri	84.56	100	76.58	78.25	77.81	78.86	78.07	77.98	77.81	72.37	72.28	73.95	69.21	69.91	71.67	71.4	72.02	70.35	73.25	72.54
H. gabriellae	78.25	76.58	100	81.84	80.18	82.82	82.81	83.35	82.91	75.09	74.39	75.61	70.44	71.08	72.54	72.37	74.39	73.95	74.56	74.74
P. pygmaeus	78.95	78.25	81.14	100	92.28	85.61	84.91	85.35	85.09	73.6	74.82	77.11	72.54	71.84	73.16	74.39	74.74	71.49	74.65	74.3
P. abelii	78.16	77.81	80.18	92.28	100	85.09	84.04	85.44	85.35	73.86	74.47	76.93	72.37	72.46	72.72	73.68	74.65	72.37	74.56	74.12
G. gorilla	78.42	78.86	82.82	85.61	85.09	100	87.46	88.26	88.52	75	75.61	77.54	72.89	73.01	74.74	75.09	75.79	74.47	76.84	76.58
H. sapiens	78.07	78.07	82.81	84.91	84.04	87.46	100	88.07	88.95	73.95	74.91	77.28	72.63	71.67	72.54	74.74	74.82	72.54	74.91	75.44
P. tragladyte	78.68	77.98	83.35	85.35	85.44	88.26	88.07	100	94.92	75.09	75.79	78.07	71.93	72.39	73.33	74.74	74.39	73.51	75.61	75.09
P. paniscus	78.16	77.81	82.91	85.09	85.35	88.52	88.95	94.92	100	74.39	76.23	77.19	72.1	72.48	73.33	74.12	74.39	73.16	75.35	74.65
S. sciureus	72.81	72.37	75.09	73.6	73.86	75	73.95	75.09	74.39	100	79.39	82.28	71.93	72.28	72.11	72.98	75.18	71.4	73.51	73.68
C. donacophi	72.72	72.28	74.39	74.82	74.47	75.61	74.91	75.79	76.23	79.39	100	82.54	71.58	73.16	72.02	73.07	75.61	73.07	74.47	75
A. caraya	73.86	73.95	75.61	77.11	76.93	77.54	77.28	78.07	77.19	82.28	82.54	100	71.58	72.46	73.95	75.26	77.46	74.21	75.88	75.53
D. boliviensis	69.56	69.21	70.44	72.54	72.37	72.89	72.63	71.93	72.11	71.93	71.58	71.58	100	74.12	72.72	75.7	77.02	76.05	74.82	75.7
A. spinosissin	71.23	69.91	71.08	71.84	72.46	73.01	71.67	72.39	72.48	72.28	73.16	72.46	74.12	100	72.54	74.82	77.11	74.74	77.81	77.81
L. edwardsi	70.7	71.67	72.54	73.16	72.72	74.74	72.54	73.33	73.33	72.11	72.02	73.95	72.72	72.54	100	78.25	75.61	74.65	75.96	75.44
L. catta	72.02	71.4	72.37	74.39	73.68	75.09	74.74	74.74	74.12	72.98	73.07	75.26	75.7	74.82	78.25	100	78.51	76.14	77.63	77.89
L. tartigradis	73.07	72.02	74.39	74.74	74.65	75.79	74.82	74.39	74.39	75.18	75.61	77.46	77.02	77.11	75.61	78.51	100	80.09	80.61	82.11
G. demidoff	71.14	70.35	73.95	71.49	72.37	74.47	72.54	73.51	73.16	71.4	73.07	74.21	76.05	74.74	74.65	76.14	80.09	100	83.33	82.63
G. crassicaud	73.07	73.25	74.56	74.65	74.56	76.84	74.91	75.61	75.35	73.51	74.47	75.88	74.82	77.81	75.96	77.63	80.61	83.33	100	91.32
O. garnettii	73.25	72.54	74.74	74.3	74.12	76.58	75.44	75.09	74.65	73.68	75	75.53	75.7	77.81	75.44	77.89	82.11	82.63	91.32	100

The intersection between a species on the Y axis and a species on the X axis is the percent similarity of the Cyt B gene for the two species.

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