

Are Birds Bats? Uniprot and BLAST Analysis

See lecture questions 75, 95, 98, 122-123, 127, 137

Annotating Text	
<input type="checkbox"/>	<u>UNDERLINE</u> concepts you think might be useful for understanding or solving the problem
<input type="checkbox"/>	<div style="border: 1px solid black; display: inline-block; padding: 2px 5px; margin-right: 5px;">Box</div> information you think might be helpful for designing your investigation
<input type="checkbox"/>	← Write notes in the left margin
<input type="checkbox"/>	→ Write questions and answers in the right margin
Each paragraph (including each step of the procedures) must have something underlined or boxed, AND have something written in the margins (a question and/or note).	

How does an evolutionary biologist decide how closely related two different species are? The simplest way is to compare the physical features of the species (their “morphologies”). This method is very similar to comparing two people to determine how closely related they are. We generally expect that brothers and sisters will look more similar to each other than two cousins might. If you make a family tree, you find that brothers and sisters share a common parent, but you must look harder at the tree to find which ancestor the two cousins share. Cousins do not share the same parents; rather, they share some of the same grandparents. In other words, the common ancestor of two brothers is more recent (their parents) than the common ancestor of two cousins (their grandparents), and in an evolutionary sense, this is why we say that two brothers are more closely related than two cousins.

Similarly, evolutionary biologists might compare salamanders and frogs and salamanders and fish. More physical features are shared between frogs and salamanders than between frogs and fish, and an evolutionary biologist might use this information to infer that frogs and salamanders had a more recent common ancestor than did frogs and fish.

This methodology certainly has problems. Two very similar looking people are not necessarily related, and two species that have similar features also may *not* be closely related. Comparing morphology can also be difficult if it is hard to find sufficient morphological characteristics to compare. Imagine that you were responsible for determining which two of three salamander species were most closely related. What physical features would you compare? When you ran out of physical features, is there anything else you could compare?

Instructions:

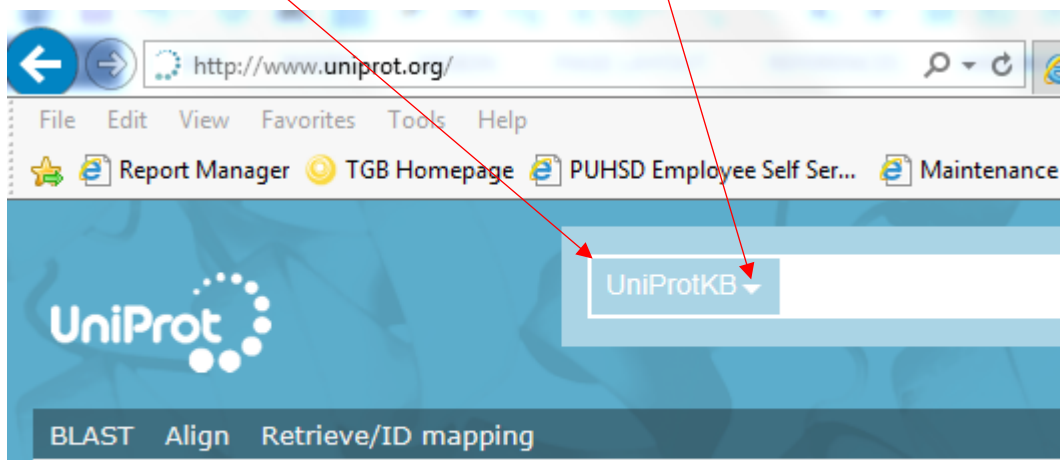
Fill in data table 1 and answer the questions

Table 1: Morphological comparison of birds, bats, and other non-bat mammals			
Feature	Birds	Bats	Other mammals
Presence of hair			
Presence of feathers			
Presence of mammary glands			
Presence of wings			
Homoeothermic			
4-chambered heart			

1. What other morphological features could you use to compare these organisms?
2. Explain why you think bats are more closely related to birds or other mammals.

1) Go to <http://www.uniprot.org/>

Make sure UniProtKB is selected (click on drop down menu if necessary)



The mission of UniProt is to provide the scientific community with a comprehe

- 2) Type in **hemoglobin beta** and then click search on the far right of the page



- 3) Use the right hand scroll bar to scroll through the entries. Find 2 for bats, 2 for birds, and 2 for any other mammal. Make sure it is for the hemoglobin subunit beta without a number, alpha, or other hemoglobin subunit. Write both the common name and scientific names in Table 2.

	Hemoglobin subunit beta	HBB	Homo sapiens (Human)
	Hemoglobin subunit beta-1/2	HBB1 HBB2	Oryctolagus cuniculus (Rabbit)
	Hemoglobin subunit beta-2	hbb2	Xenopus laevis (African clawed frog)
	Hemoglobin subunit beta-A		Capra hircus (Goat)
	Hemoglobin subunit gamma-1	HGB1, PRO2979	Homo sapiens (Human)

- 4) Click on the box to the far left of the organism you want to use in your comparison, scroll back to the top and then add to your basket.

<input checked="" type="checkbox"/>	P02117	HBB_AN SAN		Hemoglobin subunit beta	HBB
<input checked="" type="checkbox"/>	P68873	HBB_PANTR		Hemoglobin subunit beta	HBB

in beta"

About UniProtKB Basket 3

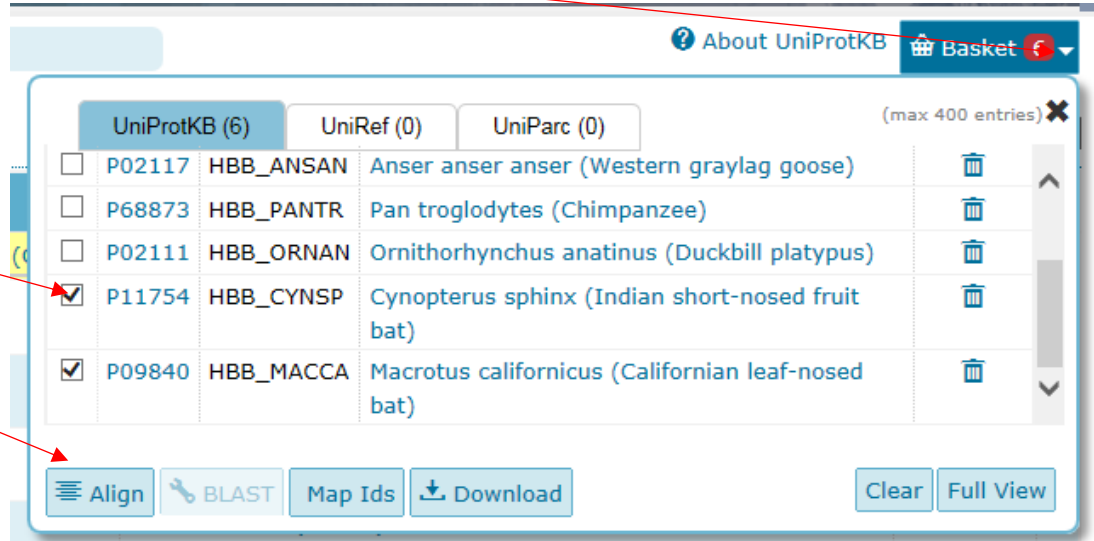
LAST Download Add to basket Columns

1 to 250 of 2,564 Show 250

Entry	Entry name	Protein names	Gene names	Organism	Length
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- 5) Continue until you have all 6 organisms in your basket
6) Add the common names of your organisms to data table #2

7) Click on the drop down arrow to view your basket



8) Select two organisms

9) Click Align and wait patiently

Interpreting results:

```
P11754 HBB_CYNXP 1      VHLSGEEKSAVTSLWGGKVKVDEVGGEALGRLLVVYPWTQRFFDSFGDLSSASAVMGNKV      60
P09840 HBB_MACCA 1      VHLTGEEKSTVSALWGGKVNVEEIGGEALGRLLVVYPWTQRFFDSFGDLSSPSAVFGNAKV      60
***:*****:*.:.*****:*.:.*****:*****:*****:*****:*****:*****:*****
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Each letter represents an amino acid. An asterisk (*) means the amino acid is identical, a colon (:) means the two amino acids have very similar properties, a period (.) means the two amino acids have somewhat similar properties, and a space indicates the amino acids are different

- 10) Scroll down to find the percent the sequences are identical and record in data table 2
- 11) Calculate the percent identical **AND** similar positions by adding similar positions to identical positions and dividing the sum by the number of amino acids in the protein
- 12) Repeat steps 7-11 until you have compared all organisms

Running time	26.8 seconds
Identical positions	131
Identity	89.726%
Similar positions	14

Table 2: The distance matrix for Part I (do not enter percentages for the grayed boxes)

<i>% identical</i> <i>% identical</i> <i>or similar</i>	Bat #1	Bat #2	Bird #1	Bird #2	Other mammal #1	Other mammal #2
Bat #1	100%					
Bat #2		100%				
Bird #1			100%			
Bird #2				100%		
Other mammal #1					100%	
Other mammal #2						100%

3. Use the distance matrix to construct a cladogram

4. Justify the cladogram above. A justification has 3 components: 1) scientific knowledge and/or theory; 2) specific data from your analysis related to the knowledge; and 3) and explanation of HOW the data supports the knowledge.

5. Would you expect bats to have hemoglobin beta chains more, less, or equally similar to the two bird species you chose?

6. Explain your answer to the previous question

7. How did your results compare to your expectations?

8. Explain any differences between what you expected and what the experimental results were.

PART II: "Whale Feet?"

Help a graduate student figure out what the feet of the ancestors of whales were like! She knows that whales are mammals and that, based on earlier studies, they very likely evolved from terrestrial four-legged hooved mammals (ungulates) which, over many generations and millions of years, gave rise to animals fully adapted to life in the sea: whales. She heard that whales could have evolved either from perissodactyl (odd-toed) or from artiodactyl (even-toed) ungulates, and that these two ungulate groups have distinct differences in their feet. Unfortunately, modern whales do not have feet so she cannot examine the morphology of those whales to figure out which is the more likely scenario. Help her use hemoglobin beta chain sequence data to evaluate these two hypotheses.

Procedure Part II:

Examine some specimens or pictures of whales, fish, and perissodactyl (odd-toed) and artiodactyl (even-toed) mammals, and take note of the morphological differences of these. Repeat the process used in Part I, but this time construct a distance matrix for one whale, one fish, two perissodactyl mammals, and two artiodactyl mammals.

9. Describe the morphological distinctions between perissodactyls and artiodactyls.

10. What are some examples of perissodactyl ungulates (minimum of 3)?

11. What are some examples of artiodactyl ungulates? (minimum of 3)

Table 3: The distance matrix for Part II (do not enter percentages for the grayed boxes)

% identical % identical or similar	Whale	Fish	Perissodactyl #1	Perissodactyl #2	Artiodactyl #1	Artiodactyl #2
Whale	100%					
Fish		100%				
Perissodactyl #1			100%			
Perissodactyl #2				100%		
Artiodactyl #1					100%	
Artiodactyl #2						100%

12. Draw a cladogram showing the evolutionary relationship among the organisms you chose

13. Is the whale hemoglobin more similar to the fish hemoglobin, or to the mammal hemoglobin?
14. Are whales fish? _____ Why?
15. Is the whale hemoglobin more similar to the hemoglobin of odd-toed mammals or even-toed mammals?
16. Was the hemoglobin of the whale much more similar to the hemoglobin of mammals of one type of foot than the other?
17. What problems do you see with using hemoglobin to determine which type of four-footed mammal whales evolved from? Hint: consider convergent evolution.

Part III: "Reptiles With Feathers?"

Some phylogenetic systematists (scientists who work to make the classification of organisms match their evolutionary history) complain that the vertebrate class Reptilia is improper because it should include birds. In technical terms, the vertebrate class Reptilia is *paraphyletic* because it contains some but not all of the species that arose from the most recent common ancestor of this group. Just how similar are reptiles and birds in terms of the beta-hemoglobin chain? Should birds be considered a type of reptile? You will evaluate this question in this exercise using a BLAST (Best Local Alignment Search Tool) search.

Procedure Part III:

Step 1: As in the previous two exercises, Begin by going to the UniProt database

Step 2: Find a hemoglobin beta chain for any type of crocodile. You can do this just as in the previous two exercises OR you can type "crocodile" into the search box instead. Make sure to select the hemoglobin *beta* chain without a number after it.

Step 3: A BLAST (Best Local Alignment Search Tool) search takes a particular sequence and then locates the most similar sequences in the entire database.

A BLAST search will result in a list of sequences with the first sequence being most close to the one entered and the last sequences being least similar.

The easiest way for us to do a BLAST search is using links within UniProt as follows:

Once you have found the hemoglobin beta chain of a crocodile, add it to your basket as you have done previously. Within your basket, select your species of crocodile and then click on “Blast” at the top of the page. It may take a few minutes for the results to appear.

Step 4: Scroll down to Alignments. There will be a list of sequences in order of similarity. List those species in Table 4 beginning with the first most similar species that is **not a crocodile**. To find the common name, hover your mouse over the scientific name.

If no common name comes up, click on the scientific name to see the taxonomy of the organism (Aves is the name of the class for birds).

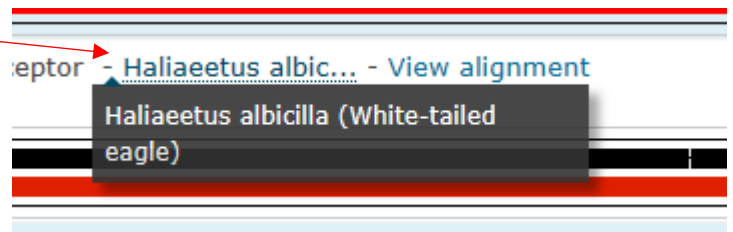
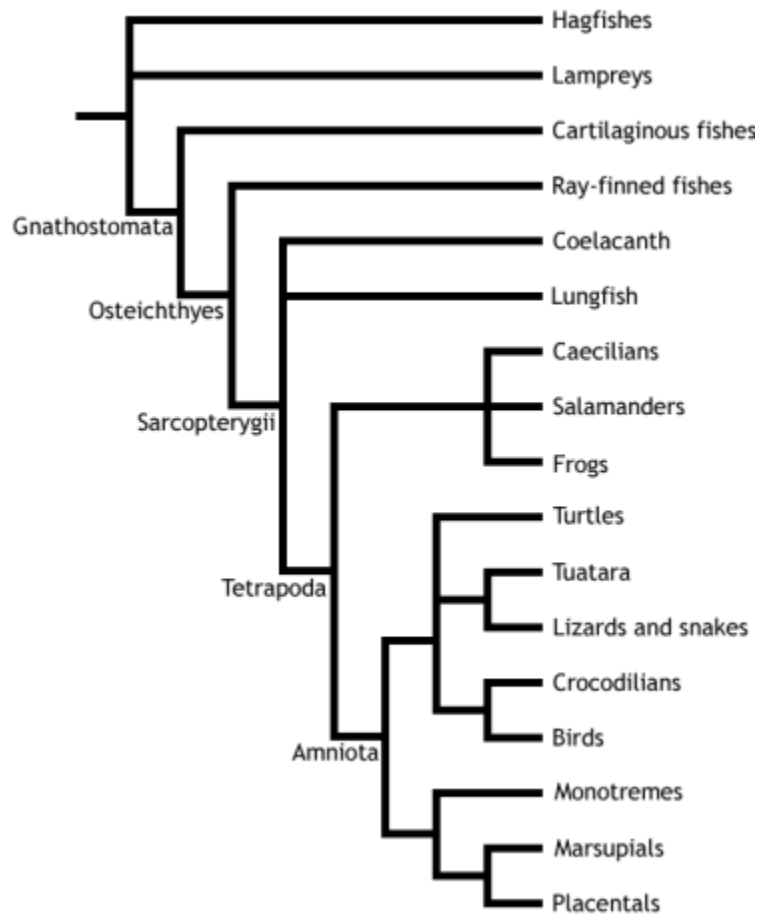


Table 4: results of a BLAST search on the crocodile hemoglobin beta sequence		
Similarity	Species common name	Sequence Identity
Starting with the 1 st non crocodile species		
1 st most		
2 nd most		
3 rd most		
4 th most		
5 th most		
6 th most		
7 th most		
8 th most		
9 th most		
10 th most		

18. What type of organism is most closely related to crocodiles? (birds or reptiles)

Many phylogenetic systematists believe that the names of taxa should include ALL the relatives of the most recent common ancestor of the group (in technical terms, they believe that the group should be monophyletic). A paraphyletic group contains the latest common ancestor, but excludes one or more of the extant relatives. A polyphyletic group contains 2 or more extant relatives, but excludes the latest common ancestor.

Reptilia is a taxon that includes organisms such as turtles, lizards, and crocodiles. Birds and mammals are not considered reptiles.



19. Is Reptilia a monophyletic, paraphyletic, or polyphyletic group?

20. Justify the cladogram above. A justification has 3 components: 1) scientific knowledge and/or theory; 2) specific data from your analysis related to the knowledge; and 3) and explanation of HOW the data supports the knowledge.