Name _____Sample Answer Sheet_____ Date___10/13/01_

The following sample answers are intended only as a guide. Note especially the HINTS and special notes alerting you to variations to be expected and special circumstances to consider...
The answers could vary for any of the sections that involve using the online databases. These online databases are updated (new sequences may be added) and answers can vary depending on which sequences the students actually choose to answer the questions!

TABLES FOR DATA ENTRY, QUESTIONS: Investigating Evolutionary Questions Using Online Databases

PART I TABLES AND QUESTIONS

1.1) What morphological features do bats share with mammals? With birds? Fill in **Table 1**.

TABLE 1: Morphological comparison of birds, bats, and other non-bat mammals

Feature	Birds	Bats	Other mammals
Presence of hair	No	Yes	Yes
Presence of feathers	Yes	No	No
Presence of mammary glands	No	Yes	Yes
Presence of wings	Yes	Yes	No
Homeothermy	Yes	Yes	Yes
Four chambers in heart	Yes	Yes	Yes

1.2) (other morphological features to compare?):__Presence of teeth (modern birds lack them), presence of a beak, lay eggs (or bare live young), presence of diaphragm to help lungs function (birds lack a diaphragm), which digits make up the wing of a bat versus the wing of a bird?

1.3) (bats more similar to birds, or to mammals?)__Except for the wing itself, bats share only some of the features that define birds, but share all the features that define mammals

TABLE 2: A list of the species used for Part I

Species	Common Name	Scientific Name
Bat species #1	Indian short-nosed fruit bat	*Cynopterus sphinx*
Bat species #2	California big-eared bat	*Macrotus californicus*
Bird species #1	White stork	*Ciconia ciconia*
Bird species #2	Domestic pigeon	*Columba livia*
Mammal species #1	Lowland gorilla	*Gorilla gorilla gorilla*
Mammal species #2	Horse	*Equus caballus*

TABLE 3: The distance matrix for Part I (No need to fill the x'd boxes; this just repeats information)
Hint, there are some common mistakes that students may make. First, it is easy to fail to copy the entire sequence. If the percent similarity looks very odd, you may want to make the student recopy and repaste their sequences into lalign. Also, some students might by mistake pick a hemoglobin subunit that is not the beta subunit. They might accidentally pick the fetal hemoglobin subunit or the alpha hemoglobin subunit of an animal (For example, the sequence P02082 is the Hemoglobin beta fetal chain (Hemoglobin gamma chain) for Capra hircus (Goat).) Also, a few sequences are fragments and not the entire beta chain: Q9TT34 is only a fragment of the Hemoglobin beta chain of Pongo pygmaeus (Orangutan).) Although this is probably uncommon, there are a few sequences in lalign where there are number of X's in the sequence of amino acid, again indicating that the researcher failed to sequence the entire chain. The one case I know of is: P02076, Hemoglobin beta chain - Ovis orientalis musimon (Mouflon).

	Bat #1	Bat #2	Bird #1	Bird #2	Mammal #1	Mammal #2
Bat #1	100%	89.7	68.5	69.9	85.6	84.9
Bat #2	XXXX	100%	66.4	67.1	84.2	82.9
Bird #1	XXXX	XXXX	100%	86.3	68.5	65.8
Bird #2	XXXX	XXXX	XXXX	100%	68.5	69.2
Mammal #1	XXXX	XXXX	XXXX	XXXX	100%	82.9
Mammal #2	XXXX	XXXX	XXXX	XXXX	XXXXXX	100%

ANSWER THE FOLLOWING QUESTIONS (based on Table 3):

3.1) Which two species in the above table have the most similar beta-hemoglobin chains?

The two bat species.

3.2) Which two species in the above table have the *least* similar beta-hemoglobin chains?

The stork and the California big-eared bat.

3.3) For **bat #1,** make a list of species that have the *most* similar beta-hemoglobin sequence to the *least* similar:

a. (most similar): California Big-Eared Bat 89.7 % identity
b. Lowland Gorilla 85.6 % identity
c. Horse 84.9 % identity
d. Domestic Pigeon 69.9 % identity
e. (least similar): White Stork 68.5 % identity

3.4) For **bat #2**, make a list of species that have the *most* similar beta-hemoglobin sequence to the *least* similar:

a. (most similar): Indiana short-nosed fruit bat 89.7 % identity
b. Lowland Gorilla 84.2 % identity
c. Horse 82.9 % identity
d. Domestic Pigeon 67.1 % identity
e. (least similar): White Stork 66.4 % identity

3.5) Does this information seem consistent with the hypothesis that bats are mammals? Yes

3.6) Are bats more closely related to other mammals than to birds? Yes Why do you say this?
Bats share many more characteristics with mammals than with birds. For example, they have hair, mammary glands, and they don't lay eggs. The hemoglobin beta chain of bats also seems more similar to mammals than to birds.

PART II DATA TABLES and QUESTIONS (from Procedure)

TABLE 4: Species used for Part II

Species	Common Name	Scientific Name
Whale species	Minke whale (Lesser rorqual).	*Balaenoptera acutorostrata*
Fish species	Atlantic cod	*Gadus morhua*
Odd-toed mammal #1	Horse	*Equus caballus*
Odd-toed mammal #2	White rhinoceros	*Ceratotherium simum*
Even-toed mammal #1	Hippopotamus	*Hippopotamus amphibius*
Even-toed mammal #2	Pig	*Sus scrofa*

4.1) Examples of perissodactyls (at least 3) horses, asses (donkeys) and zebras, tapirs, Rhinoceros

4.2) Examples of artiodactyls (at least 3) camels, llamas, alpacas, vicugnas, guanacos, pigs, hogs, Tayassuids, hippopotamus, camels, llamas, alpacas, vicugnas, guanacos, mouse deer, chevrotain, giraffes and okapis, Musk deer, deer, pronghorn antelope, gazelles, African antelope, buffalo, mountain goats, yaks, and domesticated species such as cattle, sheep, and goats (There are yet others that aren't listed here!)

4.3) Morphological distinctions (differences) between perissodactyls and artiodactyls:

Artiodactyls are even-toed mammals. By "even-toed", we mean that they stand on two toes (the third and fourth digits). In contrast, perissodactyls are odd-toed mammals. Most perissodactyls stand on three toes for their hind feet. (However, they *may* stand on four for their front feet.) In some perissodactyls (horses), the animals stand on just one toe.

TABLE 5: Distance matrix for Part II (No need to fill the x'd boxes; this just repeats information)

The minke whale sequence is probably the only whale sequence your students will find.

	Whale species *(minke)*	Fish species *(cod)*	Odd-toed mammal #1 *(horse)*	Odd-toed mammal #2 *(rhino)*	Even-toed mammal #1 *(hippo)*	Even-toed mammal #2 *(pig)*
Whale species	100%	43.8	84.2	84.9	88.4	84.2
Fish species	XXXX	100%	42.5	41.8	41.8	40.4
Odd-toed mammal #1	XXXX	XXXX	100%	94.8	83.6	82.2
Odd-toed mammal #2	XXXX	XXXX	XXXX	100%	84.2	80.8
Even-toed mammal #1	XXXX	XXXX	XXXX	XXXX	100%	87.0
Even-toed mammal #2	XXXX	XXXX	XXXX	XXXX	XXXX	100%

Hint*, students can check to make sure they have picked an artiodactyl or perissodactyl by looking at the taxonomy of the animal they choose in SWISS-PROT. This appears on the SWISS-PROT screen before you retrieve the FASTA formatted sequence. All artiodactyls will have the following beginning to their taxonomy:*

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; ***Cetartiodactyla***

All perissodactyls will have the following beginning to their taxonomy:

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; ***Perissodactyla***

Students may be clever enough to figure out themselves that whales are classified in SWISS-PROT as artiodactyls.

5.1) Is the whale hemoglobin more similar to the fish hemoglobin or the mammal hemoglobin? ___Mammal_
(Are you convinced that whales are not fish!? Why?)
Minke whale beta hemoglobin chain shares less than 50% of the amino acids with those of the Atlantic cod. The Minke whale shares more amino acids with other animals for the hemoglobin beta chain.

5.2) Is the whale hemoglobin more similar to the hemoglobin of odd-toed mammals or even-toed mammals?
OK, yes the students may find this frustrating! The answer depends on which animals you pick. In the animals chosen here, if we average the two odd-toed similarities and the two even-toed similarities, you would find that the Minke whale shares more amino acids with the even-toed mammals. You might get the opposite answer if you did not choose the hippos but chose a reindeer instead. If you pick sheep and hippos as your even-toed animals, then both hemoglobin sequences of the even-toed animals are more similar to the whale than are the odd-toed sequences. I use this to illustrate that comparing molecules is not always going to give you the "right" answer, and I ask students to discuss why not. What is the solution? Perhaps to look at many different molecules (not just hemoglobin). If you look at just the titles of the scientific papers which use molecular data to try to place whales, you find that many molecules have been used, and that scientists are trying to bring together all that data to come to a consensus about the correct origin of whales. The recent fossil data has been perhaps more helpful than the molecular data! Another solution might be to look at many artiodactyls and perissodactyls. This is a solution that you and your class can actually undertake. At the time I did the exercise, here are the perissodactyls and artiodactyls that I could find. I've listed them just by their sequence abbreviations and put the percent similarity to the minke whale in parentheses:
Artiodactyls:
HBB_ALCAA (86.8)
HBB_BISBO (86.1)
HBB_BOSGF (88.2)
HBB_BOSMU (86.8)
HBB_BOVIN (87.5)
HBB_CAMDR (84.9)
HBB_HIPAM (88.4)
HBB_LAMGL (84.2)
HBB_OVIMU (fragment; 75.0)
HBB_PIG (84.2)
HBB_RANTA (83.3)
HBB_SHEEP (86.1)
HBB_TRAST (88.9)
HBB_TURTR (91.8)
Perissodactyls
HBB_CERSI (84.9)
HBB_EQUHE (84.2)
HBB_HORSE (84.2)
HBB_RHIUN (83.6)
The students are unlikely to pick HBB_TURTR as this is a dolphin. Even so, the species with the greatest similarity to whales in terms of HBB (hemoglobin beta subunit) are artiodactyls. If you look at the names of the scientific papers referenced you see that hippos are considered a close relative of whales and the hippo does score a high similarity for the HBB.

5.3.a) Was the hemoglobin of the whale much more similar to the hemoglobin of mammals of one type of foot than the other or just a little more similar?

No, the whale beta chain of hemoglobin is typically somewhere between 82-89% similar to the hemoglobin beta chains of hooved animals. *Another lesson from this is that you must pick your molecule carefully if you want to use molecular information to assess similarities. The hemoglobin molecule may not be variable enough among ungulates to be useful in trying to tell whether a whale is even- or odd- toed.*

5.3.b) With this in mind, what problems do you see with using your answer to 5.2 to conclude from which type of four-footed mammal whales evolved?

I would conclude that I need to examine other molecules and fossils to be sure whether whales belong with the artiodactyls or perissodactyls.

Return to the second paragraph under the **Procedure** for Part II.

5.4) If your conclusion is different, propose some reasons why.

See the answers above.

PART III DATA TABLE and QUESTIONS

TABLE 6: Results of a BLAST search on the crocodile beta-hemoglobin sequence

Similarity	Species name & name of protein
First most similar (do not use crocodile)	Hemoglobin beta chain - *Alligator mississippiensis* (American alligator)
Second most similar	Hemoglobin beta chain - *Caiman crocodilus* (Spectacled caiman)
Third most similar	Hemoglobin beta chain - ***Turdus merula*** **(Blackbird)**
Fourth most similar	Hemoglobin beta chain - ***Passer montanus*** **(Tree sparrow)**
Fifth most similar	Hemoglobin beta chain - ***Phalacrocorax carbo*** **(Great cormorant)**
Sixth most similar	Hemoglobin beta chain - ***Accipiter gentilis*** **(Goshawk)**
Seventh most similar	Hemoglobin beta chain - *Chrysemys picta bellii* (Western painted turtle)
Eighth most similar	Hemoglobin beta chain - ***Anas platyrhynchos platyrhynchos*** **(Northern mallard)**
Ninth most similar	Hemoglobin beta chain - ***Vultur gryphus*** **(Andean condor)**
Tenth most similar	Hemoglobin beta chain - ***Eudyptes crestatus*** **(Rockhopper penguin)**

6.1) Were any of those species birds? __Yes_(see list, in bold)__

6.2) One unusual reptile is the tuatara, whose scientific name is *Sphenodon punctatus.* How similar is the tuatara to the crocodile?

_____They are both reptiles.

6.3) Does it appear in your list of ten? If not, how far down on the BLAST search list does it occur, fifteenth, twenthieth, etc.? (Hint: *Sphenodon punctatus* is abbreviated as "sphpu" in the NCBI blast search list.)

_____tuatara is 25th in the list_____

6.4) Most importantly, which species are more similar to the crocodile? (birds, or other reptiles?)

___Although the caiman and alligator are similar to crocodiles, some birds are more similar to the crocodile than the turtle or the tuatara_in terms of the beta hemoglobin subunit_____

Many phylogenetic systematists believe that the names of taxa should include ALL the relatives of the most recent common ancestor of that group (in technical terms, they believe that the group should be "monophyletic"). If Reptilia is monophyletic, then all reptiles should be more closely related to the crocodile than any other non-reptilian group. If any other non-reptile is more closely related than a reptile, then the group is paraphyletic.

6.5) Do the molecular data suggest that Reptilia is paraphyletic, or monophyletic? Explain.

_____ __ Paraphyletic. If it were monophyletic, then turtles and the tuatara ought to be more similar to the crocodile than any birds. Of course, this assumes the hemoglobin beta chain is reflecting true evolutionary similarity of these organisms.

PART IV: QUESTIONS (if assigned):

IV-1) What animal came up that was most similar and that you recognized? Go look up your unknown animal in a textbook or at the library.

_____let students choose_

IV-2) What type of animal was it?

_____

IV-3) Did the BLAST search help you predict what type of animal it was? _____

IV-4) How important and of what use is it that the taxonomy and classification of organisms reflect the evolutionary relationships of organisms?

Different biologists might give you different answers to this question. Some of the advantages of having your taxonomy reflect evolutionary relationships is that then the names of an organism truly reflect the organism's geneaology. Furthermore, if the name reflects evolutionary relationships, then you may be able to gather information about that organism simply from the name (for example, if you know an organism belongs with the birds, you know that it lacks a diaphragm for bringing air into its lungs). You may also be able to infer what kinds of diseases or pathogens may affect that organism (since sometimes pathogens affect organisms of particular taxa).