Exploratory Activities for Understanding Evolutionary Relationships Depicted by Phylogenetic Trees: United but Diverse

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ABSTRACT

Evolution explains both the unity and the diversity of all organisms, and developing students' ability to represent and communicate evolutionary relationships is an important component of a complete biology education. We present a series of student-centered, exploratory activities to help students develop their tree-thinking skills. In these activities, students use complementary phenotypic and molecular data to explore how to build phylogenetic trees and interpret the evolutionary relationships they represent. This learning module is designed to engage students in the process of science, provide them with active learning experiences using online bioinformatics tools, and foster their appreciation for the evolutionary connections across the tree of life.

Key Words: *tree-thinking; cladogram; DNA sequences; evolution; bioinformatics; inquiry.*

○ Introduction

Understanding the evolutionary relationships among organisms is a central goal of biology (Darwin, 1859; Dobzhansky, 1973; Schluter, 2000; Grant & Grant, 2011) and a key component of a complete biology education (Baum et al., 2005; Gregory, 2008; Meisel, 2010; Young et al., 2013). Phylogenetic trees are one of the most important tools that evolutionary biologists use to depict the hypothesized relationships among a set of species. Because phylogenies illustrate the key evolutionary concept that all living organisms are connected through common ancestry, understanding phylogenies is critical for understanding evolution (Baum & Offner, 2008).

Numerous studies have shown that students have difficulty interpreting phylogenetic trees and understanding the evolutionary relationships they represent (Meir et al., 2007; Gregory, 2008; Halverson, 2011; Catley et al., 2013; Dees et al., 2014; Blacquiere & Hoese, 2016; Kummer et al., 2016). For example, many students misinterpret phylogenetic trees by reading the order of species along the tips (usually from left to right) to infer relatedness, and therefore make the common mistake of assuming that "primitive" species on the left give rise to "advanced" species on the right (Meir et al., 2007; Omland et al., 2008; Meisel, 2010). Similarly, many students get distracted by the shape of the tree and erroneously assume that species that appear in close proximity at the tips of the tree are more closely related than those further apart (Baum et al., 2005; Meir et al., 2007; Baum & Offner, 2008). The correct way to interpret a phylogenetic tree is as a set of nested hierarchies: trees can be rotated around any node and still depict the same evolutionary relationships. Teaching students to focus on the relative branching pattern of the tree helps emphasize that evolution is indeed a branching process, and that there is no single, linear narrative to describe the divergence of species (Baum et al., 2005; Meisel, 2010).

Science educators have argued that developing students' tree-thinking skills should be a priority for biology curricula (Baum et al., 2005; Catley, 2006; Meisel, 2010), and more educational resources are needed to train students to accurately read phylogenetic trees and understand what they communicate (O'Hara, 1997; Baum et al., 2005; Meisel, 2010; Halverson, 2011). Additionally, research indicates that student performance and conceptual understanding increases in classes that incorporate inquiry-based instruction (Minner et al., 2010; Freeman et al., 2014). In particular, the integration of web-based resources allows students to gather up-to-date information in virtually any subject area and become actively engaged in the learning process through exploration (Kuzoff et al., 2009; Satterthwait, 2010). Particularly among contemporary students who have grown up in a technology-driven world, the use of online resources has the potential to enhance classroom activities and invigorate science education (Mistler-Jackson & Songer, 2000; Jang, 2009; Kuzoff et al., 2009). We capitalized on the wealth of online bioinformatics resources to develop a series of inquiry-based activities aimed at improving students' tree-thinking skills. The learning module described here will help students recognize the evolutionary relationships among organisms, understand how these relationships are determined, and appreciate that phylogenetic trees are hypotheses that are subject to revision following new discoveries and analyses.

○ Alignment with *Next Generation Science Standards*

The activities described in this module allow students to engage in several Science and Engineering Practices of the *Next Generation Science Standards*, or NGSS (NGSS Lead States, 2013). Specifically, students will gain experience developing and using models; analyzing and interpreting data; constructing explanations; and obtaining, evaluating, and communicating information. By participating in the same practices used by scientists and engineers, students gain a more meaningful learning experience and a better understanding of the relevance of science, technology, engineering, and mathematics to their everyday lives (NGSS Lead States, 2013). This module also presents several NGSS Crosscutting Concepts that will help students organize interrelated knowledge from various scientific fields and develop a more coherent and scientifically based view of the world (National Research Council, 2012). In particular, the module helps to unite central concepts related to patterns, systems and system models, structure and function, and stability and change. Finally, this module addresses several NGSS Disciplinary Core Ideas that reflect unifying principles in life sciences, including structure and function, variation of traits, evidence of common ancestry and diversity, natural selection, and adaptation. These core ideas are essential for understanding the central organizing principle that evolution explains both the unity and diversity of life on Earth. We list the specific student activities in the module that are aligned with each NGSS dimension in Table 1.

○ Details of the Module

Learning Objectives

The goal of this learning module is to develop students' skills in building phylogenetic trees and interpreting the evolutionary relationships they represent. Students will gain firsthand experience in the construction of phylogenetic trees using both morphological and molecular data, and will use interactive worksheets and online phylogenetic resources to explore how phylogenetic trees are testable hypotheses that are updated over time following the addition of new data. Having completed the module, students will recognize that there is still uncertainty about the true evolutionary

Table 1. Alignment of student activities with *Next Generation Science Standards* (NGSS).

NGSS Dimension	NGSS Topics & Codes	Student Activities
Science and Engineering Practices	• Developing and using models • Analyzing and interpreting data • Constructing explanations • Obtaining, evaluating, and communicating information	• Construct phylogenetic trees as a visual representation of the evolutionary relationships among organisms. • Search online bioinformatics resources (NCBI website) to collect genetic data. • Use online phylogenetics software (Phylogeny.fr) to align DNA sequences and construct molecular phylogenies. • Make a prediction about the evolutionary relationships among present-day animals, and then test it using genetic data and phylogenetic analyses. • Evaluate the impact of new data by adding a "Discovery" species to the phylogenetic tree. • Compare the advantages of different types of data (morphological vs. molecular) for determining the evolutionary relationships among organisms.
Crosscutting Concepts	• Patterns • Systems and system models • Structure and function • Stability and change	• Group organisms based on their shared characteristics. • Construct phylogenetic trees to model evolutionary relationships. • Explain the relationship between DNA and protein production. • Compare if/how the phylogenetic tree changes with the addition of new data (the "Discovery" species).
Disciplinary Core Ideas	• Structure and function (LS1.A) • Variation of traits (LS3.B) • Evidence of common ancestry and diversity (LS4.A) • Natural selection (LS4.B) • Adaptation (LS4.C)	• Explain the relationship between DNA and protein production for the hemoglobin molecule used in the phylogenetic analyses. • Evaluate the variation in nucleotide sequences across the different animals on the Alignment page of Phylogeny.fr. • Observe that the nucleotide sequences for the hemoglobin gene vary among species, but that there are also many overlaps. • Identify the shared, derived characteristics among a group of organisms that make them adapted to their particular environments. • Consider how similar environments and lifestyles may lead to the evolution of similar phenotypic characteristics among distantly related species through the process of convergent evolution.

relationships among organisms, and that new evidence continues to improve our understanding of the tree of life.

Overview

This module was designed as a story in which students are applying for an internship at a local college. Stories help capture students' attention and keep them engaged in activities because they appeal to the human desire for goals (Wilson, 2002; Schultheis & Kjelvik, 2015). The module is presented in two sections: (1) a "Diagnostic Assessment" in which students investigate the relationships among fictitious alien species using solely morphological data; and (2) an "Application" in which students use online resources to gather real DNA sequences on present-day animals, construct a phylogenetic tree based on these genetic data, and then revise their tree after the addition of a new species. An educator's guide with step-by-step instructions for the entire module (Supplemental File 1), ready-made student worksheets and activity cards (Supplemental Files 2–4), handouts for students on how to use the online resources (Supplemental Files 5 and 6), and a complete educator's key (Supplemental File 7) are all provided as Supplemental Material with the online version of this article.

Section 1: Comparing Shared Characteristics among Fictitious Species (Days 1–2)

In the "Diagnostic Assessment" section, students compare the characteristics (i.e., phenotypes) among nine fictitious alien species using a character matrix, group the species on the basis of their shared characteristics, and then build a phylogenetic tree based on these groupings (Figure 1). The tree contains multiple gains and losses of traits across two major lineages, which are designed to challenge students' misconceptions that evolution is a linear, progressive process toward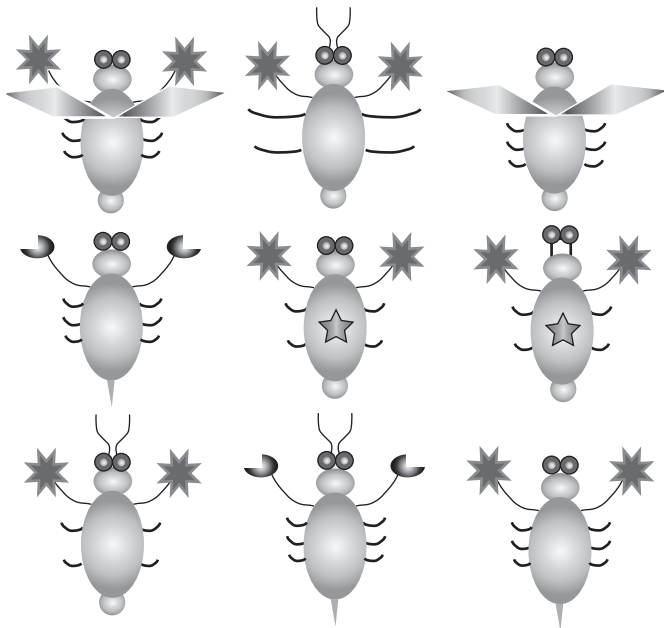 increasing complexity. This activity is designed to help students understand how shared, derived characteristics are used to infer evolutionary relationships, and that, in general, species with recent common ancestry will have more phenotypic characteristics in common.

Section 2: Using Online Tools to Construct a Real Phylogenetic Tree (Days 2–3)

In the "Application" section, students apply the tree-thinking skills they developed in the alien species activity to analyze the evolutionary relationships among 10 present-day animals: nine mammals and a bird (Figure 2). Students are asked to construct a phylogenetic tree for the 10 species based on their knowledge of shared phenotypic characteristics. Students then search the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov) to collect DNA sequences for the hemoglobin alpha protein for all 10 species, and use these molecular data to construct a phylogenetic tree with the web-based phylogenetic alignment and analysis software Phylogeny.fr (http://www.phylogeny.fr; Dereeper et al., 2008). Allowing students to explore computational resources used by scientists to collect genetic data, align DNA sequences, and construct molecular phylogenies helps them appreciate the importance of computational technologies for evolutionary analyses and scientific research.

Lastly, to mirror the discovery of a new species or the incorporation of new data, students are asked to select one of six possible "discovery" mammals to add to their phylogenetic tree: bat, platypus, hedgehog, tarsier, walrus, or whale. These organisms were intentionally selected to challenge students' understanding of
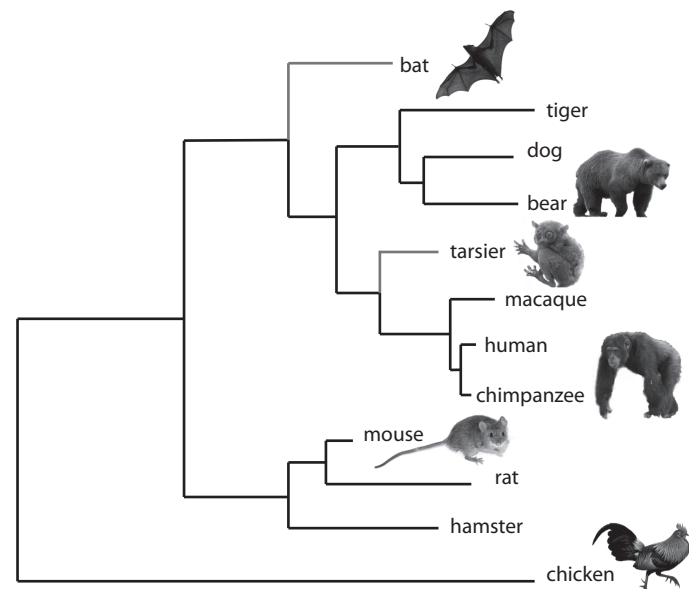
Figure 2. Phylogenetic tree depicting the evolutionary relationships among the animals used in the "Application" section of the module. The relative branching pattern was established using the online phylogenetic software Phylogeny.fr, based on the DNA sequences for the hemoglobin alpha protein. The original 10 species are indicated in black, and the addition of two "discovery" species is illustrated in red. All images are from Wikimedia Commons. Photo credits (top to bottom): Hasitha Tudugalle, Anbeast25, mtoz, Hans Hillewaert, George Shuklin, CK Subramanya.

Figure 1. Alien species used in the "Diagnostic Assessment" section of the module. Students are asked to group species on the basis of shared characteristics to infer their evolutionary relationships.

evolutionary relationships and foster discussion about convergent evolution. Students are asked to predict the position of the new species on their existing tree, and then test their prediction by collecting the DNA sequence data for the 11th species and constructing an updated phylogenetic tree. By engaging in the iterative process of reexamining and revising their evolutionary hypotheses, students gain experience with the process of science.

Target Student Audience

The primary target audience for this module is high school students enrolled in an Advanced Placement (AP) Biology course, an International Baccalaureate (IB) Biology program, or a higher-level evolution elective. However, the module can easily be adapted for a variety of student levels. For example, instructors of lower-level biology courses can distribute the DNA sequences of the animals in the "Application" section to the students directly if the task of collecting DNA sequence from the NCBI website may be too challenging. Ready-made handouts with the DNA sequences for the hemoglobin alpha protein of all 10 species in Card Set B and the six "discovery" species are available in the Supplemental Material (Supplemental Files 8 and 9). Alternatively, instructors of an undergraduate evolution course can expand the module by building a three-dimensional phylogenetic tree to tackle the misconception of relatedness that two-dimensional trees may convey, or by engaging the students in further discussions about the applications of phylogenetics in diverse disciplines (see "Beyond the Module" in Supplemental File 1).

Prior to starting these activities, students should be familiar with binomial nomenclature, homologous and analogous structures, evolutionary histories, and the basic methodology of building and interpreting phylogenetic trees. Students should work in pairs and will need approximately three 45-minute class periods to complete both the "Diagnostic Assessment" and "Application" sections.

Educator Comments

This module was assessed in a classroom of 20 high school seniors at KIPP NYC College Prep High School in the Bronx, New York. It was used as a summative assessment following a series of lessons on common ancestry, evolutionary relatedness, and phylogenetics. Students found the activities engaging, and the educator found that the module improved the students' overall understanding of evolution. Although some students were initially hesitant about "making a mistake" when asked to place animals on the phylogenetic tree, they enjoyed being able to predict and test their hypotheses about the evolutionary relationships of familiar and unfamiliar organisms. Importantly, some of the "mistakes" that students made about the arrangement of animals on the tree revealed common misconceptions about phylogenies, which led to valuable classroom discussions to address these misconceptions. Students also found it exciting to use the online resources and real genetic data. The inclusion of these technologies elicited an appreciation for scientists who regularly analyze large-scale molecular data, and for the speed at which large amounts of information can be processed with modern computers. Finally, the process of revising the phylogenies after the inclusion of new data led to fruitful discussions about tree-thinking and gave students firsthand experience with the iterative nature of science.

Conclusions

Developing students' tree-thinking skills should be a major goal for biology education in order to help them organize knowledge about biological diversity (Baum & Offner, 2008). The learning module presented here provides students with practical experiences using online technologies to explore phylogenetic trees and the evolutionary relationships they represent. The activities are designed to address several common misconceptions about phylogenetic trees, and to challenge students to reexamine and revise their predictions about evolutionary relationships using multiple types of data. By allowing students to collect, analyze, and evaluate real data using authentic bioinformatics tools, this module engages students in important scientific practices that will be applicable throughout their lives. We hope that these activities, or variations of them, will be useful to educators and spark student excitement about the evolutionary connections among all organisms.

Supplemental Material

The following resources are available with the online version of this article.

- Supplemental File 1: Educator's Guide
- Supplemental File 2: Student Handout
- Supplemental File 3: Card Set A – Alien Species
- Supplemental File 4: Card Set B – Present-Day Animals (including the six Discovery Species)
- Supplemental File 5: Resource no. 1 – How to Use NCBI
- Supplemental File 6: Resource no. 2 – How to Use Phylogeny.fr
- Supplemental File 7: Educator's Key to Student Handout
- Supplemental File 8: DNA Sequences for 10 Original Species (optional handout for lower level)
- Supplemental File 9: DNA Sequences for 6 Discovery Species (optional handout for lower level)

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UNITED, BUT DIVERSE

At a Glance

Learning Objective

Build phylogenetic trees and interpret the evolutionary relationships that they represent.

Student Objectives

Construct phylogenetic trees using complementary phenotypic and molecular data.

Use online resources to explore genomic information and molecular phylogenies.

Recognize that uncertainty exists about the true evolutionary relationships among organisms.

Acknowledge that new information continues to improve our understanding of the Tree of Life.

Introduction

This module is designed as a story in which students are applying for an internship at a local college. It is presented in two sections: a "Diagnostic Assessment" and an "Application".

In the first section, students investigate the relationships among fictitious alien species by comparing shared characteristics using morphological data.

In the second section, students use online resources to gather real DNA sequences on present-day organisms, construct a phylogenetic tree based on this genetic data, and then revise their tree after the addition of a new "Discovery" species.

Targeted Audience:

High school students in grades 11 and 12 (but can be adapted for higher or lower levels)

Duration of Exercise

Three to four class periods (45 minutes each)

Materials Needed

Student handout (One copy per student)
Card Sets A and B (One copy of each per pair)
Discovery Species Cards (Copies dependent on class size)
Online Resources #1 and 2 (One copy of each per pair)
Computer/Laptop
Colored Pencils
Scissors

Alignment with Next Generation Science Standards

The activities described in this module align with several Next Generation Science Standards (NGSS). Below, we highlight the NGSS dimensions that are most relevant to this module, and the specific skills that would demonstrate understanding of the core ideas.

Students who demonstrate understanding can:

HS-LS-1-1   Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.

HS-LS-3-2   Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

HS-LS-4-1   Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.

HS-LS-4-2   Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproductions, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.

HS-LS-4-3   Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.

HS-LS-4-4   Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

Science and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Developing and using models **Analyzing and interpreting data** **Constructing explanations** **Obtaining, evaluating and communicating information**	**Structure and function (LS1.A)** **Variation of traits (LS3.B)** **Evidence of common ancestry and diversity (LS4.A)** **Natural Selection (LS4.B)** **Adaptation (LS4.C)**	**Patterns** **Systems and system models** **Structure and function** **Stability and change**

Terms to Know

Analogous trait – A character that is similar in structure and function between species, but was not present in the most recent common ancestor. Analogous traits are the result of convergent evolution.

Binomial nomenclature – A formal system of naming species of organisms using a two-part Latin format (genus and species) that was instituted by Carolus Linnaeus in the 18th century. For example, the binomial name, or scientific name, for leopard is *Panthera pardus*.

Clade – A group of organisms that consists of a common ancestor and all of its descendants.

Cladogram – a branching diagram depicting the evolutionary relationships between groups of organisms.

Convergent evolution – the process in which organisms that are not closely related to one another independently evolve similar characteristics due to adaptations to similar environments or ecological niches.

Genotype – The genetic makeup of an organism.

Genus – A taxonomic rank that ranks above species and below family. In the system of binomial nomenclature, genus is the first term used to denote a species of organisms (e.g. *Panthera* for leopard).

Homologous trait – A character that is similar in structure and function between species, and is derived from the same character in the most recent common ancestor.

Most recent common ancestor (or MRCA) – The youngest common ancestor shared by two lineages. On a phylogenetic tree, the node at which two lineages meet represents the MRCA for those taxa.

Outgroup – A species or group of species that diverged *before* the lineage of the group of species that is being studied (the ingroup).

Phenotype – The set of observable characteristics of an organism that results from the interaction of its genotype and the environment.

Phylogenetic tree – A branching diagram that represents the evolutionary history of a group of organisms.

Phylogeny – The evolutionary history of a species or group of species.

Shared derived characteristic (or synapomorphy) – An evolutionary novel characteristic that distinguishes a clade from other organisms. It is present in the most recent common ancestor and then shared among the descendent species.

Species - The basic unit of biological classification and taxonomic rank of an organism. In the system of binomial nomenclature, the species name is the second term used to denote a species of organisms (e.g. *pardus* for leopard), and is also known as the specific epithet.

Educator's Instructional Suggestions

Section 1: Diagnostic Assessment – "Do you have skills to be an intern?"

A checklist of the tasks to complete is found on the first page of the student packet.

Teacher's discretion: student pairs may complete tasks together as a class or at their own pace.

Have Section 2 "Application" handouts and resources ready for pairs who finish quickly.

MATERIALS:

CARD SET A: Alien Species

1. (Optional) Laminate sheets for future use.

2. Before class, cut and organize one deck of alien cards for each pair of students.

3. Student worksheets may be collated together or printed as separate handouts.

ACTIVITY:

a) TASK #1 – Arrange the Alien cards using prior knowledge of Biology to hypothesize the evolutionary relatedness among the species.

b) Teacher can circulate around the room and question choices made by the students. *Why did you place this alien next to this one?*

c) TASK #2 – Complete the character matrix by noting which phenotypic characteristics are present or absent in each alien species.

d) TASK #3 – Fill in the phylogenetic tree by labeling the evolution of new (derived) characteristics at the appropriate arrows, and identifying the serial number for each species in the appropriate box.

e) Announce to students that they should leave room next to serial numbers to fill in the scientific name. (See Task #4)

f) TASK #4 – Follow the steps in the dichotomous key to identify the alien species.

g) TASK #5 – Answer the analysis questions. (See Educator's Key for answers and notes)

h) Debrief answers as a whole class.

i) Have student pairs submit for "approval" from Dr. Wallace-Darwin. (That's you!)

j) Students who finish quickly may work on level-appropriate multiple choice and/or open response questions for homework.

Section 2: Application – "You earned the internship!"

A checklist of the tasks to complete is found on the first page of the Application section (DNA border).

Teacher's discretion: Student pairs may complete tasks together as a class or at their own pace.

Have copies of RESOURCE #1 and RESOURCE #2 ready.

MATERIALS:

CARD SET B: 10 present-day animal species

1. (Optional) Laminate sheets for future use.

2. Before class, cut and organize one deck of animal cards for each pair of students.

3. Student worksheets may be collated together or printed as separate handouts.

DISCOVERY SPECIES CARDS

1. Six Discovery Species are included on the last sheet of CARD SET B.

2. Make as many copies as needed so that each pair of students receives one Discovery Species.

3. (Optional) Place one species in one envelope. Have a student pair select one envelope from your stack for their Discovery Species. *For example, 15 pairs of students (30 in total) should have at least 15 envelopes from which to select.*

ACTIVITY:

a) TASK #1 – Arrange the species cards using prior knowledge of Biology to hypothesize the evolutionary relatedness among the ten animals.

b) TASK #2 – In the box for Tree A, draw a phylogenetic tree that depicts the predicted evolutionary relationships.

c) TASK #3 – <u>Computers with internet access are needed.</u> Before using this module in the classroom, check that your school has access to the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). See your school administrator or technology specialist if necessary.

> **If students are searching the database for DNA sequences:**
>
> - Students must read RESOURCE #1 carefully and not skip any steps.
>
> - With this resource, students will use the NCBI website to look up the DNA sequence for the hemoglobin alpha protein for each species.
>
> - Open a Word Doc or Google Doc to paste all 10 species' sequences.
>
> - Do not close any tabs. You may need them later.
>
> **If educator is distributing DNA sequences:**
>
> - Educators can distribute the DNA sequences of the ten species to the students directly if the task of collecting DNA sequence from the NCBI website may be too challenging.
>
> - A file with the DNA sequences of hemoglobin alpha for all 10 species can be found in the supplementary materials.
>
> - The educator should email the DNA file to each student pair for them to input in TASK #4.

d) TASK #4 - <u>Computers with internet access are needed.</u> Prior to use in the classroom, check that your school has access to phylogenetic alignment and analysis website Phylogeny.fr. See your school administrator or technology specialist if necessary.

> **STUDENTS:** Must read RESOURCE #2 carefully. Students will build a computer-generated phylogenetic tree by inputting the collected FASTA files from NCBI and compare it to their predicted phylogeny (Tree A).

> **EDUCATOR:** Practice using Phylogeny.fr prior to administering in class for easier troubleshooting during the exercise. Feel free to contact the corresponding author (Erin McCullough) if needed.

e) TASK #5 – Students copy the generated tree into the box for TREE B.

> *Note: The branch lengths and red numbers indicate confidence estimates, but are not important for Tree B analysis.*

> - Students answer the accompanying analysis questions. (See Educator's Key for answers.)

f) TASK #6 – Announce to pairs that an 11th species has been discovered!

> - Have each student pair select an envelope containing a discovery species.

> - Write the species on the line provided in the student packet.

> - <u>PREDICT</u> the phylogentic position of the 11th species in relation to the original ten species, and draw a new branch for this species in TREE B using a colored pencil.

g) TASK #7 – Students should:

> - Use RESOURCE #1 to find the DNA sequence for the discovery species on NCBI.

> - Add the DNA sequence for the 11th species to the existing DNA sequence file.

> - Follow the instructions on the second page of RESOURCE #2 to add the name and DNA sequence for the 11th species.

> - Click "SUBMIT" to generate an updated phylogenetic tree

h) TASK #8 – Answer the analysis questions. (See Educator's Key for answers and notes.)

Beyond the Module – Extensions of Learning

1) Create a three-dimensional phylogenetic tree mobile

This activity tackles the misconception of relatedness that two-dimensional trees may convey. This extension allows students to physically pivot the character nodes to see the relationships more clearly.

2) Research: What sorts of information can be extracted from molecular data (i.e. DNA sequences)? Medical? Genetic? Historical?

3) Brainstorm: What are some other applications for phylogenetic trees?

Instructors may want to discuss how phylogenetics has become an important tool in a number of diverse disciplines, including Conservation biology (illegal whale hunting), Medicine (disease transmission events), and Agriculture (pesticide sensitivity).
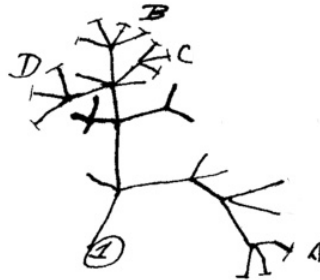
Phylogenetic Trees: United, but Diverse

Illustration from Charles Darwin's notebook (1837)

SCENARIO: The Biology Department at your local college is looking to hire paid interns who will assist in the development of the Evolutionary Relationships course (Bio 327). You and your lab partners are definitely interested in this opportunity and attend the information session.

You meet Dr. Wallace-Darwin, a Science Education research fellow, who is screening potential applicants for intern positions. She states that only applicants who successfully complete an initial diagnostic assessment will be offered one of the internships. Still interested, you request the assessment materials.

Section 1: Diagnostic Assessment – Alien Phylogenetics

Using CARD SET A, complete the following tasks and submit them for approval to Dr. Wallace-Darwin. Good Luck!

Task #1 – Arrange the cards based on shared characters to establish a hypothesis about the relationships between these species.
Task #2 – Complete Character Matrix 📝
Task #3 – Complete Phylogenetic Tree 📝
Task #4 – Complete Dichotomous Key 📝
Task #5 – Complete Analysis Questions
Request Teacher Feedback. Approved _____ Revised _____
Submit completed files for Dr. Wallace-Darwin's approval.

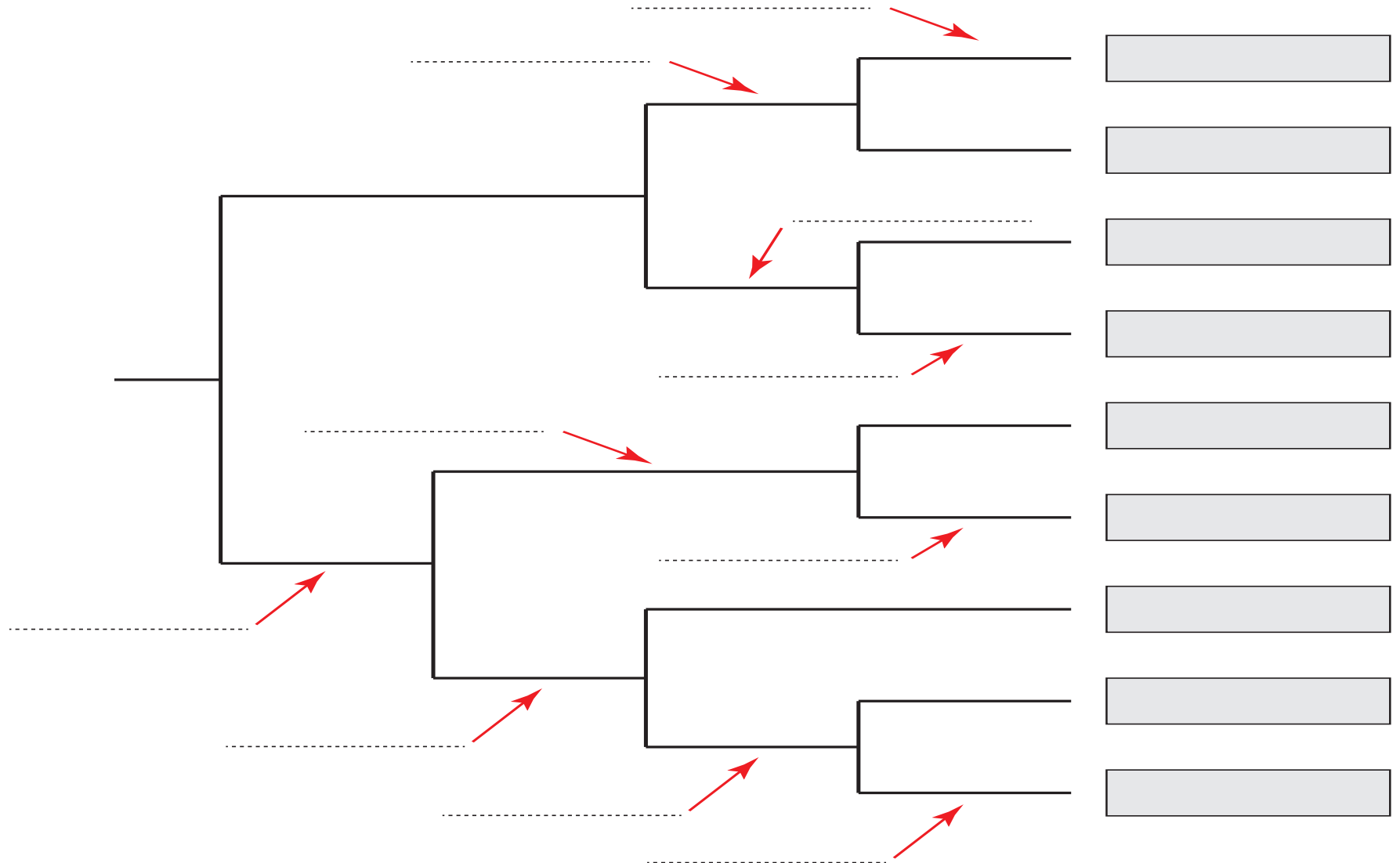📝 **= Indicates that a worksheet is needed**

Name: _____   **Character Matrix**

For each species, mark an "X" for each character present. Using the information in this matrix, arrange the species based on their shared characters to fill in the phylogenetic tree below.

CHARACTER	A2E	A7V	A4O	A6L	A1U	A3T	A9I	A5O	A8N
SIX LEGS									
WINGS									
STINGER									
STAR BELLY									
CLAWS									
LONG LEGS									
LOSS OF ARMS									
EYE STALKS									
ANTENNAE									

Instructions: Using the information from your character matrix, arrange the alien species based on their shared characters. Identify the evolution of new (derived) characters and label them at the appropriate branch on the tree. At the tip of each branch, identify the species using the dichotomous key provided.

Name: _____   **Dichotomous Key**

Instructions: A dichotomous key is an organized set of couplets of mutually exclusive characteristics that is used to identify biological organisms. Use the key below to identify each alien species. Write the genus species by the appropriate serial number at the end of this key.

1a – Has 4 legs..**Go to STEP 2**
1b – Has 6 legs...**Go to STEP 5**

2a – Has antennae...**Go to STEP 3**
2b – Has no antennae..**Go to STEP 4**

3a – Has long legs...*Globocorpus lata*
3b – Has short legs.. *Globocorpus auribus*

4a – Has star belly and no eye stalks.. *Globocorpus stellans*
4b – Has star belly and eye stalks.. *Globocorpus longoculus*

5a – Has wings...**Go to STEP 6**
5b – Has a stinger..**Go to Step 7**

6a – Has arms... *Hexapedes elegans*
6b – Has no arms... *Hexapedes volantis*

7a – Has claws... Go to Step 8
7b – Has no claws... *Hexapedes aculeus*

8a – Has antennae... *Hexapedes odoror*
8b – Has no antennae... *Hexapedes ungula*

A1U: _____   **A6L:**_____

A2E: _____   **A7V:**_____

A3T:_____   **A8N:**_____

A4O:_____   **A9I:**_____

A5O:_____

Analysis Questions of Diagnostic Assessment
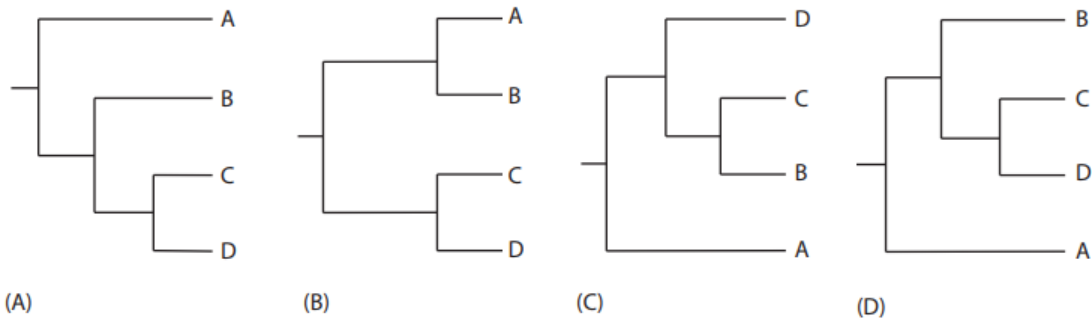
1. The phylogenetic tree that you completed in the lab investigation is also known as a:

    A) cladogram
    B) evolutionary diagram
    C) flow chart
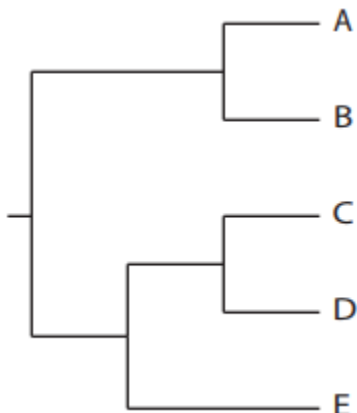   D) genetic map
    E) morphological diagram

2. Which of the following best explains what a cladogram is?

    A) a map showing the geographical location of species
    B) a visualization of the chromosomal similarities between species
    C) a diagram of the analogous similarities between species
    D) a diagram of the evolutionary relatedness of species with branches not drawn to scale
    E) a visualization of the location of genes on chromosomes

3. Which two phylogenetic trees (cladograms) depict the same evolutionary relationships between species A, B, C and D? Explain your answer.

(A)   (B)   (C)   (D)

4. Draw a new phylogenetic tree that depicts the same evolutionary relationships as the tree below. Your new tree must change the order of species in at least two positions.

STATE UNIVERSITY AT YORKVILLE
DEPARTMENT OF BIOLOGY

Dear Applicant:

Congratulations! Based on your performance, you have been selected as an intern for BIO 327: Evolutionary Relationships. We look forward to your participation and your valuable input.

Our department is gathering data on students' abilities to construct and interpret phylogenetic trees using molecular data. Using your knowledge of Biology, complete each activity and answer the accompanying analysis questions. Thank you in advance for your time and effort in helping us improve this course.

<u>Section 2: Application – Phylogenetic tree construction</u>

Task #1 – Using the ten species from CARD SET B, evaluate the phenotypic characteristics to predict the evolutionary relationships among these ten species.

Task #2 – Based on your prediction in Task #1, draw a phylogenetic tree in Tree A that illustrates the relationships among these species.

Task #3 – Collect DNA sequences for the hemoglobin alpha protein for all 10 species using the NCBI website. See Resource #1.

Task #4 – Analyze the DNA sequences to build a phylogenetic tree using the web-service Phylogeny.fr. See Resource #2.

Task #5 – Draw this phylogenetic tree in Tree B and complete the analysis questions.

Task #6 – Choose an 11th "discovery" organism and draw its predicted location on Tree B.

Task #7 – Collect the hemoglobin alpha DNA sequence for this species from NCBI, add it to previous data, and reanalyze the phylogenetic relationships using Phylogeny.fr.

Task #8 – Draw the updated phylogeny in Tree C.

Task #9 – Submit your completed application to Dr. Wallace-Darwin for evaluation.

Phenotypic vs. Molecular Data

Tree A: Construct a phylogenetic tree for 10 species based on phenotypic characteristics.

Tree A Analysis Questions:

a) Identify at least three characteristics that all 10 species have in common.

b) Explain how shared, derived characteristics are used to infer evolutionary relationships.

c) Select a group of closely related species from your phylogenetic tree and explain why you have grouped them together.

d) Select two species that are distantly related on your phylogenetic tree and describe at least two phenotypic characteristics that distinguish them from one another.

e) What additional data could be incorporated to strengthen your hypothesis about the evolutionary relationships between these species?

Tree B: Draw the phylogenetic tree for the 10 species generated by Phylogeny.fr based on DNA sequences. Your tree should reflect the correct branching order of these species.

Note: The branch lengths and red numbers that indicate confidence estimates are _not_ important for Tree B analysis. However, these data points may be needed in the future.

Tree B Analysis Questions:

a) Hemoglobin is an oxygen-transport protein that is found in the red blood cells of almost all vertebrates. It has four subunits: two alpha subunits and two beta subunits. Explain how this protein is produced from the DNA sequences you just collected.

b) Identify the similarities and differences between the phenotypic phylogeny (Tree A) and molecular phylogeny (Tree B)?

c) The phylogeny you built using molecular data correctly assigns the nine mammalian species to their respective orders (i.e., primates, carnivores, and rodents), and identifies the chicken as an evolutionarily distant outgroup. Are the specific branching patterns within these three orders the same as your original predictions in Tree A? Are the species in these orders equally related to one another? Explain.

Now choose a Discovery Species _____
Using a colored pencil, <u>predict where the Discovery Species</u>
<u>would fit in Tree B.</u> Draw it in.

d) Explain your reasoning for placing the Discovery Species in this phylogenetic position.

Tree C: Draw the revised phylogenetic tree generated by Phylogeny.fr after adding the DNA sequence of the 11th species.

For this analysis, *consider* the confidence estimates for each node, which are indicated in red.

Tree C Analysis Questions:

a) Is the phylogenetic placement of your Discovery species consistent with your original prediction? If not, identify an additional phenotypic characteristic(s) that supports the updated molecular phylogeny.

b) On the Phylogeny.fr screen, click on the 3. Alignment tab. What information is presented? How is it relevant to understanding the evolutionary relationships between species?

1. Overview   2. Data & Settings   3. Alignment

c) Today, you compared the evolutionary relationships among species using the DNA sequence for the hemoglobin alpha protein. If we repeated this analysis using the DNA sequence of another protein, would you predict the evolutionary relationships to stay the same? Why or why not?

d) You constructed a phylogenetic tree based on molecular data for a single protein. Would it be beneficial to repeat this analysis using molecular data from more than one protein? If so, why?

e) The numbers in red indicate the confidence estimates for each node. If you repeated the analysis using multiple proteins, would you predict most of the values to increase or decrease? Why?

f) Two species are that are distantly related based on the analysis of multiple DNA sequences also share a set of very similar phenotypic characteristics, such as the presence of wings in both bats and chickens. Consider and discuss what evolutionary process could lead to this pattern?

f) In this activity, you used several analyses and types of data to explain the evolutionary relationships among a set of present-day species. How do these activities model the process of science?
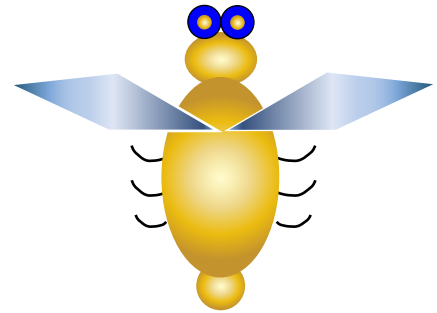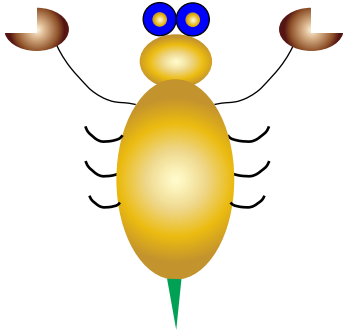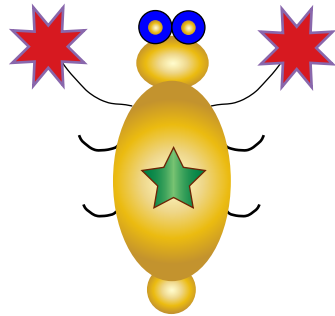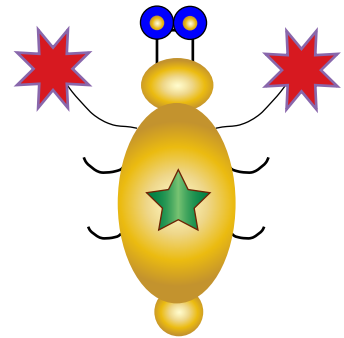
SET A - Alien Insects
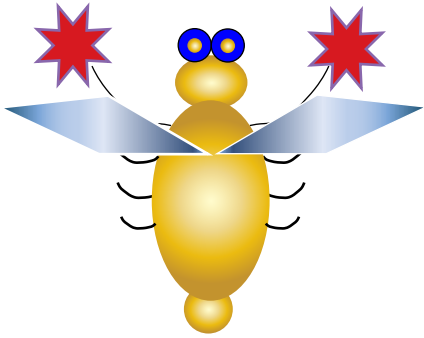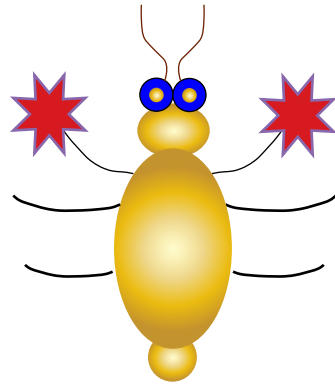
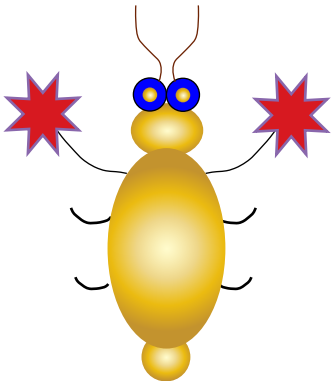A1U
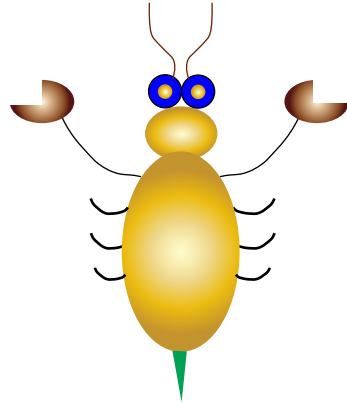A2E
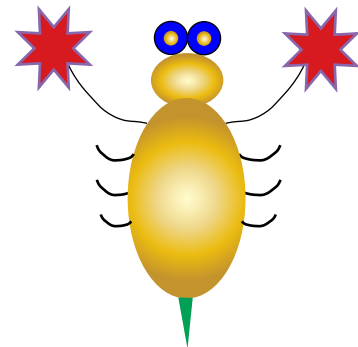A3T
A4O
A5O
A6L
A7V
A8N
A9I

Card Set B: **10-Card Set:** **Tiger, Dog, Polar Bear, Mouse, Macaque, Hamster, Chicken, Chimpanzee, Rat, Human**	**Tiger** (*Panthera tigris*) 	**Dog** (*Canis lupus*)
Polar Bear (*Ursus maritimus*) 	**Mouse** (*Mus musculus*) 	**Macaque** (*Macaca mulatta*)

Hamster (*Cricetulus griseus*)	**Chicken** (*Gallus gallus*)	**Chimpanzee** (*Pan troglodytes*)
Brown Rat (*Rattus norvegicus)*	**Human** (*Homo sapiens*)	**Discovery Species 6-Card Set:** Hedgehog, Tarsier, Whale, Platypus, Bat, Walrus

Walrus (*Odobenus rosmarus*)	**Tarsier** (*Carlito syrichta*)	**Platypus** (*Ornithorhynchus anatinus*)
Hedgehog (*Erinaceus europaeu*)	**Killer Whale** (*Orcinus orca*)	**Bat** (*Myotis lucifugus*)

DISCOVERY SPECIES

RESOURCE #1: Using the Website NCBI.

https://www.ncbi.nlm.nih.gov/

1) What is NCBI? A free web service focused on providing genomic resources further the National Institute of Health's mission of "uncovering new knowledge".
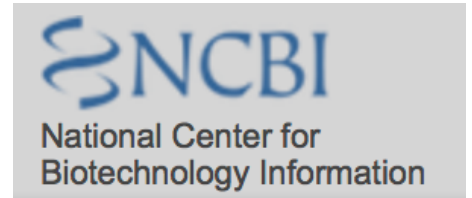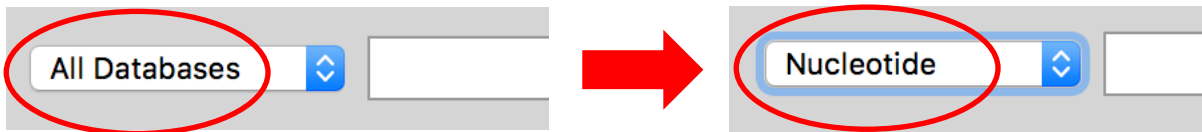

2) How do I use NCBI?

a) For our purposes, you will be searching for the **DNA sequence** of Hemoglobin alpha of your specific species.

→ We want to access a specific database to find the nucleotides of our DNA sequence.

→ Click the dropdown menu for "All Databases" and select **NUCLEOTIDE.**

b) In the blank box to the right, type **Hemoglobin alpha**, your species' scientific name and **mRNA**. mRNA stands for messenger RNA and is needed for our search. An example using mouse is provided below.

Common name	Scientific name
Dog	*Canis lupus*
Tiger	*Panthera tigris*
Mouse	*Mus musculus*
Hamster	*Cricetulus griseus*
Polar Bear	*Ursus maritimus*
Macaque	*Macaca mulatta*
Rat	*Rattus norvegicus*
Human	*Homo sapiens*
Chicken	*Gallus gallus*
Chimpanzee	*Pan troglodytes*
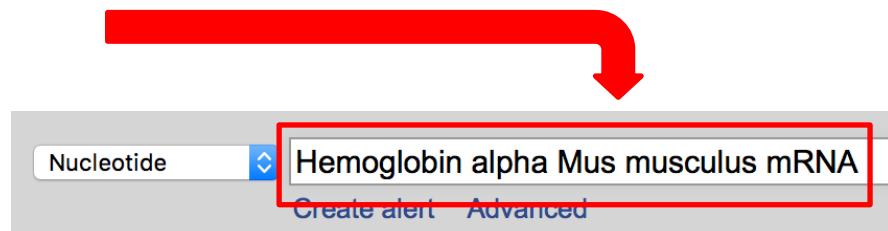
c) Click SEARCH

*Special note for searching Human (*Homo sapiens*)

Because there is so much genetic information available online for humans, you will need to take an extra step after searching "Hemoglobin alpha Homo sapiens mRNA".

In the top right corner on the NCBI website, there should be a list of the number of results from your search, organized by species. Click on the number next to *Homo sapiens*.

This will restrict your search results, so it will be easier to find the correct nucleotide sequence. You are now ready to continue.

Results by taxon

Top Organisms [Tree]
Homo sapiens *(4709)*
Mycobacterium tuberculosis *(2115)*
Pseudomonas aeruginosa *(677)*
Klebsiella pneumoniae *(553)*
Bordetella pertussis *(542)*
All other taxa *(5110)*
More...

d) Select the result where **"hemoglobin alpha"** or **"hemoglobin alpha 1"** is mentioned in the results and click **FASTA**.

☐ **Mus musculus hemoglobin alpha**, adult chain 1 (Hba-a1), **mRNA**
29. 569 bp linear **mRNA**
Accession: NM_008218.2 GI: 145301577
Protein   PubMed   Taxonomy
GenBank   FASTA   Graphics

e) The next screen will show the DNA sequence for Hemoglobin alpha for your specific species. An example is shown below. **COPY ONLY** the nucleotides

GenBank   Graphics

>NM_008218.2 Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1), mRNA

```
GACACTTCTGATTCTGACAGACTCAGGAAGAAACCATGGTGCTCTCTGGGGAAGACAAAAGCAACATCAA
GGCTGCCTGGGGGAAGATTGGTGGCCATGGTGCTGAATATGGAGCTGAAGCCCTGGAAAGGATGTTTGCT
AGCTTCCCCACCACCAAGACCTACTTCCCTCACTTTGATGTAAGCCACGGCTCTGCCCAGGTCAAGGGTC
ACGGCAAGAAGGTCGCCGATGCTCTGGCCAATGCTGCAGGCCACCTCGATGACCTGCCCGGTGCCCTGTC
TGCTCTGAGCGACCTGCATGCCCACAAGCTGCGTGTGGATCCCGTCAACTTCAAGCTCCTGAGCCACTGC
CTGCTGGTGACCTTGGCTAGCCACCACCCTGCCGATTTCACCCCCGCGGTGCATGCCTCTCTGGACAAAT
TCCTTGCCTCTGTGAGCACCGTGCTGACCTCCAAGTACCGTTAAGCTGCCTTCTGCGGGGCTTGCCTTCT
GGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGAAA
AAAAAAAAA
```

f) **In a separate document,** type a "greater than" symbol (>) and then the name of the species. A Microsoft Word document or Google doc are acceptable.

g) **PASTE** the DNA sequence underneath. An example for mouse is shown below:

> Mouse *Mus musculus*

```
GACACTTCTGATTCTGACAGACTCAGGAAGAAACCATGGTGCTCTCTGGGGAAGACAAAAGCAACATCAA
GGCTGCCTGGGGGAAGATTGGTGGCCATGGTGCTGAATATGGAGCTGAAGCCCTGGAAAGGATGTTTGCT
AGCTTCCCCACCACCAAGACCTACTTCCCTCACTTTGATGTAAGCCACGGCTCTGCCCAGGTCAAGGGTC
ACGGCAAGAAGGTCGCCGATGCTCTGGCCAATGCTGCAGGCCACCTCGATGACCTGCCCGGTGCCCTGTC
TGCTCTGAGCGACCTGCATGCCCACAAGCTGCGTGTGGATCCCGTCAACTTCAAGCTCCTGAGCCACTGC
CTGCTGGTGACCTTGGCTAGCCACCACCCTGCCGATTTCACCCCCGCGGTGCATGCCTCTCTGGACAAAT
TCCTTGCCTCTGTGAGCACCGTGCTGACCTCCAAGTACCGTTAAGCTGCCTTCTGCGGGGCTTGCCTTCT
GGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGAAA
AAAAAAAAA
```

Do not let your word processor program change the "greater than" symbol (>) to a different type of bullet. If it does, change it back to the simple > symbol.

h) **Repeat** for each species until all ten DNA sequences are in one document.

Let's Build a Phylogenetic Tree! SEE RESOURCE #2

RESOURCE #2: Using the Website Phylogeny.fr

www.phylogeny.fr

1) What is Phylogeny.fr? A free, simple-to-use web service dedicated to reconstructing and analyzing phylogenetic relationships with molecular sequences.

2) How do I use Phylogeny.fr?

a) For our purposes, you will be using the "One Click" option of phylogenetic analysis.

→ Scroll down below the genie lamp and select **"One Click"**
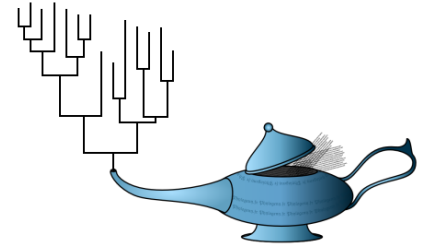
Phylogeny analysis

"One Click"
Paste your set of sequences and let the software make decisions on your behalf (Each step is optimized for your data).

"Advanced"
Manually set parameters for the various steps.

"A la Carte"
Create your own phylogeny workflow using more programs available.

b) You may title your work, **"Phylogenetic Tree w/Molecular Data"** in the analysis box here:

1. Overview	2. Data & Settings

Name of the analysis (optional):

c) The file you created that contains the DNA sequences for Hemoglobin alpha is known as a FASTA file. You will now use this FASTA file to build a phylogenetic tree.

→ **Copy all of the data in your FASTA file, starting with the > symbol and scientific name of your first species, and ending with the last nucleotide of your last species.**

→ **Paste all of the data in the box and click SUBMIT.**

Upload your set of sequences in FASTA, EMBL or NEXUS format from a file:
Choose File No file chosen
Or paste it here (load example of sequences)

d) Wait patiently. Each nucleotide essentially represents a character (like in the matrix of the aliens) and the program runs an algorithm to determine the maximum likelihood tree.

e) Draw the phylogenetic tree in **TREE BOX B**.

Where does the Discovery Species fit in?

See your student guide and **RESOURCE #2** for further details.

Go back to your existing Phylogeny.fr window and click **Data & Settings**
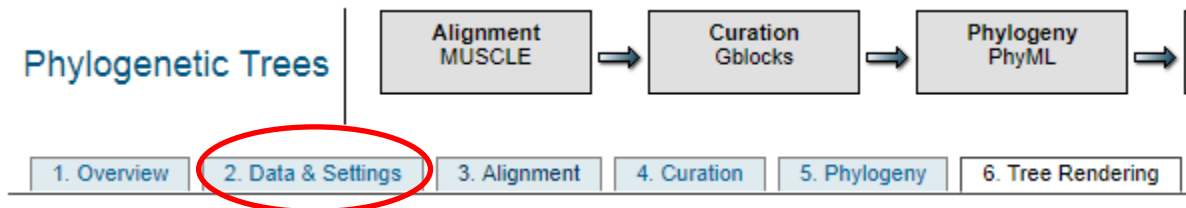
f) In the box, underneath the last DNA Sequence, skip a space and add a > and the name of the discovery species.

For example: > Hedgehog *Erinaceus europaeus*

g) Using Resource #1, **SEARCH** the DNA sequence for Hemoglobin alpha of the discovery species, and then **PASTE** the DNA sequence underneath the last species in your original list.

h) Click SUBMIT and view the altered phylogenetic tree.

i) Copy the tree into the box for **TREE C** and answer the Analysis Questions.

Phylogenetic Trees: United, but Diverse

Illustration from Charles Darwin's notebook (1837)

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> Request Teacher Feedback. Approved _____ Revised _____
> Submit completed files for Dr. Wallace-Darwin's approval.

📝 **= Indicates that a worksheet is needed**

Character Matrix

Instructions: For each species, mark an "X" for each character present. Using the information in this matrix, arrange the species based on their shared characters to fill in the phylogenetic tree below.

CHARACTER	A2E	A7V	A4O	A6L	A1U	A3T	A9I	A5O	A8N
SIX LEGS			✖		✖	✖	✖		✖
WINGS					✖	✖			
STINGER			✖				✖		✖
STAR BELLY				✖				✖	
CLAWS			✖						✖
LONG LEGS	✖								
LOSS OF ARMS						✖			
EYE STALKS				✖					
ANTENNAE	✖	✖							✖

<u>Instructions:</u> Using the information from your character matrix, arrange the alien species based on their shared characters. Identify the evolution of new (derived) characters and label them at the appropriate branch on the tree. At the tip of each branch, identify the species using the dichotomous key provided.

long legs

antennae

star belly

eyestalks

wings

loss of arms

six legs

stinger

claws

antennae

A2E - *Globocorpus lata*

A7V - *Globocorpus auribus*

A5O - *Globocorpus stellans*

A6L - *Globocorpus longoculus*

A1U - *Hexapedes elegans*

A3T - *Hexapedes volantis*

A9I - *Hexapedes aculeus*

A4O - *Hexapedes ungula*

A8N - *Hexapedes odoror*

Dichotomous Key

Instructions: A dichotomous key is an organized set of couplets of mutually exclusive characteristics that is used to identify biological organisms. Use the key below to identify each alien species. Write the genus species by the appropriate serial number at the end of this key.

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3a – Has long legs...*Globocorpus lata*
3b – Has short legs.. *Globocorpus auribus*

4a – Has star belly and no eye stalks.. *Globocorpus stellans*
4b – Has star belly and eye stalks.. *Globocorpus longoculus*

5a – Has wings..**Go to STEP 6**
5b – Has a stinger..**Go to Step 7**

6a – Has arms.. *Hexapedes elegans*
6b – Has no arms.. *Hexapedes volantis*

7a – Has claws.. Go to Step 8
7b – Has no claws.. *Hexapedes aculeus*

8a – Has antennae.. *Hexapedes odoror*
8b – Has no antennae... *Hexapedes ungula*

A1U: ___*Hexapedes elegans*___   A6L: __*Globocorpus longoculus*___

A2E: ___*Globocorpus lata*___   A7V: _*Globocorpus auribus*___

A3T: ___*Hexapedes volantis*___   A8N: _*Hexapedes odoror*___

A4O: ___*Hexapedes ungula*___   A9I: _*Hexapedes aculeus*___

A5O: ___*Globocorpus stellans*___
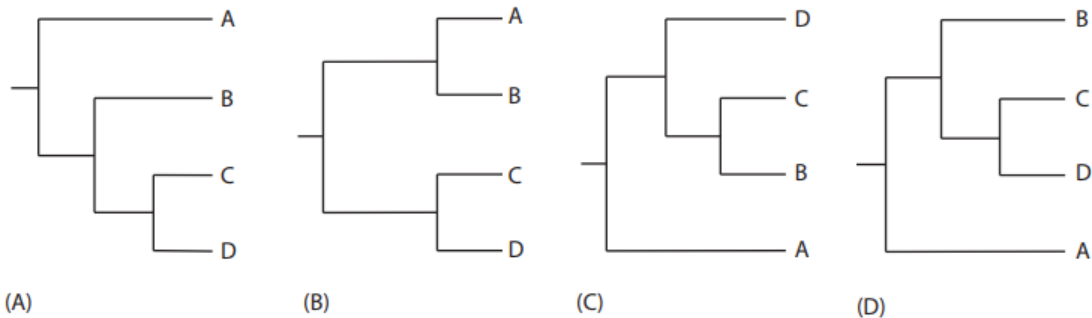
Analysis Questions of Diagnostic Assessment

1. The phylogenetic tree that you completed in the lab investigation is also known as a:

   A) cladogram
   B) evolutionary diagram
   C) flow chart
   D) genetic map
   E) morphological diagram

2. Which of the following best explains what a cladogram is?

   A) a map showing the geographical location of species
   B) a visualization of the chromosomal similarities between species
   C) a diagram of the analogous similarities between species
   D) a diagram of the evolutionary relatedness of species with branches not drawn to scale
   E) a visualization of the location of genes on chromosomes

3. Which two phylogenetic trees (cladograms) depict the same evolutionary relationships between species A, B, C and D? Explain your answer.

(A)   (B)   (C)   (D)

A and D, because they have the same relative branching pattern.

4. Draw a new phylogenetic tree that depicts the same evolutionary relationships as the tree below. Your new tree must change the order of species in at least two positions.
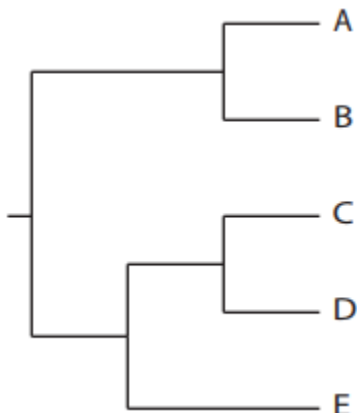
Multiple examples exist as long as the relative branching pattern is the same.

STATE UNIVERSITY AT YORKVILLE
DEPARTMENT OF BIOLOGY

Dear Applicant:

Congratulations! Based on your performance, you have been selected as an intern for BIO 327: Evolutionary Relationships. We look forward to your participation and your valuable input.

Our department is gathering data on students' abilities to construct and interpret phylogenetic trees using molecular data. Using your knowledge of Biology, complete each activity and answer the accompanying analysis questions. Thank you in advance for your time and effort in helping us improve this course.

Section 2: Application – Phylogenetic tree construction

Task #1 – Using the ten species from CARD SET B, evaluate the phenotypic characteristics to predict the evolutionary relationships among these ten species.

Task #2 – Based on your prediction in Task #1, draw a phylogenetic tree in Tree A that illustrates the relationships among these species.

Task #3 – Collect DNA sequences for the hemoglobin alpha protein for all 10 species using the NCBI website. See Resource #1.

Task #4 – Analyze the DNA sequences to build a phylogenetic tree using the web-service Phylogeny.fr. See Resource #2.

Task #5 – Draw this phylogenetic tree in Tree B and complete the analysis questions.

Task #6 – Choose an 11th "discovery" organism and draw its predicted location on Tree B.

Task #7 – Collect the hemoglobin alpha DNA sequence for this species from NCBI, add it to previous data, and reanalyze the phylogenetic relationships using Phylogeny.fr.

Task #8 – Draw the updated phylogeny in Tree C.

Task #9 – Submit your completed application to Dr. Wallace-Darwin for evaluation.

NAME: _____   **Phenotypic vs. Molecular Data**

Tree A: Construct a phylogenetic tree for 10 species based on phenotypic characteristics.

Student answers will vary. Students may correctly group the nine mammalian species according to their order (primates, rodents, and carnivores) and may also identify the chicken as the outgroup.

Tree A Analysis Questions:

a) Identify at least three characteristics that all 10 species have in common.

All of the animals are vertebrates, so it may be useful to discuss key, shared characteristics among vertebrates, including, but not limited to:
Bilateral symmetry, head, amniotic egg, hinged jaw, forelimbs and hind limbs, vertebrae

b) Explain how shared, derived characteristics are used to infer evolutionary relationships.

Recently evolved characteristics that are shared among organisms are one piece of evidence that suggest they have evolved from a common ancestor. These similarities can be used to group based on their evolutionary relatedness.

c) Select a group of closely related species from your phylogenetic tree and explain why you have grouped them together.

The main purpose of this question is to encourage students to identify specific characteristics as evidence for their hypothesized groupings. The species contained in this exercise contain species from three mammalian orders (primates, carnivores, and rodents) and one evolutionary outgroup (birds), so students may also successfully identify species that belong to the same order, and describe some of the shared characteristics for that order.

d) Select two species that are distantly related on your phylogenetic tree and describe at least two phenotypic characteristics that distinguish them from one another.

As above, the purpose of this question is to encourage students to identify specific characteristics that differ between the species in their hypothesized phylogenetic tree.

e) What additional data could be incorporated to strengthen your hypothesis about the evolutionary relationships between these species?

The purpose of this question is to highlight that additional pieces of information improve our understanding of evolutionary relationships. Some examples include molecular data (DNA protein sequences), fossil evidence, geographical distributions, and developmental patterns.

Tree B: Draw the phylogenetic tree for the 10 species generated by Phylogeny.fr based on DNA sequences. Your tree should reflect the correct branching order of these species.

Note: The branch lengths and red numbers that indicate confidence estimates are _not_ important for Tree B analysis. However, these data points may be needed in the future.

0.2
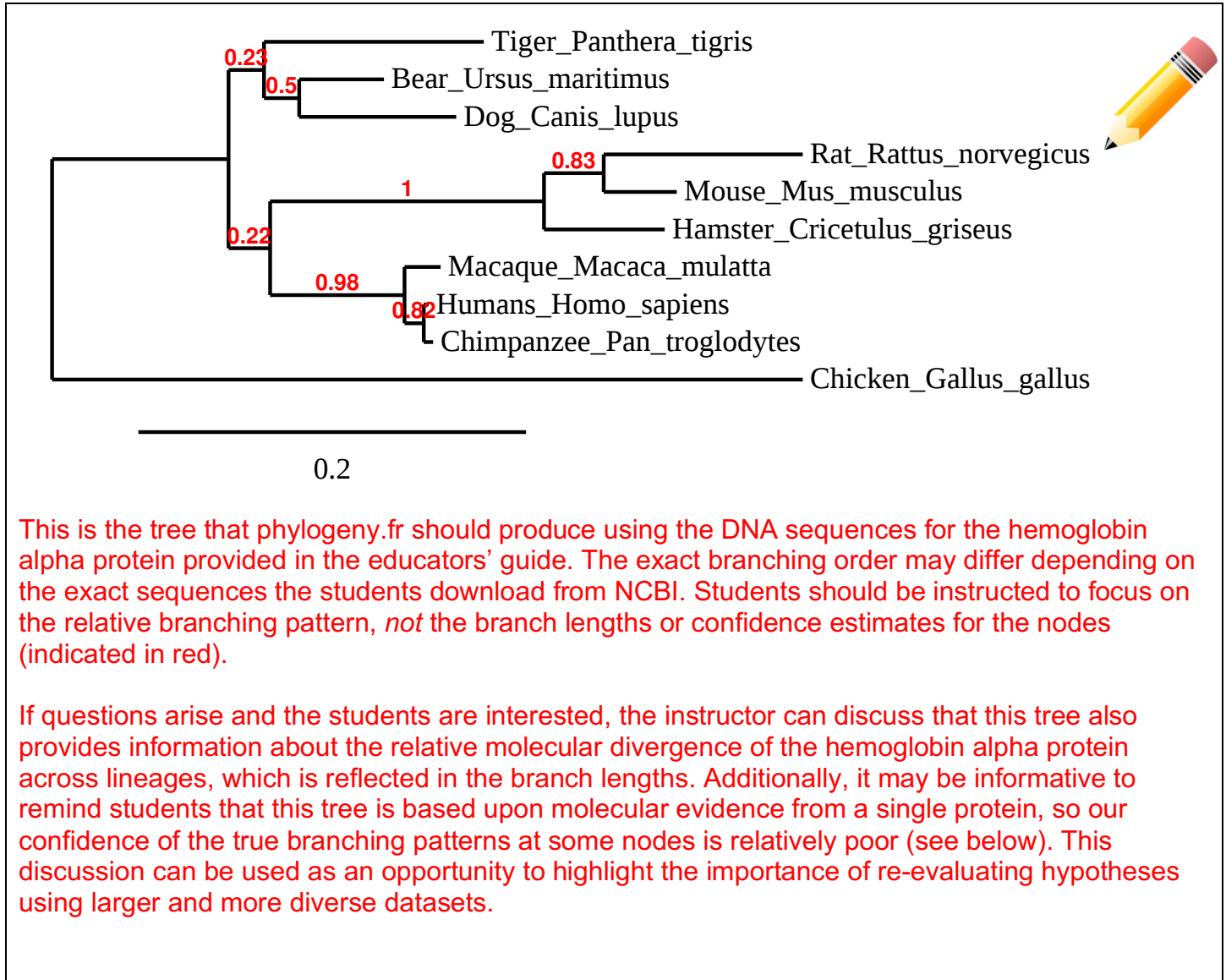
This is the tree that phylogeny.fr should produce using the DNA sequences for the hemoglobin alpha protein provided in the educators' guide. The exact branching order may differ depending on the exact sequences the students download from NCBI. Students should be instructed to focus on the relative branching pattern, _not_ the branch lengths or confidence estimates for the nodes (indicated in red).

If questions arise and the students are interested, the instructor can discuss that this tree also provides information about the relative molecular divergence of the hemoglobin alpha protein across lineages, which is reflected in the branch lengths. Additionally, it may be informative to remind students that this tree is based upon molecular evidence from a single protein, so our confidence of the true branching patterns at some nodes is relatively poor (see below). This discussion can be used as an opportunity to highlight the importance of re-evaluating hypotheses using larger and more diverse datasets.

Tree B Analysis Questions:

a) Hemoglobin is an oxygen-transport protein that is found in the red blood cells of almost all vertebrates. It has four subunits: two alpha subunits and two beta subunits. Explain how this protein is produced from the DNA sequences you just collected.

The purpose of this question is to review the basic processes of transcription and translation

b) Identify the similarities and differences between the phenotypic phylogeny (Tree A) and molecular phylogeny (Tree B)?

The purpose of this question is to encourage students to re-examine their original hypothesis after the introduction of new, complementary data. It may be common for students to successfully group the species within their correct mammal orders. However, students may not consider different degrees of relatedness between orders or amongst species within a particular order, or correctly position the chicken as the evolutionary outgroup. Additionally, the phylogenies may differ because the students mistakenly include information about biological "complexity" in deciding how to structure their tree.

c) The phylogeny you built using molecular data correctly assigns the nine mammalian species to their respective orders (i.e., primates, carnivores, and rodents), and identifies the chicken as an evolutionarily distant outgroup. Are the specific branching patterns within these three orders the same as your original predictions in Tree A? Are the species in these orders equally related to one another? Explain.

The purpose of this question is to encourage students to interpret the phylogenetic tree as a set of nested hierarchies. For example, amongst rodents, the mouse and rat are more closely related to each other than either is to the hamster.

Now choose a Discovery Species _____
Using a colored pencil, <u>predict where the Discovery Species would fit in Tree B.</u> Draw it in.

d) Explain your reasoning for placing the Discovery Species in this phylogenetic position.

The purpose of this question is to encourage students to identify specific characteristics that justify the hypothesized placement of the Discovery species.

Tree C: Draw the revised phylogenetic tree generated by Phylogeny.fr after adding the DNA sequence of the 11th species.
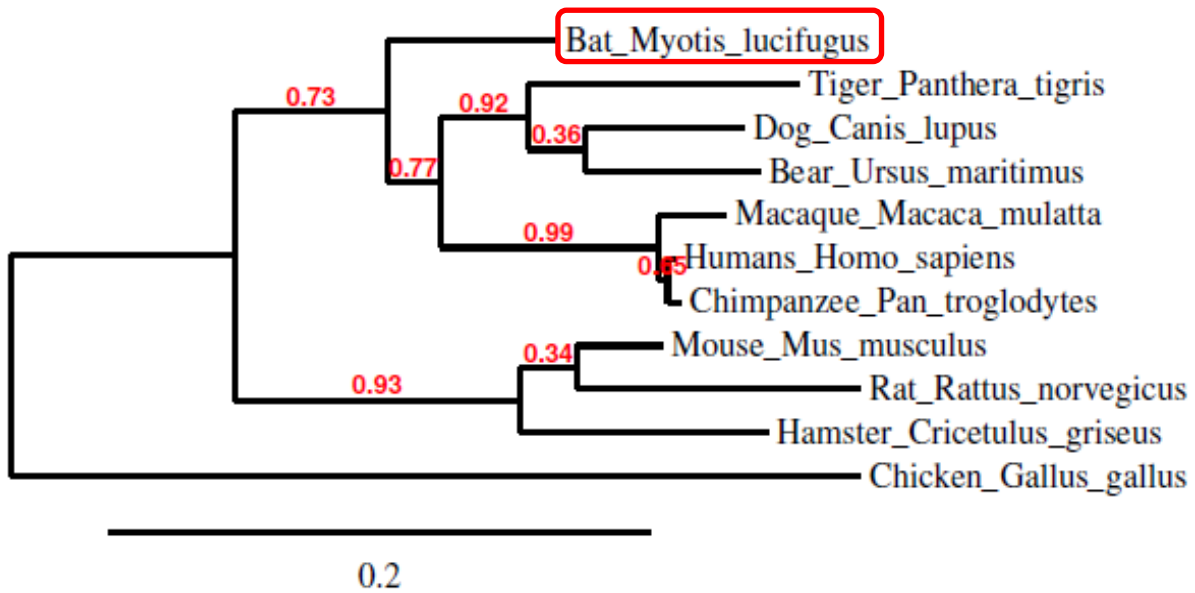
For this analysis, *consider* the confidence estimates for each node, which are indicated in red.

These are the six trees that phylogeny.fr should produce after the inclusion of DNA sequences for the hemoglobin alpha protein from the six discovery species provided in the resources.
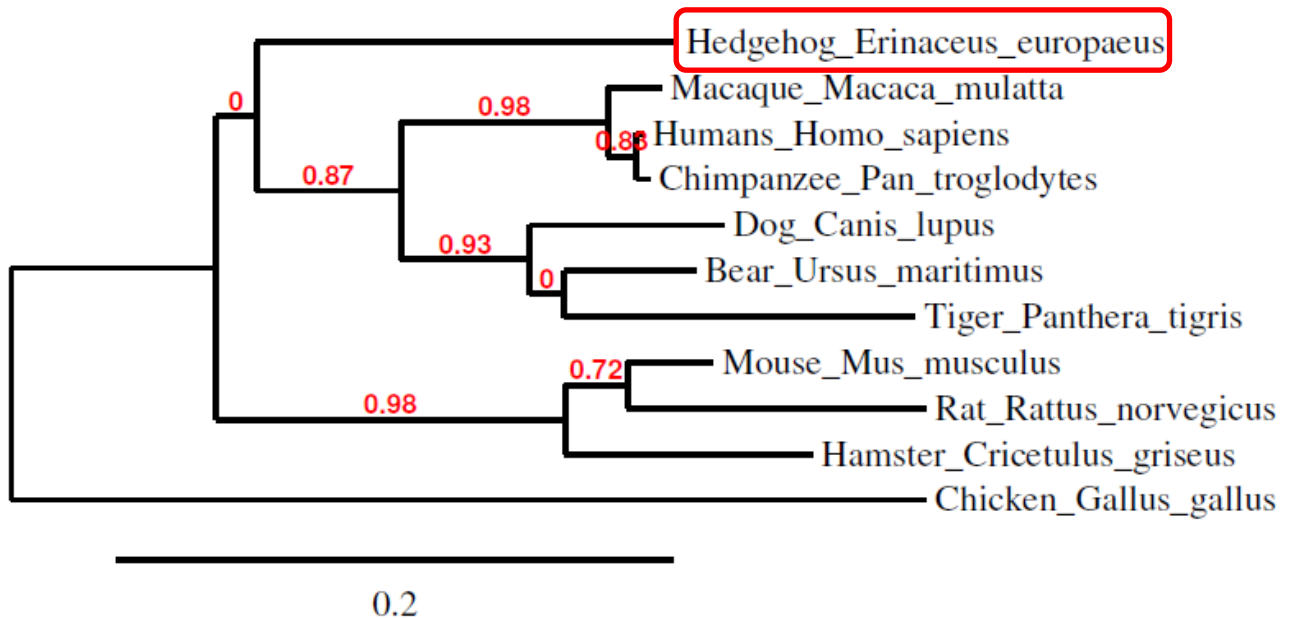
Note: because this phylogeny is based on the DNA sequences of a single protein, inclusion of more than one discovery species may alter the structure of the resulting tree.

Discovery species: Bat

0.73
0.92
0.77
0.36
0.99
Bat_Myotis_lucifugus
Tiger_Panthera_tigris
Dog_Canis_lupus
Bear_Ursus_maritimus
Macaque_Macaca_mulatta
Humans_Homo_sapiens
Chimpanzee_Pan_troglodytes
0.34
0.93
Mouse_Mus_musculus
Rat_Rattus_norvegicus
Hamster_Cricetulus_griseus
Chicken_Gallus_gallus

0.2

Discovery Species: Hedgehog

0
0.87
0.98
0.88
0.93
0
Hedgehog_Erinaceus_europaeus
Macaque_Macaca_mulatta
Humans_Homo_sapiens
Chimpanzee_Pan_troglodytes
Dog_Canis_lupus
Bear_Ursus_maritimus
Tiger_Panthera_tigris
0.72
0.98
Mouse_Mus_musculus
Rat_Rattus_norvegicus
Hamster_Cricetulus_griseus
Chicken_Gallus_gallus

0.2

Discovery Species: Platypus

Platypus_Ornithorhynchus_anatinus
Macaque_Macaca_mulatta
0.99
0.85
Humans_Homo_sapiens
Chimpanzee_Pan_troglodytes
0.23
Tiger_Panthera_tigris
0.89
Bear_Ursus_maritimus
0.16
Dog_Canis_lupus
0.78
Mouse_Mus_musculus
0.86
Rat_Rattus_norvegicus
1
Hamster_Cricetulus_griseus
Chicken_Gallus_gallus

0.1

Discovery Species: Tarsier

Tarsier_Carlito_syrichta
0.15
Macaque_Macaca_mulatta
0.98
0.82
Humans_Homo_sapiens
Chimpanzee_Pan_troglodytes
0.66
Bear_Ursus_maritimus
0.88
Tiger_Panthera_tigris
0.006
Dog_Canis_lupus
Mouse_Mus_musculus
0.6
Rat_Rattus_norvegicus
0.052
Hamster_Cricetulus_griseus
Chicken_Gallus_gallus

0.2

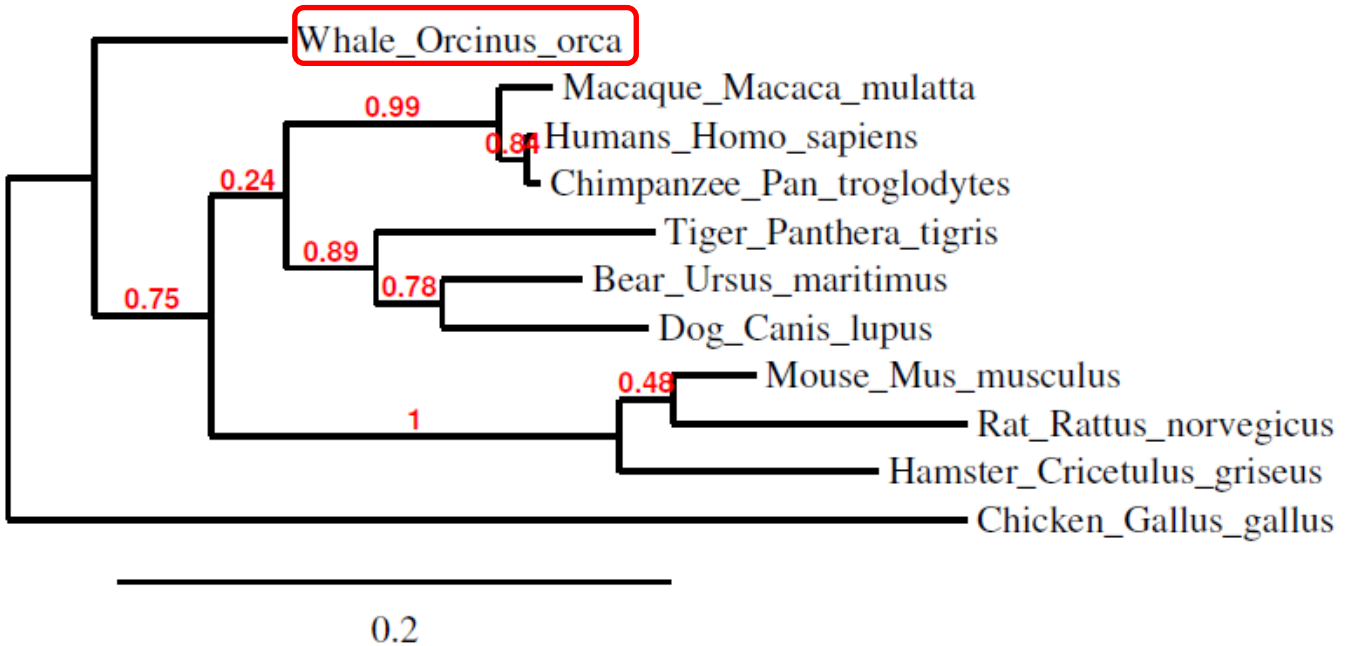**Discovery Species: Walrus**
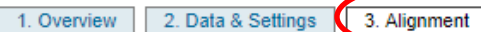

Discovery Species: Whale

Tree C Analysis Questions:

a) Is the phylogenetic placement of your Discovery species consistent with your original prediction? If not, identify an additional phenotypic characteristic(s) that supports the updated molecular phylogeny.

We intentionally selected six discovery species that would be more difficult for students to position correctly on the mammalian phylogeny using phenotypic characteristics alone. In some cases, the students may be surprised by the results of the updated tree. This may challenge their prior knowledge of the evolutionary relationships among the eleven species, and encourage them to reassess their initial prediction based on new information.

Note: Students may notice that the confidence estimates at some nodes are different in Tree C than in Tree B. The addition of a new species changes the composition of the data, and therefore changes our certainty about the relative branching pattern.

b) On the Phylogeny.fr screen, click on the 3. Alignment tab. What information is presented? How is it relevant to understanding the evolutionary relationships between species?

The alignment tab presents the nucleotide sequence alignments and highlights the nucleotide positions based on the extent to which they are shared across species. Like phenotypic traits, shared DNA sequences are evidence that species have evolved from a common ancestor and can be used to infer degrees of relatedness.

c) Today, you compared the evolutionary relationships among species using the DNA sequence for the hemoglobin alpha protein. If we repeated this analysis using the DNA sequence of another protein, would you predict the evolutionary relationships to stay the same? Why or why not?

The evolutionary relationships may or may not stay the same depending on the specific protein that is chosen. The purpose of this question is to encourage students to evaluate why the DNA sequences for some proteins might be more informative than others.

d) You constructed a phylogenetic tree based on molecular data for a single protein. Would it be beneficial to repeat this analysis using molecular data from more than one protein? If so, why?

In general, more information should lead to more accurate predictions about true evolutionary relationships. In this case, using molecular data from multiple proteins should result in a phylogeny with stronger support, particularly among closely-related species or poorly-resolved nodes.

e) The numbers in red indicate the confidence estimates for each node. If you repeated the analysis using multiple proteins, would you predict most of the values to increase or decrease? Why?

The purpose of this question is to highlight that the results from phylogeny.fr include a measure of our confidence in the relative branching pattern at each node. In general, we predict that the confidence estimates will increase with the inclusion of more molecular data.

f) Two species are that are distantly related based on the analysis of multiple DNA sequences also share a set of very similar phenotypic characteristics, such as the presence of wings in both bats and chickens. Consider and discuss what evolutionary process could lead to this pattern?

Species that are distantly related that experience similar selective forces can independently evolve similar phenotypic characteristics. This process is known is as convergent evolution. Convergent evolution can challenge our understanding of evolutionary relationships because similar phenotypic characteristics may be mistaken as shared ancestral traits. Although students may not be familiar with the term "convergent evolution", this question encourages students to consider how similar environments and lifestyles may lead to the evolution of similar phenotypic characteristics.

f) In this activity, you used several analyses and types of data to explain the evolutionary relationships among a set of present-day species. How do these activities model the process of science?

Science is an iterative process that involves re-evaluation of hypotheses using improved analytical approaches and new data. The goal of this question is for students to reflect upon the analyses they conducted, and highlight that the study of evolution also follows this process.

DNA SEQUENCES (Original 10 Species)

>Humans (Homo_sapiens)

```
ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCCTGCCGACAAGACCAACGTC
AAGGCCGCCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAGGATGTTCC
TGTCCTTCCCCACCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCCCAGGTTAAGGG
CCACGGCAAGAAGGTGGCCGACGCGCTGACCAACGCCGTGGCGCACGTGGACGACATGCCCAACGCGCTG
TCCGCCCTGAGCGACCTGCACGCGCACAAGCTTCGGGTGGACCCGGTCAACTTCAAGCTCCTAAGCCACT
GCCTGCTGGTGACCCTGGCCGCCCACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGCCTCCCTGGACAA
GTTCCTGGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTT
CTTGCCCCTTGGGCCTCCCCCCAGCCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAA
AGTCTGAGTGGGCGGCA
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>Tiger (Panthera_tigris)

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GGCCCCGCACCTTCTGGTCCCGACACAGATCAGAACCAACCATGGTGCTGTCTTCCGCCGACAAGAACAA
CGTCAAGGCCTGCTGGGGTAAGATTGGGTCCCATGCCGGCGAATATGGCGCAGAGGCCCTGGAGAGGACC
TTCTGCTCCTTCCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCCGCCCAGGTCC
AGACCCACGGCCAGAAGGTGGCCGACGCGCTGACGAAGGCCGTGGCCCACATAAACGACCTGCCTAACGC
CCTGTCGGATCTGAGCGACCTGCACGCGTACAAGCTGCGTGTGGACCCCGTCAACTTCAAGTTCCTGAGC
CACTGCCTGCTGGTGACCCTGGCCTGCCACCACCCCGAGGAATTCACCCCCGCCGTCCACGCCTCCCTGG
ACAAGTTCTTCAGCGCTGTGAGCACCGTGCTGACCTCCAAATACCGTTGAGTGAAGCCGGGGCGATCGTC
CTGAGGGCCCGGGGCCTCTTGGGCTCCGGGCACCCGAACTTCCTGATCTTTGAATAAAGTCTGAGTGGGC
TGCAA
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>Dog (Canis_lupus)

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CGAGGCCTCCCGGCCCCGCACATTTCTGGTTCTCCTCAGACTCAGAAGGAACCCACCATGGTGCTGTCTC
CCGCCGATAAGACCAACATCAAGTCCACTTGGGATAAGATTGGTGGCCACGCTGGCGACTACGGCGGAGA
GGCGCTGGACAGGACCTTCCAGTCCTTCCCCACCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCCC
GGCTCCGCCCAGGTCAAGGCCCACGGCAAGAAGGTGGCTGATGCGCTGACCACCGCCGTGGCCCACCTGG
ACGACCTGCCCGGTGCCCTGTCCGCCCTCAGCGACCTGCACGCCTACAAGCTGCGCGTGGACCCCGTCAA
CTTCAAGCTCCTGAGCCACTGCCTGCTGGTGACCCTGGCCTGCCACCACCCCACCGAATTCACCCCTGCC
GTCCACGCCTCCCTGGACAAGTTCTTCGCCGCCGTGAGCACCGTGCTGACCTCCAAGTACCGTTAAGCTG
CAGCCATGGCGGCGGGAGCCCCTGCCCTGGGCCCCTGGGTGGCCTCCTGCAACCGTGCCCTACACTTCCT
GATGTTTGAATAAAGTCTGAGTGGGCTGCAGCCTC
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>Bear (Ursus_maritimus)

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CCGCCCCGCACATTTCTGGTCCTCACAGACTCAGAAAGAAGCCACCATGGTGCTGTCTCCCGCCGACAAG
AGCAACGTCAAGGCCACCTGGGATAAGATCGGCAGCCACGCTGGCGAGTATGGCGGCGAGGCTCTGGAGA
GGACCTTCGCGTCCTTCCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAGCCCTGGCTCCGCCCA
GGTCAAGGCCCACGGCAAGAAGGTGGCCGACGCCCTGACCACCGCCGCAGGCCACCTGGACGACCTGCCG
GGCGCCCTGTCCGCTCTGAGCGACCTGCACGCGCACAAGCTGCGAGTGGACCCGGTCAACTTCAAGTTCC
TGAGCCACTGCCTGCTGGTGACCCTGGCCAGCCACCACCCCGCGGAGTTCACCCCTGCCGTCCACGCCTC
CCTGGACAAGTTCTTCAGCGCCGTGAGCACCGTGCTCACCTCCAAATACCGTTAAGCTGGAGCCGCGCGA
CCCTCCCGCTCCCGGCCTGGGGCCTCTTGCGCTCCACGCGCCTGAACTTCCCGATCTTTGAATAAAGTCT
GAGTGGGCTGCAG
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>Mouse (Mus_musculus)

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GACACTTCTGATTCTGACAGACTCAGGAAGAAACCATGGTGCTCTCTGGGGAAGACAAAAGCAACATCAA
GGCTGCCTGGGGGAAGATTGGTGGCCATGGTGCTGAATATGGAGCTGAAGCCCTGGAAAGGATGTTTGCT
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TGCTCTGAGCGACCTGCATGCCCACAAGCTGCGTGTGGATCCCGTCAACTTCAAGCTCCTGAGCCACTGC
CTGCTGGTGACCTTGGCTAGCCACCACCTGCCGATTTCACCCCCGCGGTGCATGCCTCTCTGGACAAAT
TCCTTGCCTCTGTGAGCACCGTGCTGACCTCCAAGTACCGTTAAGCTGCCTTCTGCGGGGCTTGCCTTCT
GGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGAAA
AAAAAAAAA
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>Rat (Rattus_norvegicus)

ACATTCTCCTTCTGATAGACTCAGGAAGCAATCATGGTGCTCTCTGCAGATGACAAAACCAACATCAAGA
ACTGCTGGGGGAAGATTGGTGGCCATGGTGGTGAATATGGCGAGGAGGCCCTACAGAGGATGTTCGCTGC
CTTCCCCACCACCAAGACCTACTTCTCTCACATTGATGTAAGCCCCGGCTCTGCCCAGGTCAAGGCTCAC
GGCAAGAAGGTTGCTGATGCCTTGGCCAAAGCTGCAGACCACGTCGAAGACCTGCCTGGTGCCCTGTCCA
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GCTGGTGACCTTGGCTTGCCACCACCCTGGAGATTTCACACCCGCCATGCACGCCTCTCTGGACAAATTC
CTTGCCTCTGTGAGCACTGTGCTGACCTCCAAGTACCGTTAAGCCGCCTCCTGCCGGGCTTGCCTTCTGA
CCAGGCCCTTCTTCCCTCCCTTGCACCTATACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGC

>Hamster (Cricetulus_griseus)

CTCAATACACTTCTGACTCTGACAGACTCAGGAAAGAATCACCATGGTGCTCTCTGCGGACGACAAGAAC
AACATCAAGGGTGCCTGGGGCAAGATTGGCGGCCACGGTGCTGAATATGGCGCGGAGGCCCTAGAGAGGA
TGTTCGTTACCTACCCCACCACCAAGACCTACTTCCCTCACTTTGATGTAAGCCACGGCTCTGCCCAGGT
CAAGGCCCACGGCGCAAAGGTCGCAGACGCCCTGGCCAAGGCCGCTAGCCACCTCGATGACCTGCCCGGC
ACCCTGTCCTCTCTGAGCGACCTGCACGCCCACGAGCTGCGTGTGGACCCTGTCAACTTCAAGCTCCTGA
GCCACTGCCTGCTGGTGACCCTGGCCAACCACCACCCAGCCGACTTCACCCCCGCGGTGCACGCCTCTCT
GGACAAATTCCTTGCCTCTGTGAGCACCGTCCTGACCTCCAAGTACCGTTAAACTGGA

>Macaque (Macaca_mulatta)

CACAGACTCAGAAAGAACCCACCATGGTGCTGTCTCCTGCCGACAAGAGCAACGTCAAGGCCGCCTGGGG
TAAGGTCGGCGGGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAGGATGTTCCTGTCCTTCCCCACC
ACCAAGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCTGCCCAGGTTAAGGGCCACGGCAAGAAGG
TGGCCGACGCGCTGACCCTCGCCGTGGGGCACGTGGACGACATGCCCCAAGCGCTGTCCGCGCTGAGCGA
CCTGCACGCGCACAAGCTTCGGGTGGACCCGGTCAACTTCAAGCTCCTGAGCCACTGCCTGCTGGTGACT
CTGGCCGCTCACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGCCTCCCTGGACAAGTTCCTGGCTTCTG
TGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGC
CTCCCGCCAGGCCCTCCTCCCCTCCTTGCACCGGCCCTTCCTGGTCTTTGAATAAAGTCTG

>Chimpanzee (Pan_troglodytes)

ACTCTTCTGGTCCCCACAGACTCAGAAAGAACCCACCATGGTGCTGTCTCCTGCCGACAAGACCAACGTC
AAGGCCGCCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAGGATGTTCC
TGTCCTTCCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCTGCCCAGGTTAAGGG
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GCCTGCTGGTGACCCTGGCCGCCCACCTCCCCGCCGAGTTCACCCCTGCGGTGCACGCCTCCCTGGACAA
GTTCCTGGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTT
CTTGCCCCTTGGGCCTCTCGCCAGGCCCTCCTCTCCTTCCTGCACCTGTACCCCCCCTGGTCTTTGAATA
AAGTCTGAGTGGGCGGC

>Chicken (Gallus_gallus)

GGGGCTGCCAACACAGAGGTGCAACCATGGTGCTGTCCGCTGCTGACAAGAACAACGTCAAGGGCATCTT
CACCAAAATCGCCGGCCATGCTGAGGAGTATGGCGCCGAGACCCTGGAAAGGATGTTCACCACCTACCCC
CCAACCAAGACCTACTTCCCCCACTTCGATCTGTCACACGGCTCCGCTCAGATCAAGGGGCACGGCAAGA
AGGTAGTGGCTGCCTTGATCGAGGCTGCCAACCACATTGATGACATCGCCGGCACCCTCTCCAAGCTCAG
CGACCTCCATGCCCACAAGCTCCGCGTGGACCCTGTCAACTTCAAACTCCTGGGCCAATGCTTCCTGGTG
GTGGTGGCCATCCACCACCCTGCTGCCCTGACCCCGGAGGTCCATGCTTCCCTGGACAAGTTCTTGTGCG
CCGTGGGCACTGTGCTGACCGCCAAGTACCGTTAAGACGGCACGGTGGCTAGAGCTGGGGCCAACCCATC
GCCAGCCCTCCGACAGCGAGCAGCCAAATGAGATGAAATAAAATCTGTTGCATTTGTGCTCCAG

DNA SEQUENCES (6 Discovery Species)

>Tarsier (Carlito_syrichta)

```
GCAGGATGTTCCTGTCCTTCCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCCAG
CCAGGTCAAAGGCCACGGCAAGAAGGTGGCCGACGCGCTGACCACCGCCGTAGGGCACATGGACGACATG
CCCAACGCCCTGTCCGCTCTGAGTGACCTGCACGCGCACAAGCTGCGGGTGGACCCGGTCAACTTCAAGC
TCCTGAGCCACTGCCTGCTGGTGACCCTGGCCTGCCACCACCCTGCGGACTTCACCCCTGCCGTGCATGC
CTCCCTGGACAAGTTCCTGGCCTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCCGG
GTGGCCTCCCGGGGCCCTCCTCCCTTCCCTGCACCCGCACCTTCTGGTCTCTCAATAAAGTCTGAGTGGG
TGGCAA
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>Whale (Orcinus_orca)

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ACGCTTCTGGTCCTGACCCAGACTCAGAGAGAACCCACCATGGTGCTGTCTCCCGCCGACAAGACCAACG
TCAAGGGCACCTGGGCTAAGATTGGCAACCACAGTGCAGAATATGGCGCAGAGGCCCTGGAGAGGATGTT
CATAAACTTCCCCAGCACCAAGACCTACTTCTCCCACTTCGACCTGGGACACGGCTCCGCTCAGATCAAG
GGGCACGGCAAGAAGGTGGCCGACGCGCTCACCAAAGCCGTGGGCCACATAGACAACCTGCCCGATGCCT
TGTCTGAGCTGAGCGACCTGCACGCCCACAAGCTGCGTGTGGACCCGGTCAACTTCAAGCTCCTGAGCCA
CTGCCTGCTGGTGACCCTGGCTCTCCACCTCCCCGCCGATTTCACGCCCTCGGTCCATGCCTCCCTGGAC
AAGTTCTTGGCCAGTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCCGCGATTCCTA
CCCTGGCCTGGGGCCCTCTGGCGCTCTGGGCACCCTCACTTCCCGATCTTTGAATAAAGTCTGAGTGGGC
TGCA
```

>Platypus (Ornithorhynchus_anatinus)

```
AGGGAATCTGGTCCTGACCCGATTTGGAAAGGAGCAAAGATGCTGACCGACGCCGAGAAGAAGGAAGTGA
CCGCCCTGTGGGGCAAGGCCGCCGGTCACGGCGAGGAGTACGGCGCGGAGGCCCTGGAGAGGCTGTTCCA
GGCTTTCCCCACCACCAAGACCTACTTCTCCCACTTCGACCTGAGCCACGGCTCCGCTCAGATCAAGGCT
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CCGCTCTGAGCGACCTGCACGCCCACAAGCTGAGAGTGGACCCGGTCAACTTCAAACTGCTGGCTCACTG
CATCCTGGTTGTCCTGGCCAGGCATTGCCCGGGCGAGTTCACCCCCTCCGCCCACGCCGCCATGGACAAG
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CCGCTGCCGCCTCCACCCGGCCGAACCCCTCCCCAACTCCATCATGCCCAGGCTGACCCTCGTCTTGCAA
TAAAGCTCTATCAGCGGCA
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>Hedgehog (Erinaceus_europaeus)

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TGAGTCCCGCACTTCTGATCCAACAGAGACTCAGAAAGAACTCACCATGGTGCTGTCTGCAACTGACAAG
GCCAACGTCAAGACTTTCTGGGGCAAGCTCGGCGGCCATGGCGGCGAGTATGGCGGCGAGGCTCTGGACA
GAATGTTCCAGGCCCACCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAACCCGGGCTCCGCTCA
GGTCAAAGGCCATGGCAAGAAGGTGGCAGATGCTCTGACCACTGCTGTGAACAACCTGGACGACGTTCCA
GGAGCCCTGTCTGCCCTGAGCGACCTGCACGCGCACAAGCTGCGTGTGGACCCCGTCAACTTCAAGCTCC
TGAGCCACTGTCTGCTGGTGACCCTGGCCCTCCACCACCCTGCAGACTTCACCCCCGCGGTGCACGCCTC
CCTGGACAAGTTCTTGGCTACAGTGGCCACTGTGCTGACCTCCAAGTACCGTTAAGCCACAGCCTGTCCA
GTCCCTGTCCTGCAGGGCGTCCCCAACCTCTCAATAAAGTCTGATGGGCAGCA
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>Bat (Myotis_lucifugus)

```
CGTGCCCCTTGCGCCCCGGGCATAAAAGTTCCTCACTCTCCGGGCCGGTACCCCTTCTGGTCCTCGCACA
GACCCAGAAAGAAGCAACCATGGTGCTGTCTCCCGCTGACAAGACCAACATCAAGGCCGCCTGGGACAAG
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AGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCTGCCCAGGTCAAAGGCCACGGCAAGAAGGTGGG
CGACGCCCTGGGTAACGCCGTGGCCCACATGGATGACCTGCCCGGCGCCCTGTCCGCCCTGAGCGACCTG
CACGCCTACAAGCTGCGTGTGGACCCCGTCAACTTCAAGCTGCTGGGCCACTGCCTGCTGGTCACCCTCG
CCCGGCACTACCCCGGAGACTTCAGCCCCGCCCTGCAGGCCTCGCTGGACAAGTTTCTGAGCCACGTGAC
```

TTGGGCGCTGGCCTCCAGCTATCGCTAA

>Walrus (Odobenus_rosmarus)

CCACCATGGTGCTGTCTCCCGCCGACAAGACCAACGTCAAGACCACCTGGGATAAGCTTGGCGGCCACGC
TGGCGAGTATGGCGGAGAGGCTCTGGAGAGGACCTTCATGTCCTTCCCCACCACCAAGACCTACTTCCCC
CACTTCGACCTGAGCCCCGGCTCCGCCCAGGTCAAGGCGCACGGCAAGAAGGTGGCCGACGCGCTGACCA
CCGCTGTGGCCCACATAGATGACCTGCCTGGTGCTCTGTCGGCTCTGAGCGACCTGCACGCGTACAAGCT
GCGTGTGGACCCCGTCAACTTCAAGCTCCTGAGCCACTGCCTGCTGGTGACCCTGGCCTGCCACCACCCC
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