

Post-Lab Activity

Analyzing and Interpreting Student Results

To make meaningful inferences about the evolutionary relationships among samples from different fish species, students must analyze and compare the protein banding patterns of the different fish in their gels. A typical set of results from SDS-PAGE of fish muscle proteins is shown here:

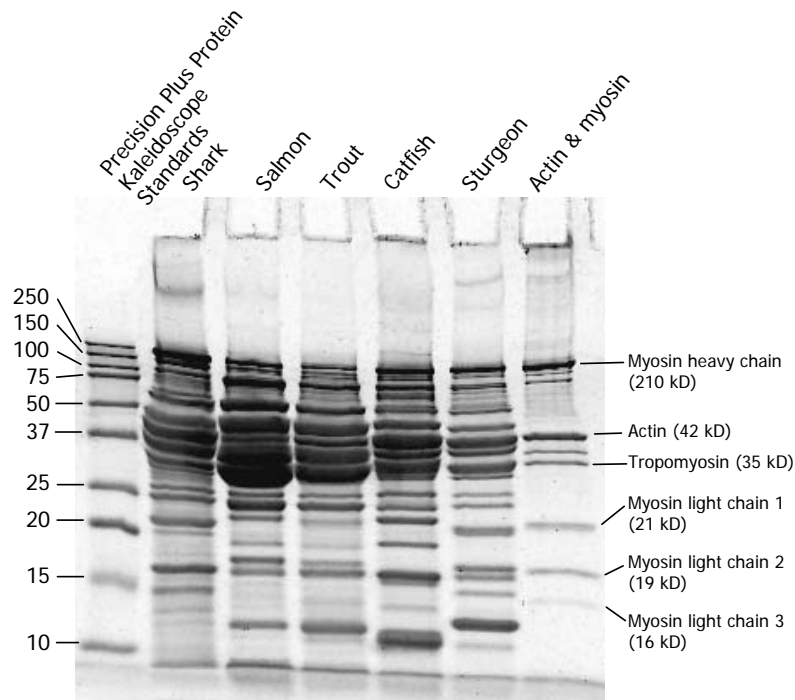


Fig. 8. 15% Ready Gel polyacrylamide gel electrophoresed at 200 V for 30 minutes, stained with Bio-Safe Coomassie stain, and destained in water.

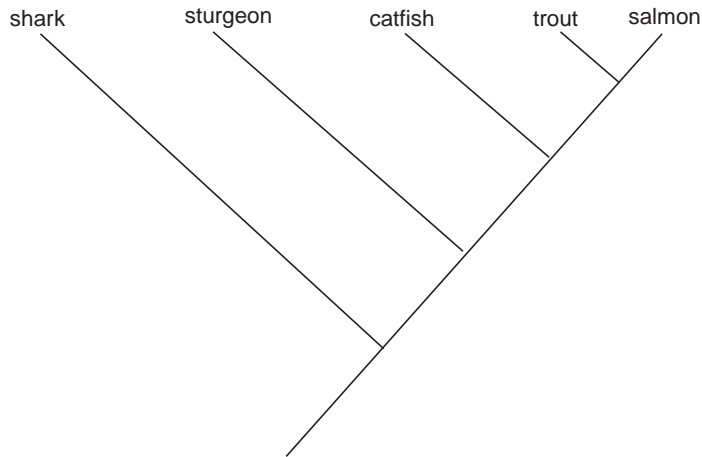
Obvious similarities and differences between protein fingerprints are easily spotted. Comparing the banding patterns of salmon and trout, it becomes clear that these are very similar. For instance, they share a common band at approximately 70 kD, as well as a pair of bands at 20–25 kD. This similarity is consistent with the provided fish evolutionary tree, as salmon and trout are both on the same branch. In contrast, a comparison of salmon with catfish reveals significant differences in their patterns. Only one of the bands of approximately 25 kD found in salmon is present in catfish.

In the post-lab activity below, students are instructed to construct a cladogram based on their own gel analysis.

Detailed Gel Analysis

This section is the "so what?" part of the activity. Students engage in a multidisciplinary approach to problem-solving to determine whether their electrophoresis data support or refute the original predictions they made during the pre-lab activity. Students create a fish family tree, or cladogram, from scratch, using their own gel results. Students determine whether their cladogram matches the evolutionary relatedness of the fish species determined by traditional morphological analysis in the evolutionary tree provided.

Each protein band that a fish has in common with another fish is considered a shared characteristic. A fish family tree, or cladogram, can be constructed based on protein bands that the fish have in common. Cladistic analysis assumes that when two organisms share a common characteristic, they also share a common ancestor with that same characteristic. This lineage can be represented on a cladogram as a node with two branches representing the descendent organisms. In this exercise your students will identify shared characteristics by listing all the different proteins in each fish muscle sample, determining which proteins (characteristics) are shared between the different fish species, and constructing a cladogram based on their own electrophoresis data.



Creating a Cladogram

Each protein band that a fish has in common with another fish is considered a shared characteristic. A protein can be identified by its molecular weight or by the distance the protein migrated through the gel. Because a cladogram may be created simply from the distance each protein band migrated through the gel, it is not strictly necessary to construct a standard curve to determine the molecular weights of the proteins in each sample. However, as a lesson in graphing, you may choose to instruct your students to create a standard curve using the protein standards they ran on their gels to determine the actual size of the protein bands in each sample.

Procedure: Construct a standard curve to calculate protein molecular weights

1. First measure the distance each of the five protein bands contained in the Precision Plus Protein Kaleidoscope prestained protein standards migrated starting from the well – from the largest (green 37 kD) protein band down to the smallest (yellow 10 kD) protein band. Accuracy to 0.5 mm is sufficient. Record the distance each protein in the prestained protein standard mixture migrated and plot that distance against the molecular mass of each.

Precision Plus Protein Kaleidoscope Prestained Standards Molecular Mass (kDa)	Distance Migrated (mm)
37	23
25	30
20	35
15	42
10	51.5

- Plot these distances in mm on the x axis against the molecular mass of the bands in kD on the y axis as a scatter plot on the logarithmic graph paper provided on page 36. Then connect the dots to produce a linear (straight line) curve, as shown in the illustration on page 36. Students can then determine the molecular masses of unknown proteins in their samples by measuring the distance each band migrated from its well of origin. By drawing a line up from the x-axis to the curve, they can estimate the molecular masses from the y-axis by extrapolation.

A graphing computer program may also be used to generate a standard curve using the prestained standards in order to calculate the molecular masses of all the unknown proteins on the gel.

- Determine the molecular masses of all the unknown proteins in the different fish samples. Instruct your students to draw a horizontal line on the dried gel (or gel image) between the 37 kD protein (green) and 25 kD protein (pink) markers – just below the fat bands that occur at around 30kD.

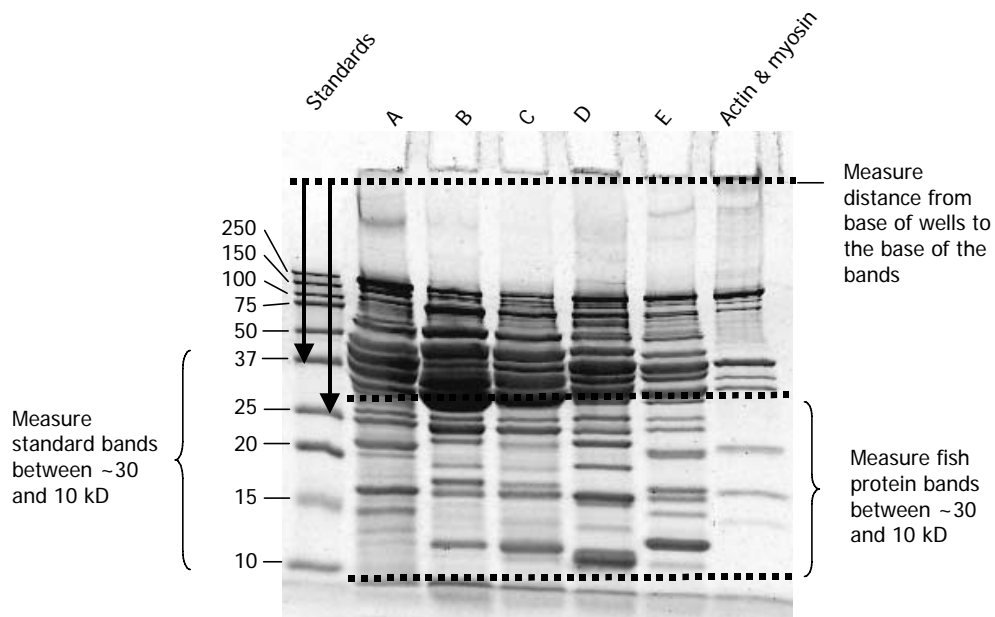


Fig. 9. Image of fish muscle proteins separated by SDS-PAGE and stained with Bio-Safe Coomassie stain. Lines illustrate measurement of bands for constructing the standard curve.

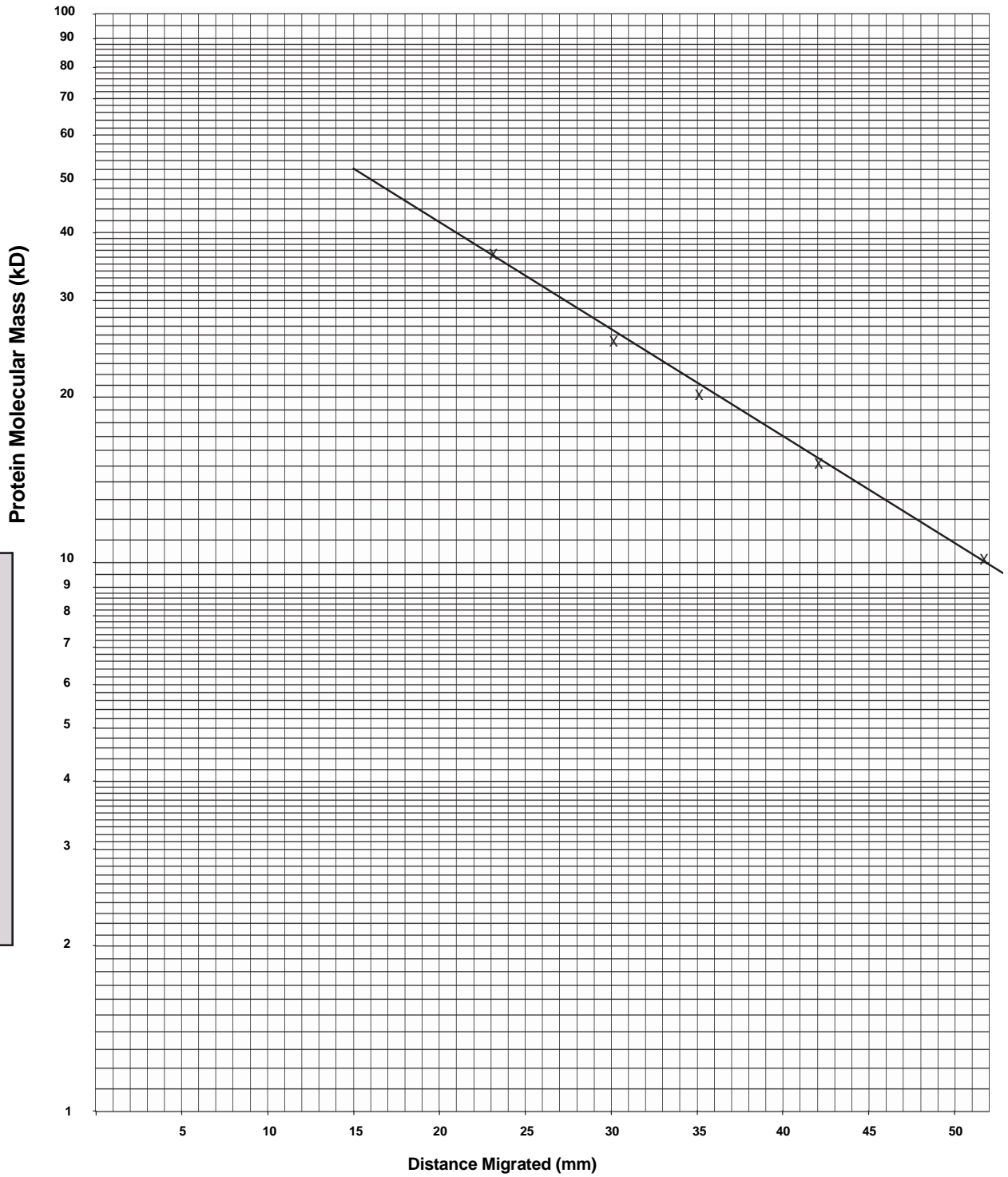


Fig. 10. Example of a standard curve drawn on two-cycle semi-logarithmic graph paper. The curve is constructed by plotting the protein molecular mass against the distance migrated.

4. Then for each band below the line (for each fish sample), students measure the distance the protein band migrated from the well of origin and write that distance down as shown on the chart below.

	Shark	Salmon	Trout	Catfish	Sturgeon
Distance protein bands migrated – in mm	25, 26.5, 29, 36, 36.5, 39, 44, 52	26, 27.5, 29, 32, 34.5, 36.5, 37.5, 40.5, 42, 45	26, 27.5, 29, 29.5, 32, 34.5, 36.5, 37.5, 40.5, 42, 45, 46.5, 51.5	26, 27.5, 29, 32, 36.5, 38, 38.5, 41, 46, 47.5	26, 27.5, 30, 30.5, 33, 35.5, 37, 39, 39.5, 42, 44, 47

5. Determine and record which fish have shared characteristics (same proteins). Students record their gel data by filling out the table as shown below. There should be a row for every measurable band size and/or distance migrated and a column for each type of fish on their gel. Then students put an X in each cell of the table where the fish has that size band and put the total count at the bottom of each column (see example below).

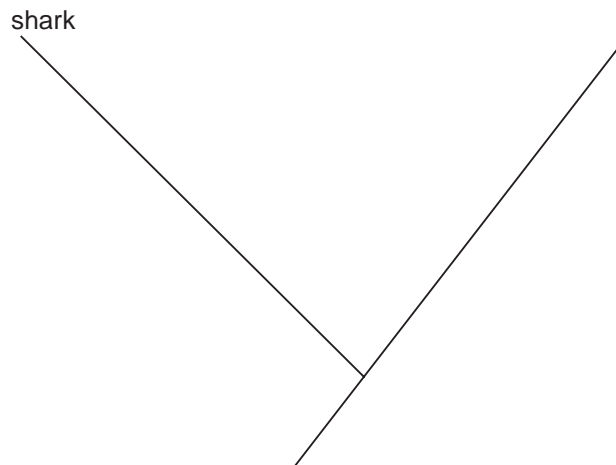
Characteristic

Distance migrated (mm)	Protein Molecular Mass (kD)	Shark	Salmon	Trout	Catfish	Sturgeon
25	32.5	X				
26	31.5		X	X	X	X
26.5	31.0	X				
27.5	30.0		X	X	X	X
28.5	29.1					
29	28.6	X	X	X	X	
30	27.6			X		X
30.5	27.1					X
32	25.6		X	X	X	
33	24.7					X
34.5	23.2		X	X		
35.5	22.2					X
36	21.7	X				
36.5	21.2	X	X	X	X	
37	20.7					X
37.5	20.2		X	X		
38	19.7				X	
38.5	19.3				X	
39	18.8	X				X
39.5	18.3					X
40.5	17.3		X	X		
41	16.8				X	
41.5	16.3					
42	15.8		X	X		X
43	14.8					
44	13.9	X				X
45	12.9		X	X		
46	11.9				X	
46.5	11.4			X		
47	10.9					X
47.5	10.4				X	
51.5	6.5			X		
52	6.0	X				
	COUNT	8	10	13	10	12

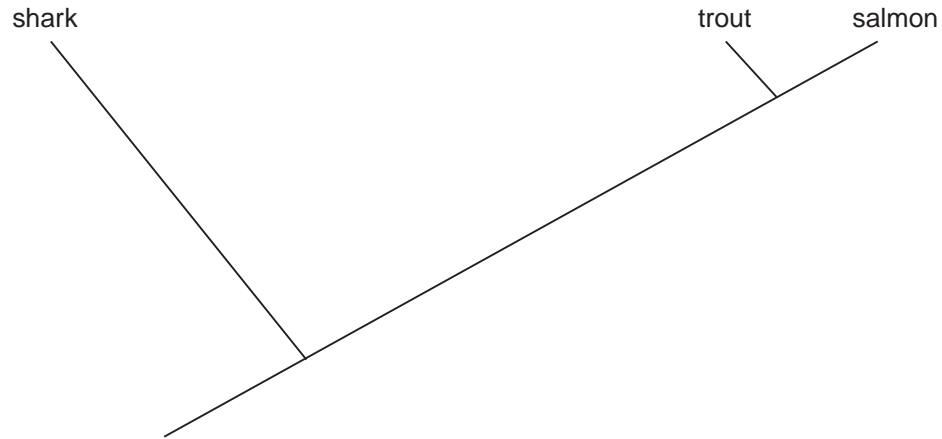
6. Find the number of characteristics shared by each of the fish. Students then create one final table that will be the basis for constructing their cladograms. In this table both the row and column headings are the types of fish analyzed. For each fish students separately compare the number of bands in common with every other fish in their gel and put those numbers into the table so that each fish is individually compared with every other fish. In this example salmon and shark have just two bands in common, while trout and salmon have ten bands in common.

	Shark	Salmon	Trout	Catfish	Sturgeon
Shark	8	2	2	2	2
Salmon		10	10	5	3
Trout			13	5	4
Catfish				10	2
Sturgeon					12

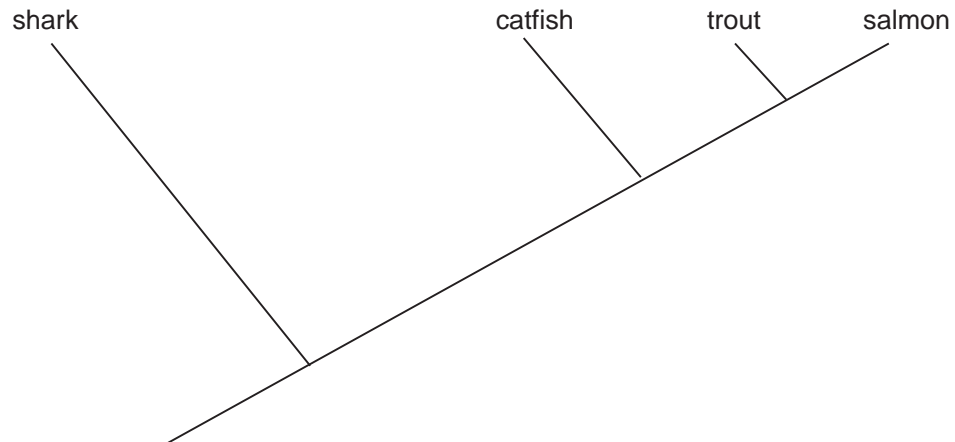
7. Now your students are ready to construct their cladograms. Construct the cladogram.
- A. First, draw a diagonal line across the page from left to right in the upward direction. This line will form the "trunk" of the cladogram. Identify the fish with the least bands in common with any of the other fish (in this example it is shark, which has only 2 bands). Draw a side branch at 90 degrees near the bottom of the trunk and label the end with the fish's name, in this case, shark. This fish is the outlier because it is the least similar to any of the others. The node where the shark's side branch forks from the trunk represents an ancestor that is common to all the fish in this analysis.



- B. Next, find the two fish with the most bands in common (in this example it is salmon and trout which have 10 bands in common). Draw a side branch off the line near the top to form a fork and label the 2 ends of the fork with the fishes names, in this case, salmon and trout (it doesn't matter which branch gets which label). The node represents a common ancestor of salmon and trout that had all those proteins.

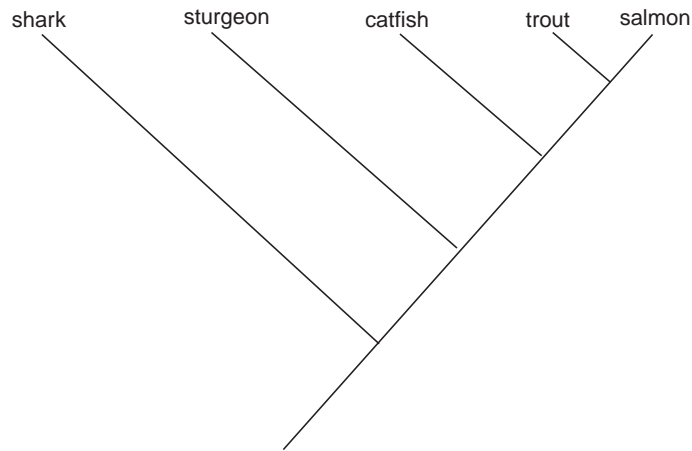


- C. Now find the fish with the next most bands in common. In this example, catfish has five bands in common with salmon and trout, which indicates it is the same cladistic distance from salmon and trout (i.e. it is not more closely related to salmon over trout or vice versa). Draw a branch further down the trunk. This node represents an ancestor that is common to salmon, trout and catfish that had these same five proteins.

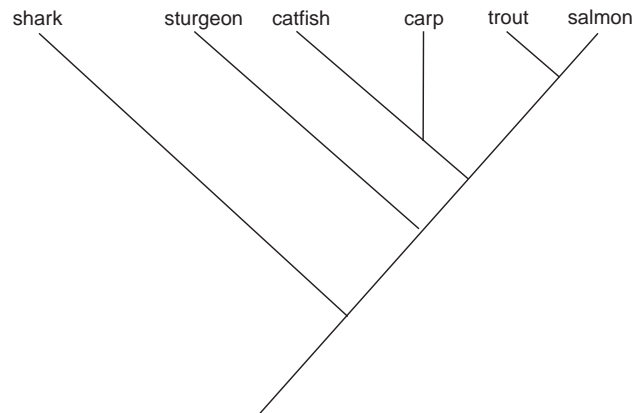


- D. The last fish to add to the cladogram in this example is sturgeon, which shares four bands with trout, three bands with salmon, and only two bands with catfish and shark. This fish may seem trickier to place than the others because it shares more characteristics with salmon and trout than it does with catfish, but catfish shares more characteristics with salmon and trout than sturgeon does.

So, to place this fish you can ask, does sturgeon share the five proteins that the common ancestor of salmon, trout, and catfish had? Answer – no. Does sturgeon share more proteins with salmon, trout, and catfish than shark? Answer – yes. Therefore, sturgeon gets its own branch in between the catfish and shark branches to indicate that it has more shared characteristics with trout, salmon, and catfish than shark, but fewer shared characteristics with salmon and trout than catfish.



Note: It has not happened in this example, however, there may be a case in which two pairs of fish are more closely related to each other than to the other fish. For example a carp would likely have more bands in common with catfish, say 8 bands (while having 5 or less bands in common with the other fish). In this case, a sub-branch would be made of the catfish branch to indicate that catfish and carp have a shared ancestor that has more shared characteristics (in this case 8 proteins) than the shared ancestor of salmon, trout, catfish and carp represented by the node on the main trunk, which has only 5 protein bands in common.



Students then compare their cladograms with the phylogenetic tree below and decide whether their molecular evidence supports or refutes published phylogenetic data.

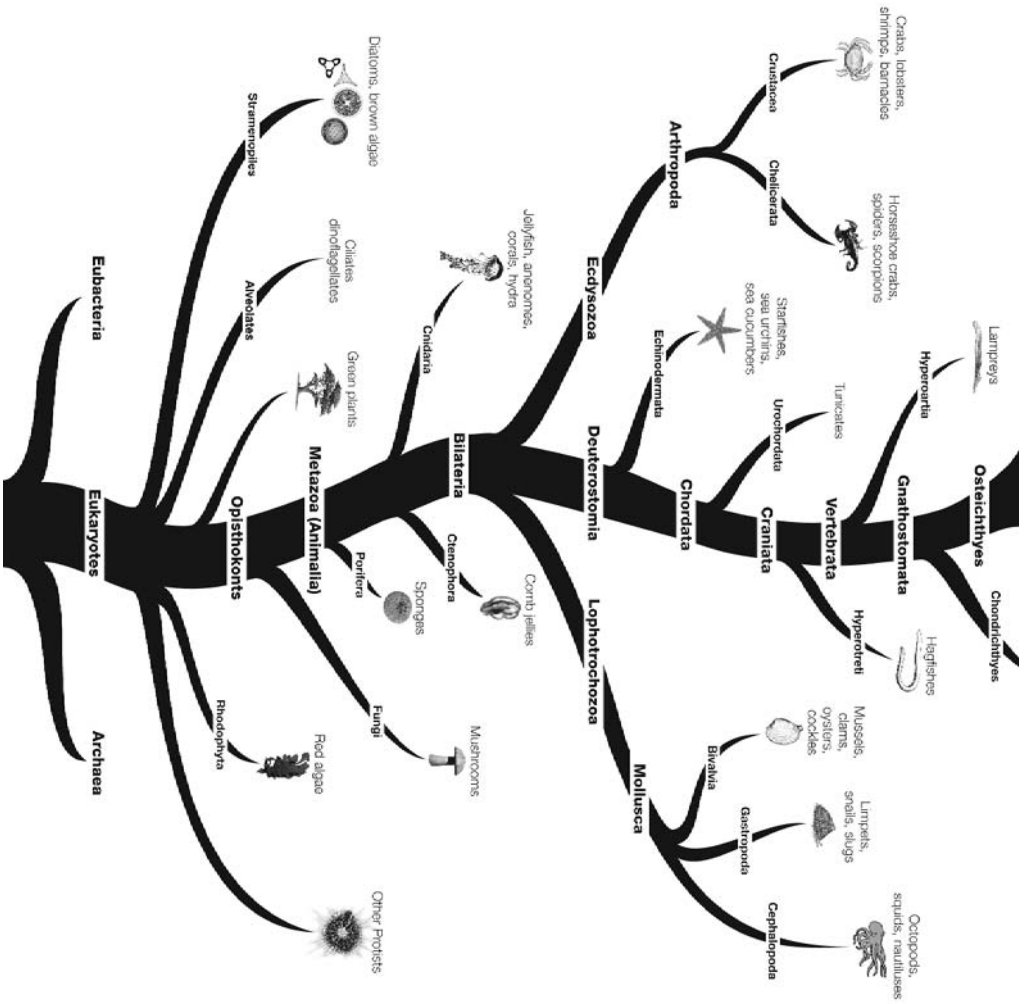


Fig. 3. Evolutionary tree showing the relationships of eukaryotes. (Figure adapted from the tree of life web page from the University of Arizona (www.tolweb.org))

