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Using Microfossils to Demonstrate Ecology and Evolution: (In Memoriam of Charlie Drewes)

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This laboratory exercise is an expansion of the workshop presented in 2005 by Dr. Charlie Drewes on Iowan microfossils. The concepts covered include microscopic manipulation, diversity indices, environmental sampling, phylogenetic trees, and the tree of life. There are three major sections: 1) identifying, sampling, and calculating a diversity index on the fossil sample, 2) comparing fossil organisms to their modern descendants, 3) creating a phylogenetic tree for both the fossils and their modern descendants. These exercises can be used individually, or combined as an ongoing project that touches on many of the topics emphasized in a first year course.

Keywords: microfossils, systematics, ecology, evolution, diversity

Introduction

In 2005, the late Charlie Drewes presented a workshop on the use of Devonian microfossils at an ABLE meeting (Drewes, 2006). During that exercise, he not only provided the knowledge and skills to manipulate these fossils, but also donated a sample of the microfossils for participants to take home with them. Using these microfossils, I have designed a multi-week exercise on ecology and evolution.

Devonian microfossils represent marine organisms from approximately 375 million years ago (Armstrong & Brasier, 2005; Boardman & Cheetham, 1991; Clarkson, 1998; Drewes, 2006; Levin, 1998; Simpson, 1949; Taylor & Lewis, 2007; Thompson & Dickinson, 1982). The fossils themselves are surprisingly small, unlike the trilobites, fern impressions and dinosaur bones typically imagined. The fossils are given to the student in microcentrifuge tubes and look to them like a small sample of gravel, until they see the sample under the dissecting microscope. Therefore, students need to learn to both find the fossils amongst other geologic detritus, as well as manipulate these fossils under a dissecting microscope. Since many of these fossils are structurally foreign to most students, this is an excellent exercise on observation. Once fossils have been isolated, the next step is to help the students come to the realization that not all organisms or parts of organisms are amenable to fossilization, thus making comparisons challenging.

These samples not only represent a wide range of anatomical parts, but also an amazing range of species types. Organisms represented in the sample include everything from algal "eggs" to jaw structures of marine worms. Initially, this fact can be utilized to talk about ecological diversity. A Simpson's Species Diversity index, generally used for ecology, can be calculated (Simpson, 1949). Additionally, this process can be used to illustrate sampling techniques since individual group means can be compared and, then, the fact that they are actually replications of the same original sample can be discussed. This also opens the door to exercises involving some basic statistics.

After an introduction on phylogenetic trees (there are many exercises available on this topic), these fossils can now serve as a basis of a tutorial on the construction of trees using actual organisms. Several pieces of information are essential for creating a tree showing the evolutionary relationships of fossil organisms: 1) the fossils themselves, 2) background information about the whole organisms the fossils represent, 3) the current theories of relationships amongst taxa, and 4) observation of modern representatives of their fossil ancestors. The lattermost of these requirements can be accomplished by supplying the students with slides and/or preserved specimens. For example, slides of green algae can show the entire organism represented in the sample only by its "eggs" and a full-grown lamprey can show how a conodont jaw might have been situated. Using all available information, the students can then build their own phylogenetic tree that can ultimately be compared to the current hypothesized tree. Students do not generally create a tree that is exactly correct, which is an excellent opportunity to discuss parsimony and how theorized trees "evolve" with new evidence.

Rarely can one example be the foundation for so many essential ideas in biology. Any one of the portions of the exercise can be used independently, or they can be combined for one complete set of ultimate goals. Fossils are easily

associated with the study of evolution, but here I will demonstrate how they can also be utilized to teach observation, sampling, diversity, statistics, systematics, geology, microscopy, micromanipulation, biological hierarchy, morphology, ecology, and potentially a myriad of other topics not yet explored in this manuscript. Tweaking of the information provided herein can allow for these fossils to be applied to all levels of Biology.

Student Outline

Student Handout 1

Species Diversity in Microfossils

Original Concept from Dr. Charles Drewes, Iowa State University

Most people are aware of common fossils such as trilobites, seashells, and even dinosaur bones, but the vast majority of fossils that can be found are actually not visible by the naked eye. Many marine organisms were smaller than the head of a pin, but still have readily identifiable structures when seen under a dissecting microscope. Many of the fossilized structures are small, dense portions (teeth and support structures) of otherwise fleshy and delicate bodies; thus in many cases, the fossils look nothing like the organism from which they came.

In any ecosystem, species are represented at varying levels of abundance depending on many factors including reproductive ability, resource availability, and survivorship curves. The sample of fossils we will use for this lab includes fossils from many parts of an ancient ecosystem and includes structures from plants, mollusks, sponges, and worms.

For this lab, you will be given less than one gram of what appears to be gravel. In fact, these small particles often contain the fossilized remains of virtually microscopic sea life that existed ~400 million years ago and were collected in Iowa courtesy of Dr. Charlie Drewes. You will begin by separating the fossils from other detritus and then continue by sorting, identifying, and counting the fossil types. From this, you will be able to calculate species diversity in the sample provided using the Simpson Index.

Procedure

1. Obtain a sample of Devonian microfossil "gravel" in a microcentrifuge tube.
2. Dump a small portion of this "gravel" into a Petri dish lined with paper.
3. View the sample under the dissecting microscope to find putative fossils.
4. Using the widget dipped in water, pick up any putative fossils and move them to other paper-lined Petri dishes, dividing them by type.
5. Refer to the laminated pictures taken from the website for identification: **http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/fossil-buttons.htm**
6. Count the number of individuals in each category you have defined.
7. Calculate the Simpson's Diversity Index (D) for your sample. (See below)

   A diversity index is a tool for combining two important qualities of a sample or ecological community: species richness and evenness. Richness is the number of species or types found in a given sample or location. Evenness measures how evenly the total number of organisms is distributed across the different species or types. More types of organisms will result in a higher diversity measurement. Likewise, a more even distribution of organisms across the different types (i.e., the community is not dominated by one or two types) results in a higher diversity. For the purposes of this lab, you will use Simpson's Diversity Index (D) to estimate the diversity of fossil types in your small sample of "gravel."

$$D = \frac{N(N-1)}{\sum n_i(n_i-1)}$$

where N is the total number of individuals of all species and n_i is the number of individuals of the *i*th species

Simpson's Index varies from a minimum of 1.00 to infinity: the higher the number, the greater the species diversity. While the number itself has no units, the diversity index from two different samples can be compared for relative diversity. For instance, if the index of one population is 2.56 and another population is 21.23, the second population is more diverse than the first.

Example: Species A = 20; Species B=5; Species C=10; Species D=45

$N = 20 + 5 + 10 + 45 = 80$
$n_A (n_A - 1) = 20(19) = 380$
$n_B (n_B - 1) = 5(4) = 20$
$n_C (n_C - 1) = 10(9) = 90$
$n_D (n_D - 1) = 45(44) = 1980$

$$D = \frac{80(80-1)}{380+20+90+1980} = 2.56$$

Questions

1. Calculate the Simpson's Index for your sample.
2. Compare your value with the other values in the class. Calculate a mean Simpson's Index for the class and discuss how your value differs from the mean.
3. Now, considering that each group's sample was actually taken from one larger sample, explain any discrepancy. Devise a way to have the most accurate index for any given sample.
4. Is the Simpson's Index, a measure of species diversity in ecology, an appropriate measure for this sample? Why or why not?

Student Handout 2

Creating a Phylogeny for the Microfossils

You have examined the microfossil sample from the Devonian period. The relics of the organisms you viewed represent some of the marine organisms from 375 million years ago (see the time scale in Fig. 1). Fig. 2 shows what the ocean floor looked like at that time. You can clear see crinoids, brachiopods, bryozoa and more. While many of these particular species may be extinct, their descendants still exist today.

You will have the opportunity to examine both microscope slides and preserved organisms that represent the modern versions of these ancestral species. This will be advantageous in two ways: 1) the new specimens can be used to envision the whole organism and not just the fossilized part and 2) having modern relatives will give a more accurate idea of some of the characteristics of their ancestors. This will make creating a phylogeny in next week's lab a bit easier for you.

The final goal of this exercise will to combine your new knowledge of the microfossils of the Devonian period with your knowledge of their descendant in order to create a phylogenetic tree of the life in a 375 million year old ocean. After you create your own tree of the organisms represented in the microfossil sample, you will compare your result to the actual tree provided by the instructor. Three useful resources are: 1) the microfossils and their associated website, 2) the modern relatives of the microfossils and 3) The Tree of Life website (**www.tolweb.org**).

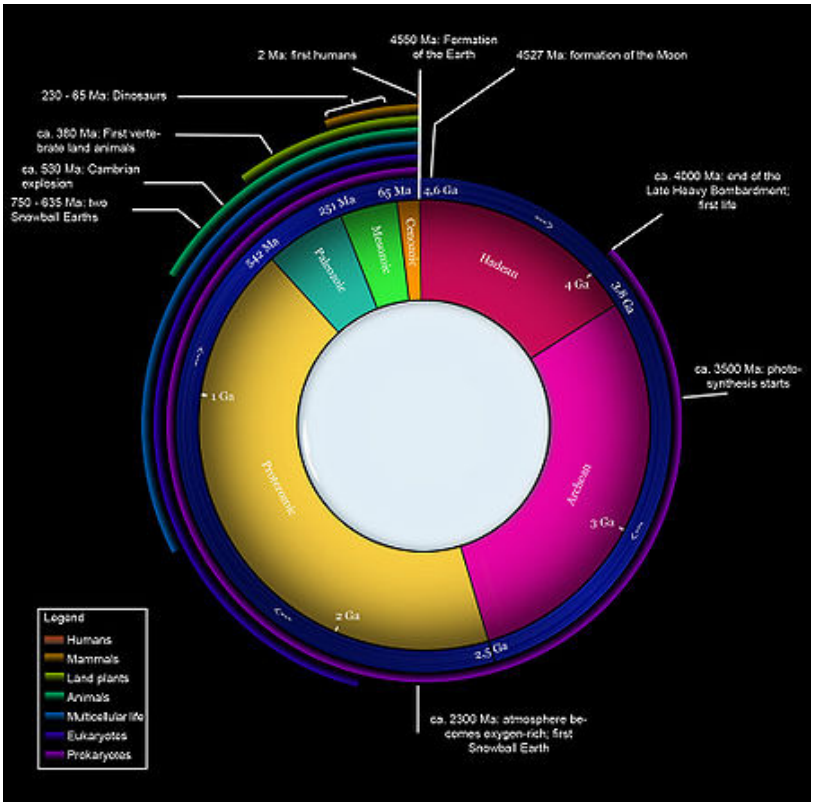


Figure 1. Geologic time. Note where on this figure the Devonian Period would be. **http://wpcontent.answers.com/wikipedia/commons/thumb/f/fe/Geologic_clock.jpg/450px-Geologic_clock.jpg**

Procedure

1) Look at the chart provided to you (Table 1) to see which microscope slides and preserved organisms are available for comparison.
2) Select at least one extant representative in each fossil category to examine more closely.
3) **Draw** and label a diagram of these selected organisms, paying particular attention to those structures that define the group to which the organism belongs.
4) **Compare** the modern representative to their fossil relative.

Figure 2. A Devonian sea bottom. **http://skywalker.cochise.edu/wellerr/students/devonian/project_files/image024.jpg**

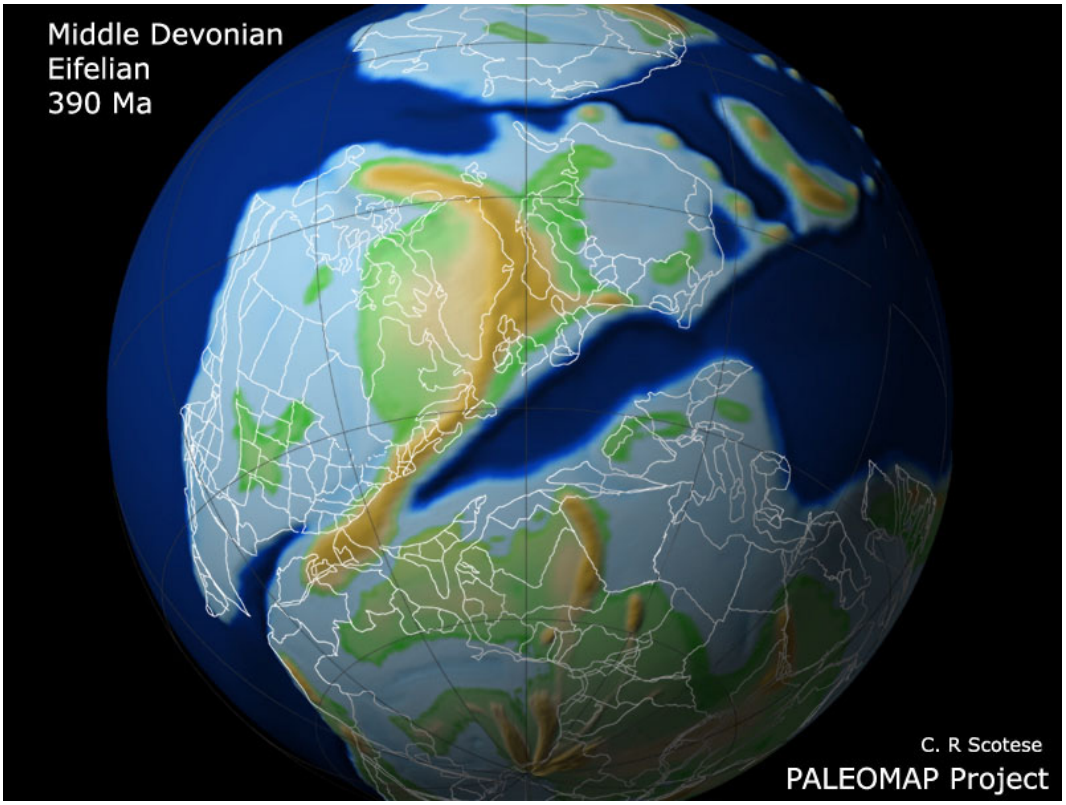


Figure 3. The Devonian world showing the outlines of the modern continents. **http://www.scotese.com/moremaps2.htm**

Table 1. Examples of existing species

Microfossil	Fossilized part	Inclusive group	Common name	Slides	Whole
Charophyte	Oogonia ("egg")	Charophyta	Green algae stonewort	*Chlamydomonas* *Volvox* (Eudorina) *Ulothrix* *Oedogonium*	Sea lettuce Acetabularia
Foraminifera	Shell	Protozoa	Amoeboid protozoa	*Amoeba* *Radiolaria* *Sarcodina* *Plasmodium* *Foraminifera*	
Sponge	Spicule	Porifera	Sponges	Sponge spicule *Grantia*	*Leucosolenia* *Grantia*
Crinoid	"Stalk"	Echinodermata (Crinoidea)	Feather stars		Sea cucumber Brittle star
Tentaculitids	Shell	**Gastropods or cephalopods???**	???		Squid
Snail	Shell	Gastropod	Snails		*Limax* Slug
Conodont	Jaw	**Chordata???**		Lampreys Amphioxus	Sea squirt Lamprey
Scolecodonts	Jaw	Annelida (Polychaeta)		Leech	Earthworm Marine worms Leech Lugworm
Bryozoa	"coral-like"	Bryozoa	Moss animals	Bryozoa "eggs"	
Ostracod	Shell	Crustacea	Seed shrimp		*Streptocephalus* Sand shrimp *Macrobrachium* Lobster Barnacle Crayfish
Brachiopod	Shell	Brachiopoda	Lampshells	Mussel	Oysters/mussels
Echinoid	Spine	Echinodermata (Echinoidea)	Sea urchins	Starfish	*Echinoderms* Sand dollar

Questions

1) What types of part of the animals are most likely to be fossilized? Why?
2) Using Fig. 3, what did Iowa look like in the Devonian Period? What did Pennsylvania look like?
3) Which fossil is most like its present day ancestor? Which one is least like its present day ancestor? Explain.
4) Do some research on the Devonian Period. Which organisms are not represented in our sample? Why would that be?

Student Handout 3

Systematics and Phylogeny Exercise

*Based on the work of Sara Morris (***http://www.wilsonsociety.org/wosmanual/8.Systematics.pdf***)*

Systematics is the study of evolutionary histories. Systematists can use many different types of characters in their study of the relationships between species including morphology, protein forms, and DNA sequences. Here we will use "morphological" characteristics of members of the "Screwidae" family to create a phylogenetic tree that defines their evolutionary relationships to each other.

When making a tree you are trying to show the evolutionary relationships between organisms. A tree may compare individuals, species, genera, etc. Regardless of what level you are comparing, each group is known as a *taxon* (plural: taxa). The taxa being compared would be shown on the ends of the branches of the tree (Fig. 1). Where the branches from two taxa connect, you get a *node*. This node represents a common ancestor for the two taxa beyond the node. A tree should be arranged so that the taxa that are most closely related have the least amount of nodes between them. For instance, in Fig. 1, Species 1 is more related to species 2 than it is to species 3 or species 4. The outgroup here has helped determine where the root of the tree is because it would have characters common to all of the taxa on this tree. A trait in the outgroup would represent the original (or ancestral) state of the trait.

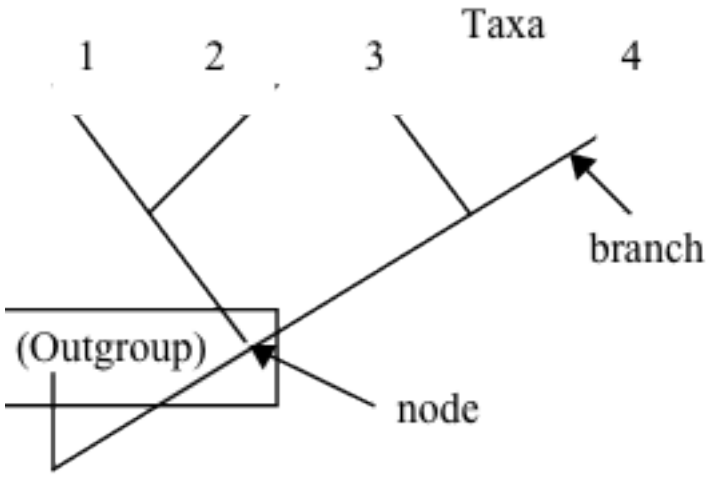


Figure 4. The basics of a tree.

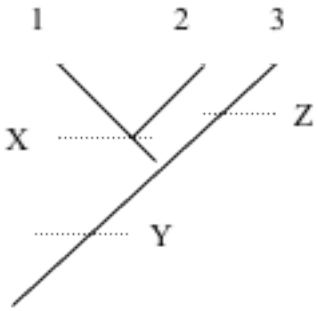
The root of the tree would be the common ancestor of everything on the tree and the branches occur when there has been an evolutionary change causing taxa to diverge. You can think of each change as a "step." We want to pick the tree that needs the least steps to create. This tree is known as the most *parsimonious*, or simplest answer. So, characters that all taxa have should arise early and characters unique to a group should arise later.

Consider the data set in Table 1.

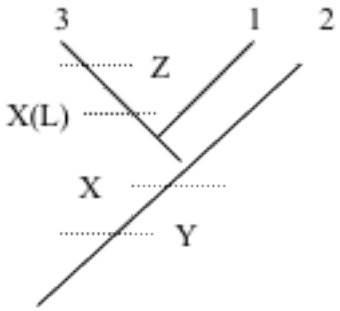
Table 1. Basic data for tree construction.

	Char X	Char Y	Char Z
Species 1	Yes	Yes	No
Species 2	Yes	Yes	No
Species 3	No	Yes	Yes

In this case, it is not hard to imagine that Character Y would have arisen before the common ancestor for these three species; character X would arise when 1 and 2 split from 3, and character Z would have arisen separately in 3, resulting in the tree on the left (Tree 1). Consider, however, that the tree on the right (Tree 2) is also possible if you allow for a trait to be lost (L) later. This second tree takes more steps than the original tree (4 versus 3), and, thus, the tree on the left is the most parsimonious answer.



Tree 1



Tree 2

Sometimes, the answer is not as clear as this answer, and sometimes, in more complex trees, more than one answer takes the same number of steps. Sometimes traits are lost, regained and lost again. This can get very difficult. That is why, in current phylogenetic methods involving numerous taxa and sometimes hundreds of characters, computer programs help build the trees based on more complex mathematical algorithms. Modern systematics (the study of phylogenies) is much more complex than this, but this exercise will be a decent introduction.

Procedure

1) As an introduction, visit the following website: **http://www.ucmp.berkeley.edu/education/explorations/tours/Trex/index.html**. Walk through the exercise and then continue below.
2) To begin, you will have to fill in Table 2 with characteristics you will define for yourself. List the "traits" across the top and then fill in the chart with a "+" if the species has the trait and a "-" if it does not. Traits must be either present or absent. Traits must be variable between species. An example trait has been filled in for you.

Table 2. Data collection table for the construction of a phylogenetic tree for the Screwdiae family.

	Phillip's Head	Trait 2	Trait 3	Trait 4	Trait 5	Trait 6	Trait 7	Trait 8
Outgroup								
Species A								
Species B								
Species C								
Species D								

3) Now, using your characters with your Screwidae species, build a Screwidae tree and label the gain and loss of the characters you have identified.
4) Using these premises, attempt to create a phylogenetic tree for the organisms represented by your microfossils.

Questions

1. Compare the tree you made for your microfossils with the "actual" tree provided to you by the instructor. How well did you do? Explain.
2. What type of characters are you limited to when creating a tree from fossils? What problems arise when trying to reconstruct a tree using fossil evidence?
3. Most modern phylogenetic trees use DNA data. What are the advantages and disadvantages to this type of data?

Materials

Sub-section	Item	Amount/2 students	Supplier	Catalog #
Diversity	Microfossils	1 ml	Yezerski	N/A
	Dissecting microscope	1	variable	N/A
	Widget	2	hand-made	N/A
	Petri dishes (60 mm)	1	VWR	25384-092
	Filter paper	1	VWR	28450-048
Microfossil Phylogeny	Slides and preserved modern organisms	varies	home institution	N/A
		varies	varies	N/A
Phylogenies	Screws	5	Home Depot	N/A

Notes for the Instructor

1. Microfossils are available as a ~10 ml sample originally obtained from Dr. C. Drewes. The author has about 70 samples available to interested parties. Students only need 0.5 – 1 ml for study. These samples should be recycled (recollected after student use and re-used indefinitely).
2. Microfossils can be obtained locally from areas that supported marine life during this time period. Methods for collection and separation are detailed in Charlie Drewes' major ABLE workshop referenced next.
3. Important fossil images from Charlie Drewes' lab can be found at: **http://www.eeob.iastate.edu/faculty/DrewesC/htdocs/fossil-buttons.htm**

   I do not control this website and it could be taken down without notice.
4. Widgets are fashioned by gluing the rubber strands used to make fly-fishing lures, to wooden craft sticks. Forceps do not work well with these tiny, hard samples. Additional details can be found on Charlie Drewes' website.
5. I generally use data from our 6-10 laboratory sections (with 5-10 pairs in each) to create a histogram of Simpson's Species Diversity calculations. Only after discussion do I reveal that these are all essentially measurements of the same sample. This leads to discussion of sample error and precision.
6. The sources of information on modern versions of the species represented in the sample include both web sites and our current collections. Any samples that we have of related organisms in our collections (ex. slides of algae and protists, a preserved lamprey, and collections from Carolina Biological or Ward's) are utilized. Different institutions will have different collections available.
7. There are numerous other exercises available to demonstrate phylogeny beyond the screw exercise demonstrated here. The main purpose is to demonstrate the principles of systematics, and any decent tutorial can be used. The systematics tutorial about T. rex (is very good as a tutorial for lower-level undergraduates. (**http://www.ucmp.berkeley.edu/education/explorations/tours/Trex/index.html**) I also recommend Hans Lemke's Major ABLE Workshop from this year, 2011.
8. The final tree of the microfossils is based on the Tree of Life website (**http://www.tolweb.org/tree**). Some of the evolutionary relationships are not well supported and slightly different final trees could be argued.
9. Student trees are often far from the actual. This is actually a great learning experience to demonstrate that in order to create phylogenetic trees, scientists must be experts on the characters of the organisms with which they are working. Also, different results can be used to illustrate parsimony and how trees can change as more evidence is collected. Discussion of molecular data could also be added.
10. All information, including PowerPoint presentations, can be obtained on request from the author.

Acknowledgements

First and foremost, Dr. Charles Drewes must be acknowledged as the primary initiator of this exercise. Additionally, Maria Gabriela Palacios must be acknowledged for locating the remaining microfossils in Dr. Drewes lab and sending them to me. Dr. Garrett Barr, Associate Professor at King's College, helped develop this current version of the laboratory exercise.

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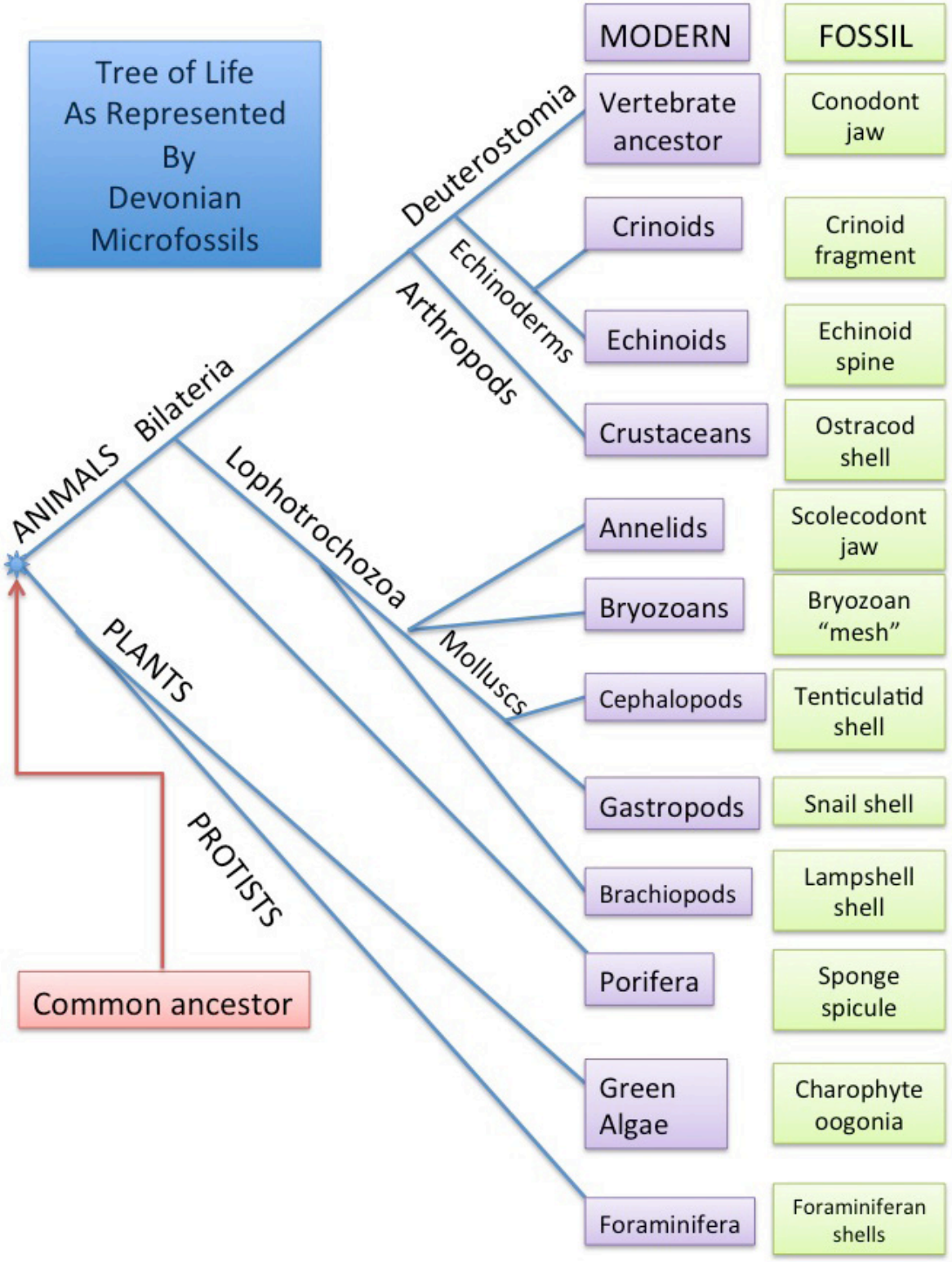
About the Author

Ann Yezerski, Ph.D. received both a M.S. and a Ph.D. in Molecular Genetics from the University of Vermont. She has been a faculty member at King's College since 1999 and has been the chairperson of the Biology Department since 2009. Dr. Yezerski teaches lecture and laboratory courses at all undergraduate levels, specializing in molecular genetics and physiology. After attending the ABLE conference in 2005, Dr. Yezerski used her newfound knowledge of microfossils to develop the above laboratory exercise first as a mini-workshop and now as a major workshop. King's College has been using and refining this exercise since 2006.

Appendix A

The final phylogenetic tree of organisms represented in the microfossil sample.



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