LAB #4: Prokaryotes and Simple Eukaryotes

BEFORE LAB

- **Read the Introduction through page 6 and skim the lab exercises below.**
- **Review chapters 24 and 25 in Sadava *et al.* from lectures 6 and 7**
- **Read the '[field guide to the tree of life]' including the '[tree guide]'**

OBJECTIVES

1. Be able to describe the diversity (metabolic, cellular, organelles, membranes, walls and shells) of prokaryotes and simple eukaryotes.
2. Understand mono-, para-, and polyphyly in the context of arranging the three domains on the tree of life.
3. Gain competence in using a compound microscope.

INTRODUCTION

Microbes

Microscopic organisms are the most abundant and diverse forms of life on earth. All multicellular organisms depend on micro-organisms either directly to provide nutrients or molecules like available nitrogen, or in the past for carrying out metabolism, either because ancient symbioses with bacteria led to oxidative respiration and photosynthesis. Over two and a half billion years ago bacteria synthesized the oxygen we now depend on in the atmosphere. Prokaryotes include diverse domains that lack a nucleus and instead contain a circular chromosome. Protists are a diverse group of unicellular, colonial, or simple multicellular eukaryotes that show tremendous cellular diversity and complexity compared to the descendent multicellular kingdoms of plants, fungi and animals.

Here is a brief glossary of terms you should be familiar with before lab:

Bacteria/Eubacteria, Archaea, Eukarya - the three domains of life. The first two are prokaryotic cells; the third includes only eukaryotic cells.

prokaryotes - organisms lacking membrane-bound organelles within cells; include the domains Bacteria and Archaea; commonly called bacteria; formerly constituted a single kingdom called Monera.

eukaryotes - organisms with a nucleus and other membrane-bound organelles (mitochondria, chloroplasts, etc.) within cells; include the domain Eukarya.

cyanobacteria - a phylogenetic group in the domain Bacteria that is photosynthetic; formerly called blue-green algae.

protist - common term including several candidate kingdoms in the domain Eukarya; most protists are unicellular, but some colonial and multicellular species are also included; formerly constituted a single kingdom Protista.

algae - common term for photosynthetic protists (can be unicellular, colonial, or multicellular); includes members of four protistan kingdoms as well as the green algae (Chlorophyta), which probably belong in the kingdom Plantae; singular is "alga."

protozoa - common term for "animal-like" (heterotrophic) protists, many of which engulf particulate food and are motile; includes members of four protistan kingdoms.

flagellates - common term for protozoa having one or more flagella (a whip-like structure used in locomotion or to produce a feeding current); includes members of four protistan kingdoms.

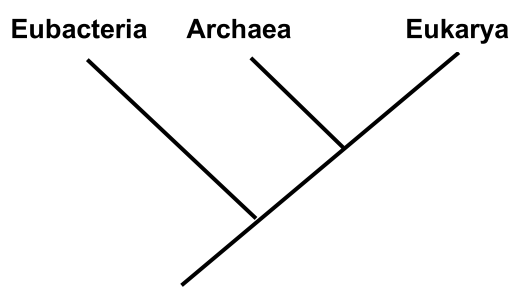
ciliates - protozoa of the phylum Ciliophora; possess cilia, which are similar to flagella but shorter and more numerous.

unicellular - an organism composed of one cell.

colonial - an organism composed of multiple cells that are loosely interconnected and may display a small degree of differentiation in structure and function; cells can survive singly.

multicellular - an organism composed of numerous cells that are highly differentiated in structure and function and are interdependent for survival.

In lecture you learned about the most basic tree of life, and the classification of the largest levels of diversity as domains or kingdoms. The Domains of Eubacteria, Archaea, and Eukaryota as described by Woese (1977) does not specify how those domains are related, which among the three are most closely related (as defined by sharing a common ancestor most recently). Below is the tree as presented in the textbook, reflecting accepted by not the most recent understanding (more on that later in the lab).

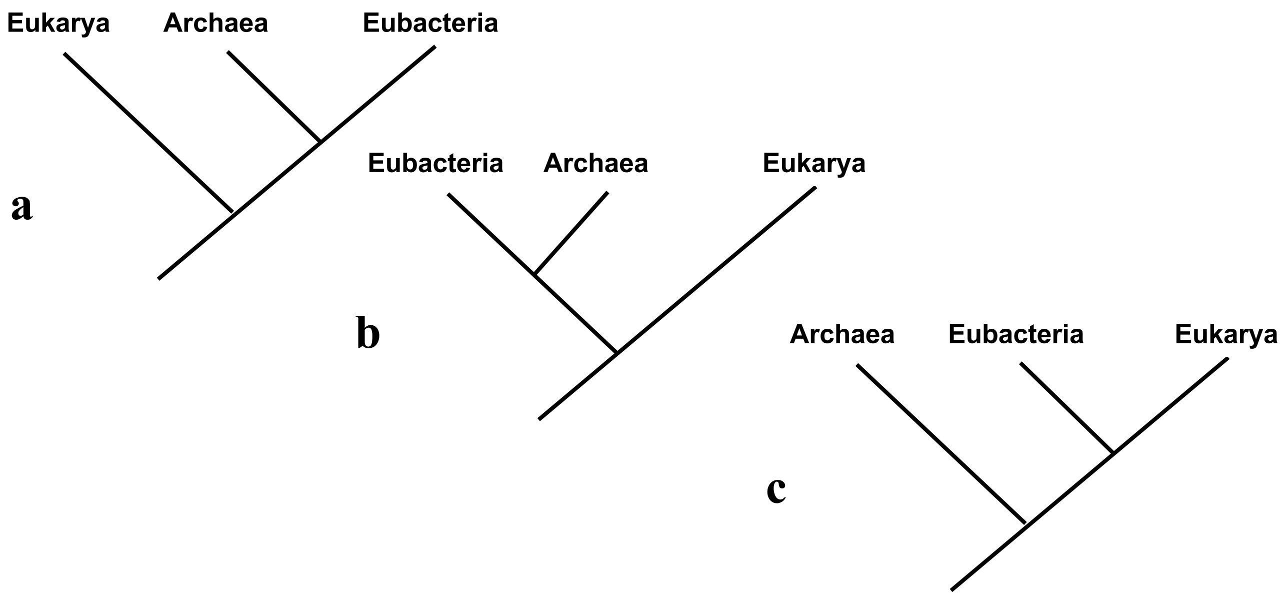


Using what you know from lecture, in the phylogeny above, are 'Prokaryotes' a monophyletic group? (yes/no) What is a monophyletic group?

____

____

Look at the three figures below. In which of them do Prokarya form a monophyletic group?



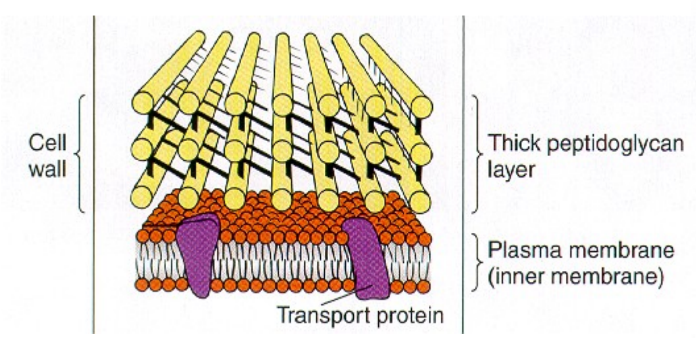
Are phylogenies ‘**a**’ and ‘**b**’ identical or different? Why?

____

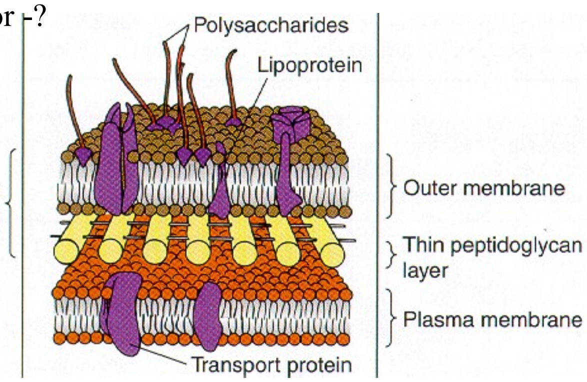
____

____

Eubacteria usually take the form of one of three basic body shapes, round cocci, rod-shaped bacilli, and helical twisted sprililli. You will observe and draw those later in lab. Bacteria are also typically classified by their staining patterns, the most common being the gram stain. The ability of bacteria to take up the violet gram stain depends on the presence and accessibility of a thick peptidoglycan wall. Bacteria with a thick peptidoglycan wall not shielded by an outer capsule/membrane can take up the gram stain, and are gram-positive (+). Bacteria with only a thin peptidoglycan layer, often insulated with an outer membrane can’t take up the stain and are gram-negative (-). Which of the bacteria below are + or -?



Gram ___________



Gram ____________

Bacteria and Archaea are also the most metabolically diverse. This means they can get energy and carbon from a diversity of sources. This is new to most of us. We are animals, all animals (metazoan) are chemoheterotrophs. This means we get both our energy and carbon from organic chemical sources (food). You are probably also comfortable with photo-autotrophs, which include various plants and algae, that get their energy from light, and their carbon from inorganic sources (they fix carbon from atmospheric CO_2 into sugars. There are many bacteria that share these metabolic pathways, but there are also bacterial chemo-autotrophs who gain energy from inorganic substances, such as hydrogen sulfide, but carbon molecules from CO_2, and photo-heterotrophs who gain energy from light but carbon from organic sources. Metabolic diversity doesn't end there, however. All plants and animals must gain nitrogen, a critical atom in all proteins, from molecules like ammonia, nitrate, or consumed proteins. Many bacteria acquire nitrogen in a similar manner, but some are able to 'fix' nitrogen from atmospheric N_2, others from nitric acid.

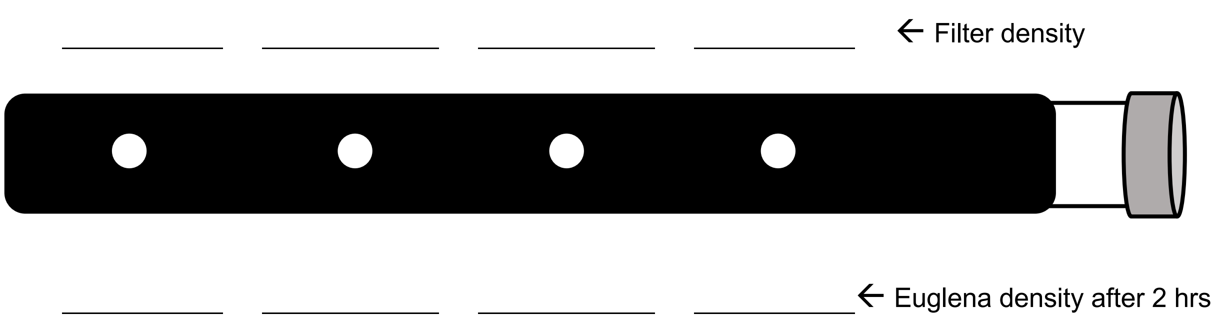
Simple Eukaryotes (Protists):

Before we get to eukaryotic diversity, we need to go over the basics of the live protist viewing tubes used in this and next week's labs. Many of the live specimens are held in flattened tubes, that collect the samples at the base for easy viewing in the compound microscopes, using specialized slide-clamps to hold them in place. Do not shake these tubes! Do not extract a droplet to place on a regular flat or depression slide. The flattened tubes are designed to collect cells near the bottom and you'll be able to see plenty directly from the tubes, at 4X and possibly 10X, often 4X is plenty for living large protists. If you don't shake them they can be re-used many times by students in each lab. Just for fun though, your lab instructor will ruin one of them to carry out an experiment on photo-taxis in euglena, a flagellate Euglenid (also called kinetoplastids). Phototaxis is the attraction and movement towards light. Your lab instructor will gently shake one display tube of Euglena, place it on its side, and construct a sheath of black construction paper, with four holes punched in a row. They will then take three squares of neutral density filter, and attach them to the black paper sheath over the holes, in a random sequence of three densities of shading and a no-filter control. Why randomize the order?

____

____

Below note the order of the filter placement:



Place the tube under a strong light source, set a timer, and in two hours removed the paper mask and note above the density/color of Euglena settlement in the tube.

(After 2 hours) your instructor will pause lab so we can observe and describe the results)

Do the *Euglena* cells appear equally distributed among the windows, or was there preferential accumulation under certain windows?

How can you describe relative density without doing actual cell counts?

Does the settlement of *Euglena* match the light intensity in each window?

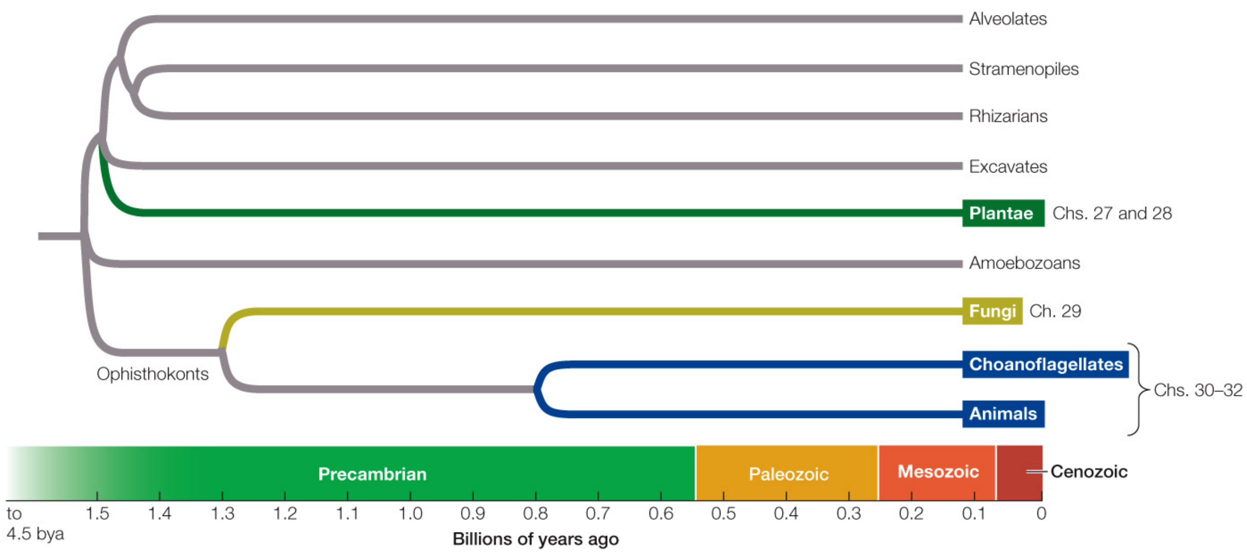
What does this tell you about phototaxis in *euglena*?

Eukaryote diversity

The larger groups of eukaryotes are defined by their cellular diversity. This includes cell walls of cellulose or chitin, or no cell wall, body form, specialized structures like flattened sacs called alveoli, the presensce of noflagella, or only one, only two, or more flagellae, or even an outer covering containing silica, calcium carbonate, or other materials. Many protist body forms fall into three basic shapes;

1) Amoeboid, irregular shape
2) Flagellate forms have one or more flagellae
3) Ciliate forms have many short cilia in rows across the body

In addition to cell structure, metabolism; heterotrophy and in particular parasitism, or photoautotrophy, often define eukaryotic groups The patterns of metabolism, especially photosynthesis, show how this trait has arisen symbiotically multiple times, and maybe been lost as well. The phylogenetic tree is not well resolved in Eukaryotes, though many of these divisions are ancient, well over a billion years old. You can see that in the phylogeny below from the textbook. Note that Stramenopiles are sometimes called heterokonts, meaning the presence of two or more flagella. This group includes photosynthetic diatoms and brown algae, but also parasitic oomycetes, which have cellulose cell walls but fungal-shaped bodies. Are they plants that lost photosynthesis, or fungi that gained some algal traits like cellulose? Excavates, sometimes called Discricristates, include Diplomonads (some serious parasites), Parabasalids (including the symbionts of termites) and Euglenizoids. Since only some euglenizoids in this group have chloroplasts with the same pigments as green plants and green algae. How might this be? Rhizaria include foraminiferans and radiolarians, groups defined both by filose pseudopods and outer coverings, of silica in radiolarians and calcium carbonate in forams. Finally the unikonts (single flagellum) include the Amoebozoans and slime molds, and opisthokonts, which include fungi and us (animals).



LIFE: THE SCIENCE OF BIOLOGY 11e, Figure 26.3

The figure above from the text has the strength of showing how ancient these eukaryotic lineages are. However, they don't just how unsure we are about the original eukaryote. That ambiguity is expressed better in the figure modified in a different text from Bauldaf 2003 below;

FIGURE 8.5. Phylogenetic tree of major evolutionary groups of eukaryotes. Branches are colored based on proposed supergroups, with *gray* indicating significant ambiguity. *Dashed lines* indicate major uncultured lineages known only from environmental surveys. *Starred* groups are possibly paraphyletic. Note the relationships among groups and the nature of the supergroups are still greatly debated.

8.5, redrawn from Baldauf S.L. et al., *Science* **300:** 1703–1706, © 2003 American Association for the Advancement of Science

Evolution © 2007 Cold Spring Harbor Laboratory Press

Observations and activities:

Start with Prokaryotes. As a warmup, draw the three body forms of eubacteria as best you can see with the compound microscope, and show the size of the cells in µm by drawing a scale bar in the square near your drawing.

____________________	____________________	____________________

Let us now organize the domains by shared traits. The phylogenies of the largest taxonomic units, the domains, or even the candidate kingdoms (superphyla) within Eukaryotes are unrooted; We don't know who the ancestor is, and can't use an outgroup to define primitive vs derived traits. However, we can make some inferences about the traits possessed by the Last Universal Common Ancestor (LUCA). Before LUCA there were probably many different life forms, different in ways it's hard to imagine (different component molecules, different ways of encoding information), but none of those left descendents still alive. LUCA is likely not the first life form, but the last one to still have living descendents. The table below shows the states for 18 traits in each of the three domains.

I) On Page two of this lab we have a phylogeny of the three domains. Redraw that phylogeny below, and using the principal of parsimony, map the characters from the table on to the tree

II) Which characters or states are unique to Archaea?

III) Which characters or states are unique to Bacteria?

IV) Which characters or states are unique to Eukarya?

V) Which characters or character states were likely present in LUCA? What criteria should we use to conclude a trait is ancestral?

Character	**Domain**		
	Bacteria	**Archaea**	**Eukarya**
1) Membrane bound nucleus	Absent, but similar structure found in one group	Absent	Present
2) Genetic material	DNA with bases A, C, T, G	DNA with bases A, C, T, G	DNA with bases A, C, T, G
3) Amino acid coding	3-letter code	3-letter code	3-letter code
4) Organelles, membrane enclosed	Absent	Absent	Present
5) Cell wall with peptidoglycan	Present	Absent	Absent
6) Membrane lipids	Ester-linked, unbranched	Ester-linked, branched	Ester-linked, branched
7) Ribosome size	70S	70S	80S
Character Domain:	**Bacteria**	**Archaea**	**Eukarya**

8) Initiator tRNA	Formylmethionine	Methionine	Methionine
9) Operons	Present	Present	Absent
10) Plasmids	Common	Occasional	Rare
11) RNA Polymerase	One	Several	Three
12) Sensitivity to antibacterials chloroamphenicol and streptomycin	Present	Absent	Absent
13) Diphtheria toxin inhibits ribosomes	Absent	Present	Present
14) Capable of methanogenesis	Absent	Present	Absent
15) Nitrogen fixer	Present	Present	Absent
16) Chlorophyll based photosynthesis	Present	Absent	Present

Cyanobacteria: "blue green algae" are important as photosynthesizers, currently, also in the past because symbioses with eukaryotes gave rise to photosynthetic algaes and plants, forming chloroplasts or plastids. They were critical ecologically for oxygenating the atmosphere, and they contributed to ancient fossil fuel deposits. They have chlorophylls and accessory pigments called phycobilins, one of the common ones, phycocyanin, gives them a blueish color and hence the name.

Many cyanobacteria fix nitrogen, and also many are multicellular, or at least colonial. These traits are linked, because the enzyme that fixes nitrogen, nitrogenase, is sensitive to oxygen, so most nitrogen fixing bacteria are heterocystic, meaning they have specialized cells for nitrogen fixing, to separate that reaction from the photosynthetic reactions that produce O_2.

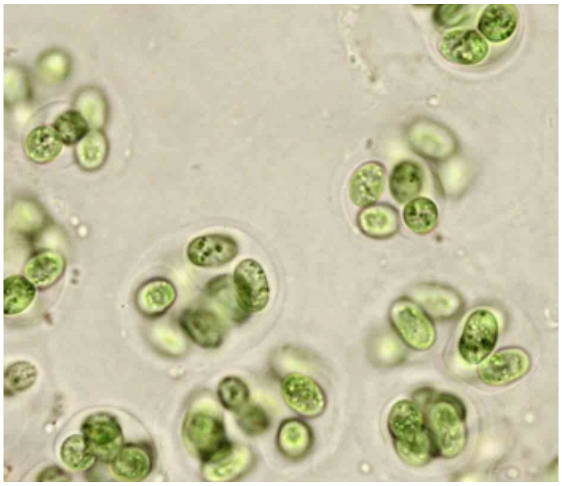
Examining living cyanobacteria cultures and prepared slides: Unicellular forms:

Gleocapsa: Examine slide #4 (*Gleocapsa*) from your slide box and prepare a wet mount of a small drop of the living culture. Your instructor will demonstrate how to prepare a wet mount. *Gleocapsa* is a common unicellular species in which the individual cells are surrounded by a clear, gelatinous coating (Perry and Morton, 1996). This gelatinous coating may be difficult to see, so adjust the lighting, and if that does not work, add a small amount of India ink to the wet mount. Sometimes one or more cells clump together within the gelatinous mass.

Why is this not an example of multicellularity? ______________________________

____

____



Gloeocapsa, Microbewiki, Kenyon.edu

Wet: Dry:

In the box to the left, draw and label what you see in the prepared and wet-mounted living *Gleocapsa* slides. How do they look different?
____
____
____

Estimate the size of your cells using the ocular micrometer, and draw the scale in the box as well.

Gleocapsa is a nitrogen fixer, but it's unicellular, and not hetercystic, so it does not have specialized cells for nitrogen fixation. How does it fix nitrogen? During the times that it can't produce oxygen, at night!

Colonial and filamentous forms:

***Nostoc*:** observe the dry-mounted slide. You should see some cells are larger with darker ends on each end called polar nodules. These called **heterocysts**, cells that fix nitrogen from gaseous N_2 to forms that can be used by plants to make proteins.

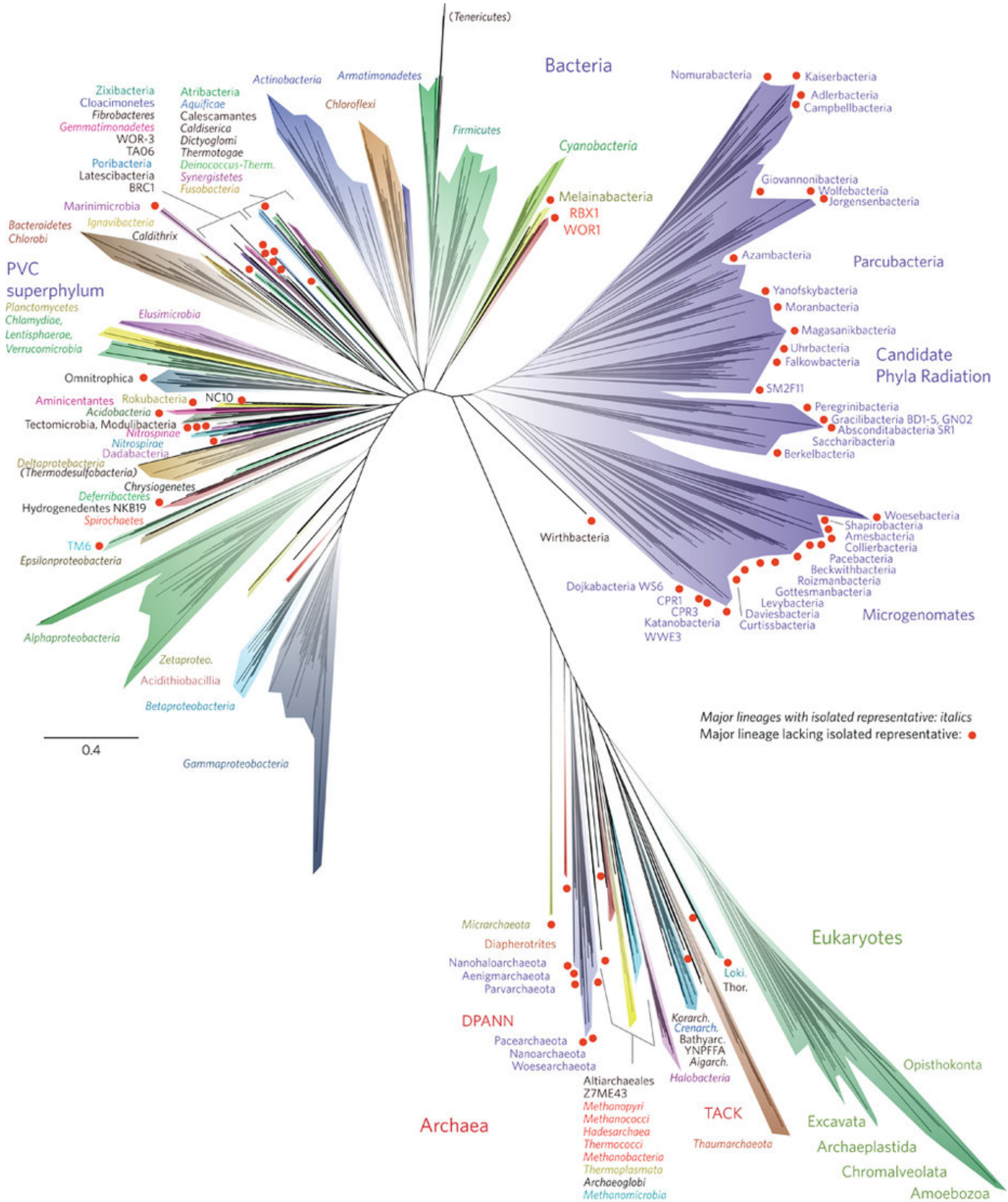
Anabena: This colonial cyanobacterium is often associated with a water fern, *Azolla*, which grows and is cultured in rice paddies. The bacteria fixes nitrogen, and the some of those nitrogenous products are released by the fern, fertilizing and improving rice growth. The benefits of *Azolla* fertilization were understood by traditional farmers long before its symbiosis with *Anabaena* was understood.

Observation of either filamentous cyanobacterium *Nostoc* or *Anabaena*. In the box, draw and label what you see in the wet-mounted (living) *Nostoc* or *Anabaena* preparation. Indicate the size of the cells as indicated in the specimen below. Note there may be a third kind of cell, akinetes (spores): these are elongate cells with thick walls. They should be rare in healthy, rapidly growing cultures

Dry	Wet

Anabena (Microbewiki, Kenyon.edu)

Let's get back to the phylogeny of all life. Here is the large scale Phylogeny by Hug *et al*. 2016:



Looking at the phylogeny above, which domain is most diverse, in terms of numbers of groups with long times since sharing common ancestry? ______________________

There's an entire group of candidate phyla that look like they are nothing but red dots. From the 'field guide' Berkeley website, what do the red dots mean? _____________________________

____

Are there any Archaean groups that share ancestors more closely with Eukarya than with other Archaeans? ___________ Name one and circle it on the phylogeny: __________________

Does this mean that Archaea is a mono-, poly-, or para-phyletic group? ______________

Why? ____

____

Simple Eukaryotes (Protists)

Protists can be defined as any Eukaryote that isn't a plant, animal, or fungus. Since those multicellular kingdoms each have close single or multicellular relatives within protists, that makes Protists a paraphyletic group. There are about 200,000 named species of Protists, and though many share similarities on a cellular level with plants, animals or fungi, many others are very distinct morphologically, and many protists have complex cells reflecting the challenges of maintaining all life functions and processes within a single cell. Here we explore a few representative protists, with a focus on ciliates, photosynthesis, and parasitism.

Alveolates is a group with both photosynthetic and heterotrophic members, and the group is defined by having flattened cavities, alveoli, near the plasma membranes of the cells.

We will look at up to three phyla today.

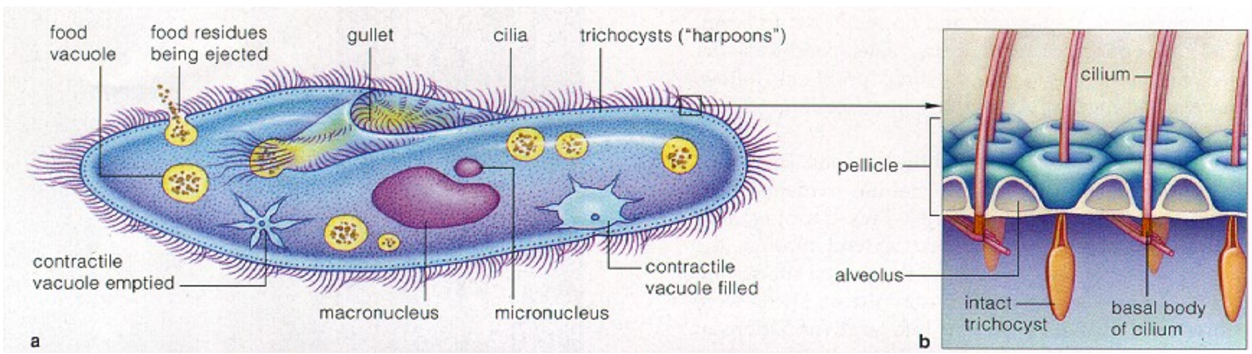
Phylum Dinoflagellata: 1100 species of free living, autotrophic (sometimes extremely abundant and toxic, responsible for red tides) and often symbiotic within other organisms.

Phylum Apicomplexa: spore forming parasitic species (formerly sporozoans), for example, *Plasmodium spp*., is the cause of malaria

Phylum Ciliata, ciliated heterotrophs, such as the well-known *Paramecium caudatum*

Ciliates: The Ciliates are a diverse group of heterotrophs, with active foragers and suspension feeders in the group. We may have both Paramecium and Vorticella live cultures for observation in the flattened demoslides. Observe both. Which is an active forager, and which is a suspension feeder?__________________ If we have paramecium in a loose culture, place a droplet in a depression slide, add a drop of proto-slo to a cover slip and place it over the depression.

Draw Paramecium. How much of its anatomy can you see compared to the figure from Starr and Taggart 10th Ed. 2004, or the large Somso model?



Ciliates have a macro-nucleus for directing cellular activities, and one or many micronuclei for reproduction and sexual reproduction via conjugation (exchange of micronuclei for meiosis). *P. caudatum* resembles a "twisted slipper" and this shape can be easily observed in wet mounts and commercially prepared slides. Notice how the external shape is maintained as the organism moves around its environment. The cilia move in coordinated waves (metachronous rhythm) which leads to directed movement.

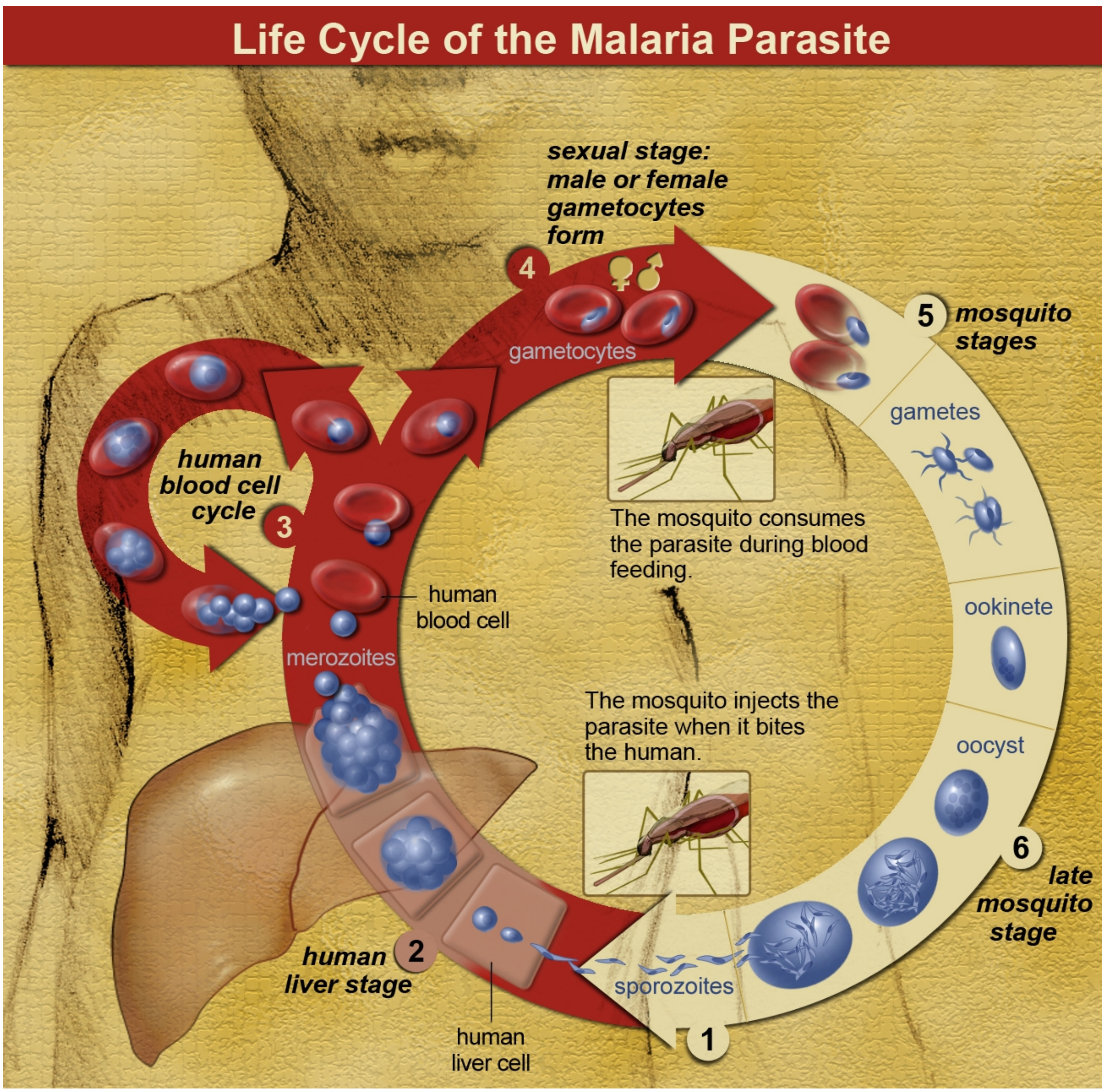
The pellicle (outer layers) of ciliates not only have cilia for movement, but trichocysts, coiled barbed filaments that can be used for defense or prey capture. They can be stimulated to extend with acids like vinegar, which can be a good demonstration in depression slides if acetic acid is available.

If the prepared slides are available, let's look at two more alveolates, the photosynthetic *Ceratium* from phylum dinoflagellates, and the parasitic Plasmodium from phylum Apicomplexa.

Dinoflagellates have two flagella in two different grooves, which are hard to see in spiky *Ceratium.* Draw what you can see, Other dinoflagellates are famous for causing toxic 'red tides' when they bloom, and others are known for their symbioses with coral.

Apicomplexans are parasites, often within cells, named for the structure that helps them penetrate and enter cells (apiplast). The most famous one is Malaria. Many parasites that cant be transmitted by air or direct contact need to use more than one species as host, in order to reinfect the original host species. The species where sexual reproduction (fusion of gametes and meiosis) takes place, is called the **definitive host,** and all other species are called intermediate hosts. Take a look at the life cycle of plasmodium below. Which is the definitive host for *Plasmodium,* mosquitos or humans? __________

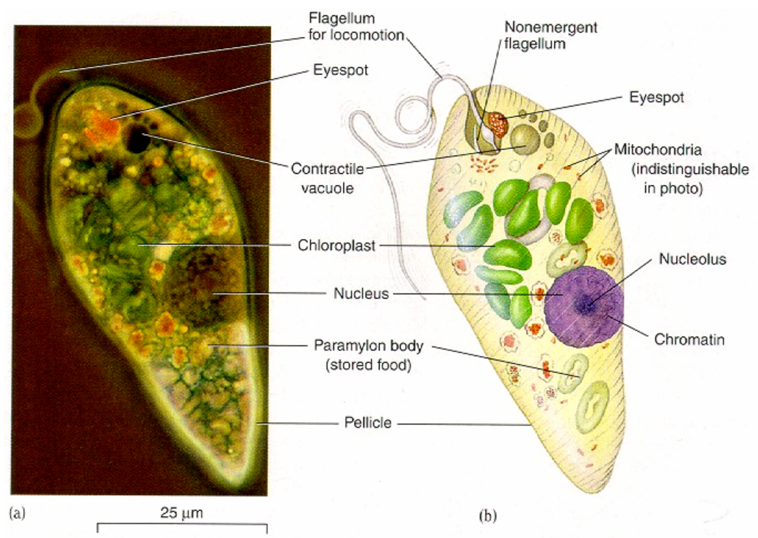
Another trait of plasmodium and other parasites is **schizogony**, repeated fission within a host cell. Schizogony can happen within mammal or bird blood cells to produce merozoites that attach more blood cells, leading to cycles of fever. Some merozoites mature into gametocytes, which are taken up by mosquitos, and then produce mega- or micro- gametes, that fuse and undergo meioisis to produce sporozoites that migrate to the mosquito salivary glands to enter the mammal or bird host, and move through the blood to attack liver cells an produce merozoites again.



Within the **Excavates / Discicristates** group are two more phyla we will observe, **Euglenozoa** and **Trypanosoma** (sometimes grouped together within Euglenozoa). Euglenids are fascinating complex cells, that photosynthesize under good light, but also consume cells heterotrophically. They have a tough but flexible outer covering called a pellicle, but not a cell wall. Also, though they have plastids with Chlorophylls a and b, they process starch differently from green algae or plants, and their plastids have three membranes, strongly supporting that an ancestral Euglena consumed a green algae, starting a new symbiosis and eventually a new cell type.

Look for both flagellar locomotion and inchworm-like euglenoid movement of the cells. Does the flagellum push or pull the cell through the water? **Describe** below your observations of both types of locomotion.

Draw and label a cell below, including as much of the external and internal structure that you can distinguish. Try to find all the features in the figure below, but draw only what you see.



The **trypanosomes** are a group of parasites responsible for multiple serious diseases, including sleeping sickness and Chagas disease. In some forms the flagellum extends alongside the body rather than out from it. Many are spread by insect vectors.

Draw the trypanosome from the prepared slide. How is it different in the bloodstream than malaria?

Amoebozoans have lobose bodies, with highly active cytoskeletons that allow them to stream, really grow, out to move and also to engulph food. These are heterotrophs that occasionally have a flagellum, and the presence of a flagellum is part of why amoebozoans are placed near the **unikonts**, that contain fungi and animals. Draw what you can see of the amoeba below.

Amoebas have lobose pseudopods for movement and prey capture, but two groups within the protist group **Cercozoa** have thin, needle-like (filose) pseudopods used for food gathering and attachment, and hard outer shells. Radiolaria have beautiful outer coverings made of silica, and Foraminiferans have almost snail-like outer shells made of calcium carbonate. The hard shells of these species make them easy to identify, in fossil deposits. Make a drawing of each

Heterokonts are a group that contains some photosynthetic phyla, and some noted heterotrophs. The algaes (diatoms, and brown algae like kelp, sargassum or fucus) are not considered closely related to green algaes and plants because of different photosynthetic pigments (Chlorophyl c but not b, flucoxanthin in diatoms). Look at sargassum and focus, multicellular, but not plants. These brown algaes have cellulose cell walls, but also unique components like alginate. **Diatoms** are incredibly important photosynthesizers producing 1/5 to ½ of all the photosynthetic oxygen on earth, and they also are known for their pillbox shaped silica-impregnated shells, or frustules. Be able to recognize them from the preserved slides. **Oomycetes** are called water molds. They have flagellated spores so even terrestrial species like the *Phytophthora infestans* that causes the late blight disease behind the Irish potato famine spread well in rainy conditions. Look at our example of *Saprolegnia* if available. They look like a fluffy fungus. The body form (absorptive tubes called hyphae) are similar to fungi. The flagellated zoospores are different, as is the cellulose cell walls vs chitinous walls in fungi. They also have diploid bodies in contrast to the haploid bodies of true fungi. Their combinations of traits is so odd that it is possible lateral transmission gave and algae some fungal traits or a fungus some algal molecules like cellulose.

Finally let's look at spirogyra. The protist group plantae contains red and green algaes, as well as land plants. We will look at the chlorophyte-plant transition next week, but for now, compare **spirogyra**, a **green algae**, with the cyanobaterial filaments you looked at before. Use words, drawings, and scale markers to describe the differences.

Spyrogyra has similar chlorophyls to all the green plants. Try to observe and draw the pyrenoid bodies, which are starch storage structures beaded onto the spiral chromosome that dominates the living cells. Some of your spirogyra will be in the process of conjugation, joining together of the cells of different filaments to exchange isogametes (gametes of the same size and structure) to fuse into new zygotes, that are released when the filaments separate. for meiosis. Finally we will look at two unrelated protists that have cellulose cell walls. **Spyrogyra** is a green algae, so well on it's way to becoming a plant, though it has none of the adaptations for terrestriality we will look at next week.