Free Living Protists

Micscape Magazine, March 2024

Ed Ward MD, Minnesota USA

Origins of this article

I was a nerdy kid, interested in science. Dad worked in a factory and the only good microscope I had was a simple stereomicroscope won at a high school science fair. Many decades later, in 2011, I bought a good used Chinese microscope. Immediately I got interested in pond water micro-critters, including protozoa. Over the years I learned a lot about using microscopes better from *Micscape* Magazine.

For a few months I've been writing about parasites, including the protozoan variety last month. I felt almost disloyal to all the friendly fresh water protozoans that have entertained me. So now I write in praise of our protist friends and relatives.

Disclaimers

I am not an expert in protozoa. Their biology and taxonomy is a realm of academic specialists. I am an amateur. I may have misunderstood and incorrectly simplified some aspects of protist biology. Please contact me (see last page for contact info) if you can set me straight.

Cover page illustration	Other illustrations
Paramecium, some smaller ciliate protozoans and some green algae protists from a cattail marsh, Red Wing, Minnesota, USA, 2016 10X objective, oblique lighting, direct projection to small USB camera sensor about doubles magnification. Image about 0.5 mm wide; *Paramecium* is "big" about 250 microns long.	This time all but 2 photomicrographs (both noted) are mine, taken with AO/Reichert microscopes with USB cameras. With a 0.5X reducer (added late 2017) my 2.5X objective images are about 5 mm across, the 4X about 3 mm, 10X about 1.1 mm, 40X about 0.3 mm (300 microns), and 100X about 125 microns. Some images adjusted in Word for brightness and contrast.

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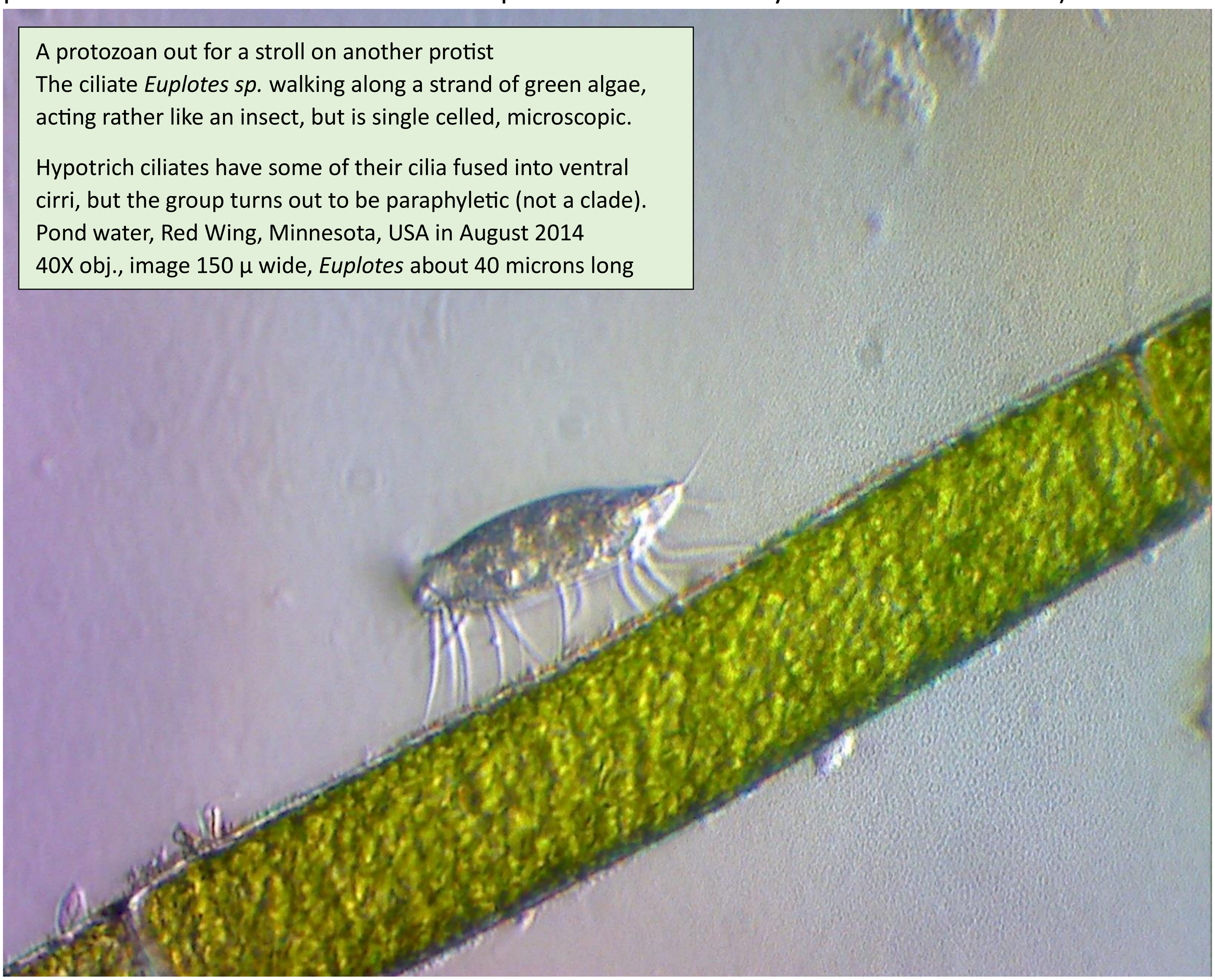
Contents

Protists are weird and wonderful relatives of ours

Last month I reviewed some protozoa that might hurt or kill you, but friendly free living protists deserve our attention and praise. You evolved from them. All of life on earth is amazing, and most of it was and still is microscopic. Arising from prokaryotic bacteria, eukaryotic ('true nucleus') single celled protists were arguably the dominate (bigger, more active) life on earth for a billion years, until animals evolved from them. Being an older group, protists show more diversity than plants and animals combined. Protists sit smack in the middle of the tree and the timeline of life. Discovered by Antonie van Leeuwenhoek with his microscopes in rainwater puddles in 1674 (ciliates) and in his diarrheal stool in 1681 (*Giardia*), protozoans can be either free living or parasitic. He called them animalcules and they really do look like tiny animals. They have complex internal and surface parts, they forage, hunt, grow, reproduce, and give the appearance of simple thought (simple preprogrammed behaviors, but watch some fascinating pond water ciliates under the microscope for 5 minutes and you'll see what I mean).



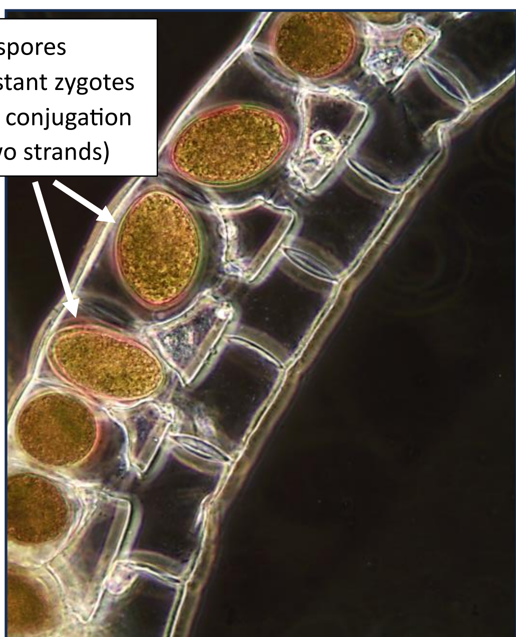
A protozoan out for a stroll on another protist
The ciliate *Euplotes sp.* walking along a strand of green algae, acting rather like an insect, but is single celled, microscopic.

Hypotrich ciliates have some of their cilia fused into ventral cirri, but the group turns out to be paraphyletic (not a clade).
Pond water, Red Wing, Minnesota, USA in August 2014
40X obj., image 150 µ wide, *Euplotes* about 40 microns long

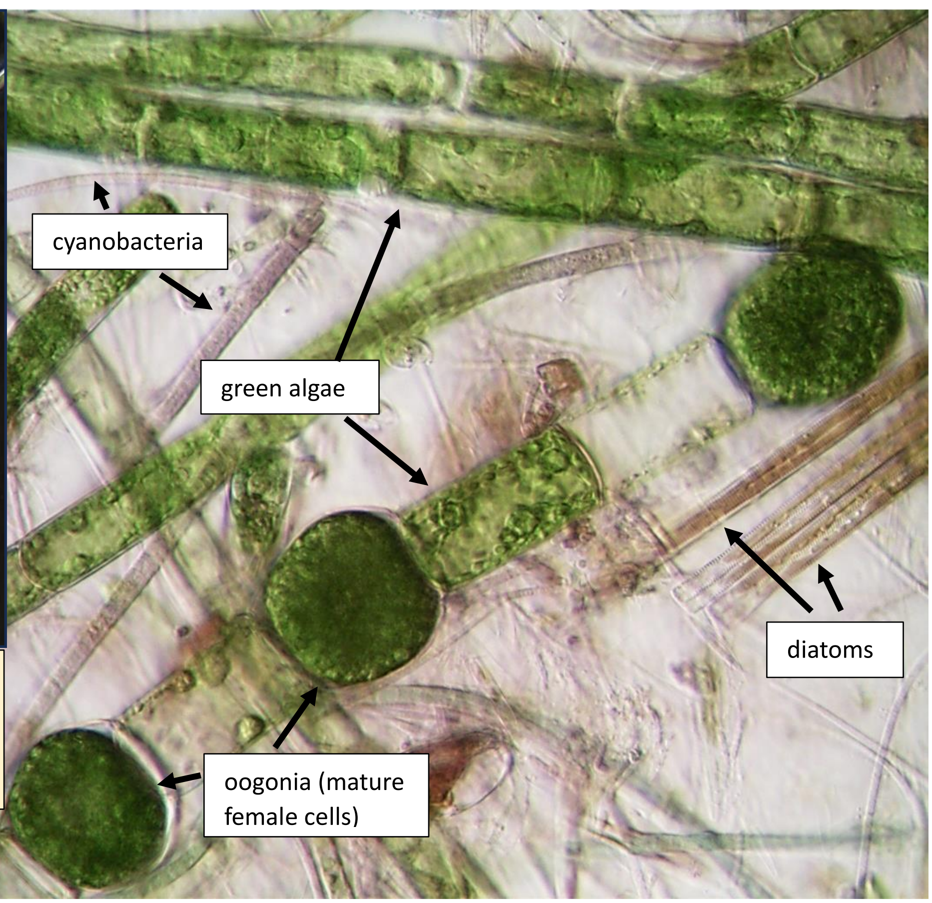
Protozoan? Protist? Protoctist?

From the time Leuwenhoek saw them in 1764, calling them "animalcules" (little animals) protozoans were considered mostly little animals (and algae were considered little plants). About a century later multiple biologists realized the single celled aquatic organisms didn't fit neatly into the Animal or Plant Kingdoms, so deserved one of their own. In 1859 Richard Owen proposed Kingdom Protozoa, in 1860 John Hogg named Regnum Primigenum (Protoctista), in 1863 T B Wilson and John Cassin suggested Primalia and in 1866 Ernst Haeckel designated Kingdom Protista. Most biologists at the time didn't accept the idea of a protozoan kingdom at the time, or distinguish prokaryotes from protists (a 1937 discovery). I still learned about animal (i.e. *Paramecium*) and plant (i.e. *Euglena*) protists in the 1970's. But the idea of a separate Kingdom for single celled eukaryotes was finally accepted by modern biologists soon after, accelerated by the discovery of DNA analysis of relatedness. Most biologists now consider all not animal or plant or fungi eukaryotes as Protista (Protozoa may be equivalent or exclude algae, and neither grouping is monophyletic).

It takes confidence to propose a big new kingdom of life, and several of those who did so were notable characters. Owen was a brilliant biologist and paleontologist, coined the name "dinosaur" and was the first president of the Royal Microscopical Society of London. Both Owen and physician James Paget independently discovered the nematode parasite *Trichinella* in muscle in 1835, but Owen denied Paget's claim. His peers all described Owen as a deceitful bastard. Charles Darwin was kinder, just noting Owen mentored no students. Ernst Haeckel was a gifted German zoologist and artist who unfortunately defended racism with evolutionary arguments. The late US biologist Lynn Margolis (ex-wife of astronomer Carl Sagan) was a modern proponent of 5 kingdoms of life (including Protoctista). She fought hard for the idea that mitochondria are symbionts inside eukaryotic cells and was vindicated by emerging DNA evidence. Iconoclastic, she also denied HIV virus causes AIDS and that 9-11 was a terrorist attack.

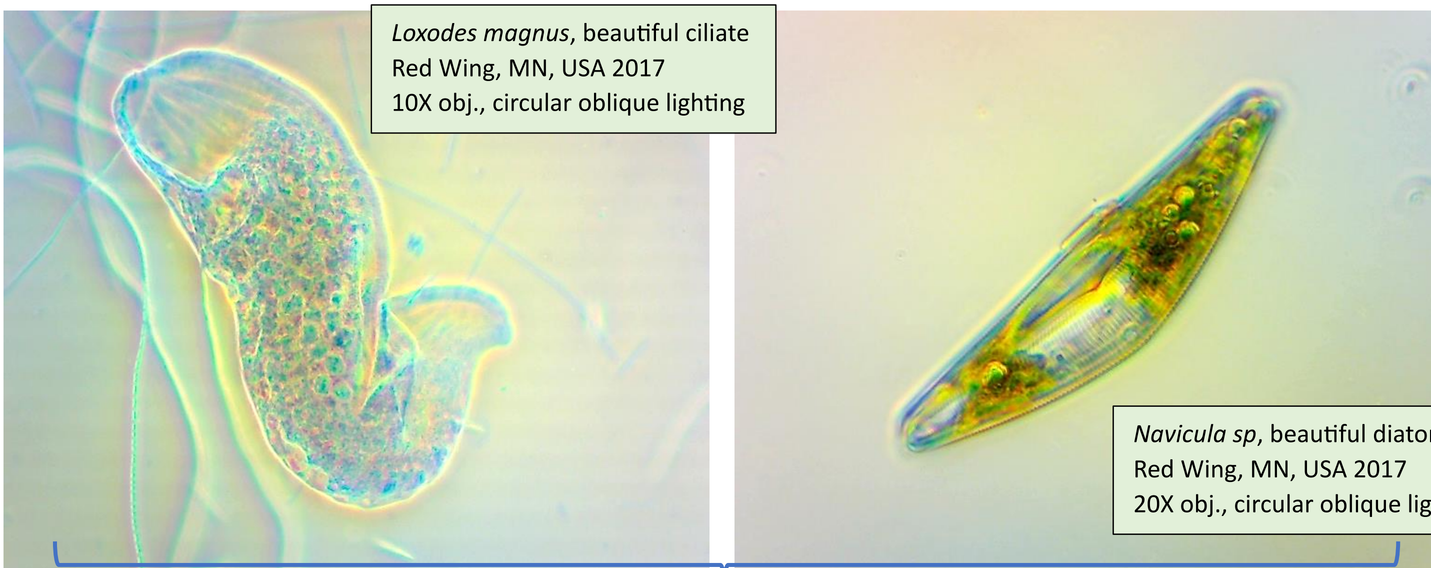


Animals inherited sex from protists. Zygospores in an old alga (likely *Spirogyra*) marsh, Red Wing, MN, USA 2018. 40X obj., phase contrast, image ~ 400 μ tall



Green and brown eukaryotic algal protists, along with a few cyanobacteria. Aquarium glass scum, Red Wing, MN, USA 2018. 40X obj., image ~ 400 μ wide

What is a protist? Most microscopists will be familiar with protozoa from pond water: amoeba crawling by shapeshifting, flagellates swimming with one or two whiplike tails, ciliates swimming gracefully using hundreds of tiny hairs, diatoms gliding in their beautiful glass houses. In my youth protozoa were considered little single celled animals, divided into groups by their means of locomotion. But in the age of genetics protist taxonomy has advanced to a state of great confusion, with many competing schemes. Note I may use protozoan and protist interchangeably to denote all single celled eukaryotes, including slime molds (a way of life of protists in several different groups) and single celled and colonial algae (including plant like giant kelp). Some authors exclude the eukaryotic algae (including diatoms) from protozoa. Other authors use Kingdom Protista for the whole group and use Protozoa for just the more "animal like" single celled organisms. Generally, all the eukaryotes that are not obviously plants, animals or true fungi get called protists. Those that remind us of animals get celled protozoa out of habit, not for good evidence. Protist taxonomy remains an ongoing mess.



Loxodes magnus, beautiful ciliate
Red Wing, MN, USA 2017
10X obj., circular oblique lighting

Navicula sp, beautiful diatom
Red Wing, MN, USA 2017
20X obj., circular oblique lighting

a "proper" animal like protozoan — one of many kinds of algae

both are protists

Like all microorganisms, we badly undercount protist species but as of 2001 there were over 213, 000 described species, including about 100,000 fossils and diatoms. There were about 92,000 described species of protozoa in the strict (excluding various algae) sense, about 25% of which were thought to exist in dependent or parasitic symbiosis with hosts. Still that leaves the majority of protozoa as potentially free living "good guys". Protists live everywhere there is moisture: lakes, rivers, oceans and soil. They are major photosynthetic producers (diatoms make about 20% of planetary oxygen), consumers of bacteria, and food for each other and for small animals. They are microscopic, mostly about 5 to 200 microns long but the ciliate *Stentor* and others can reach over 1000 microns (a mm), visible to the naked eye. Pushing the limits for a single cell, it has multiple nuclei, cytoplasmic streaming and other adaptations to being "big."

Affinities of some protists discussed in this paper

Experts don't agree on taxonomy schemes for protists, and I will be vague and probably outdated here. Clades include a common ancestor and all descendants. Genus names italicized. Note to strict cladists: if birds are dinosaurs and insects are crustaceans, then you are an opisthokont protozoan.

Domain Eukarya

Kingdom Protista

Clade Archaeplastida

includes phyla Glaucophyta, Rhodophyta (red algae), Chlorophyta (marine), Charophyta

(Charophytes are freshwater green algae, inc. *Spirogyra, Volvox, Coleochaete*; land plants arose in the charophyte clade)

SAR supergroup

Clade Stramenopiles (aka heterokonts)

inc. brown algae (Phaeophyceae, including kelp), diatoms (Bacillariophyceae)

Clade Alveolata (flattened sacs under cell membrane seen with electron microscopy)

includes phylum Ciliophora (ciliates inc. *Paramecium, Stentor, Vorticella*)

(also includes phylum Apicomplexa with many important parasites)

Clade Rhizaria (mostly amoeboid with slender pseudopods)

includes foraminifera, radiolarians and some soil amoeboids

Group Excavata (a large paraphyletic group)

superphylum Discoba (a clade with flagellate, amoeboid, cyst stages)

inc. phylum Euglenozoa (*Euglena* and relatives, some photosynthetic

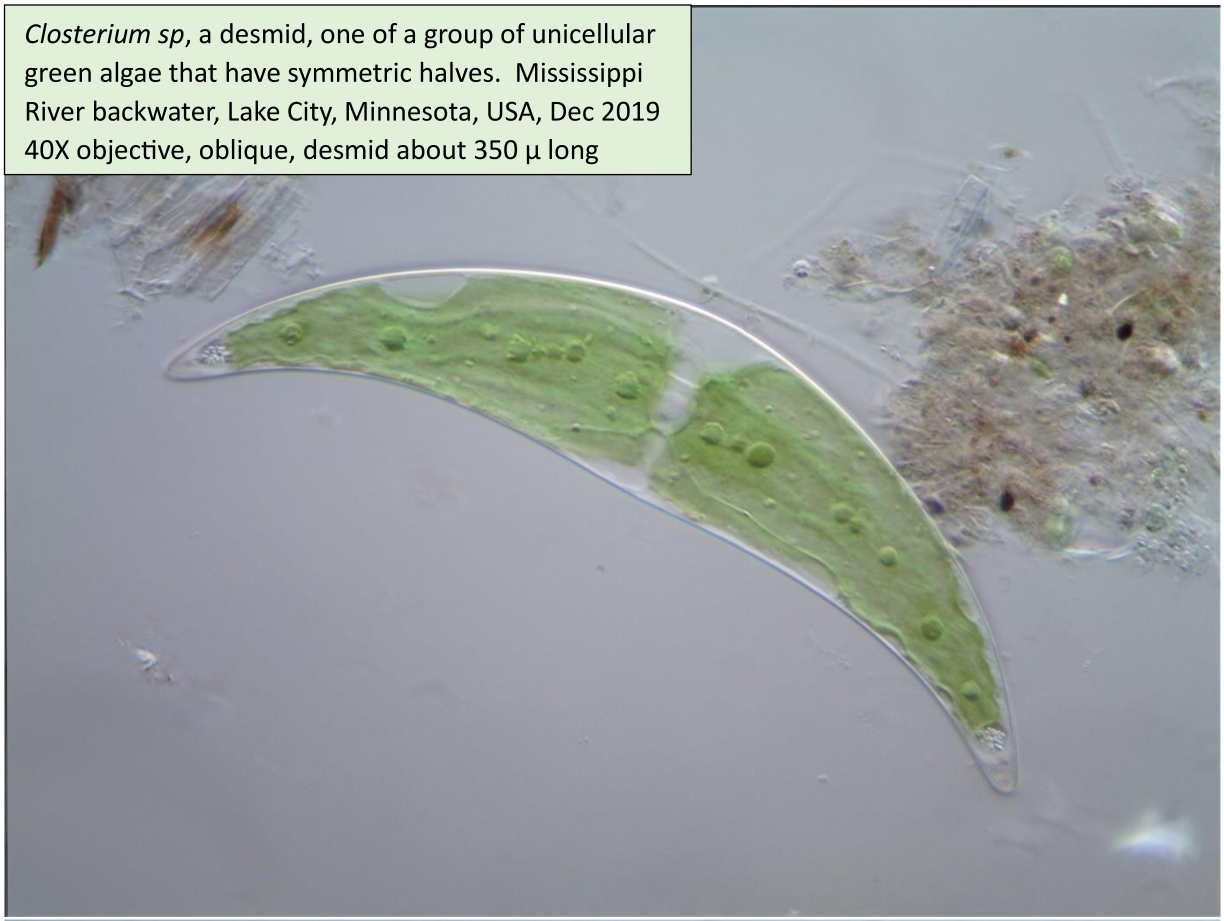
"phylum" Metamonada (amitochondriate, anaerobic inc. *Giardia*)

Clade Amorphea

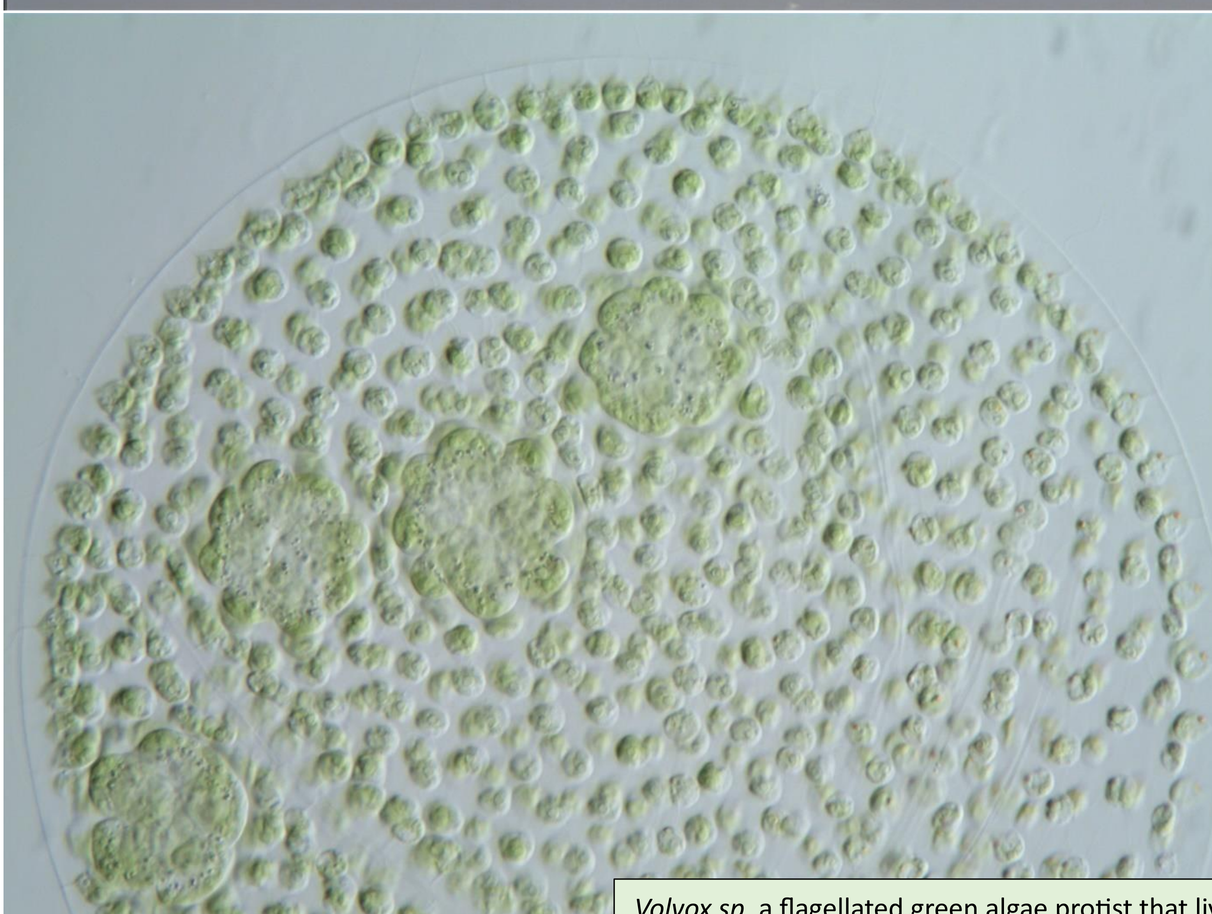
Phylum(?) Amoebozoa ("true" amoebas inc. *Amoeba, Centropyxis*)

Clade Obazoa (Opisthokonta, Breviatea, and Apusomonadida)

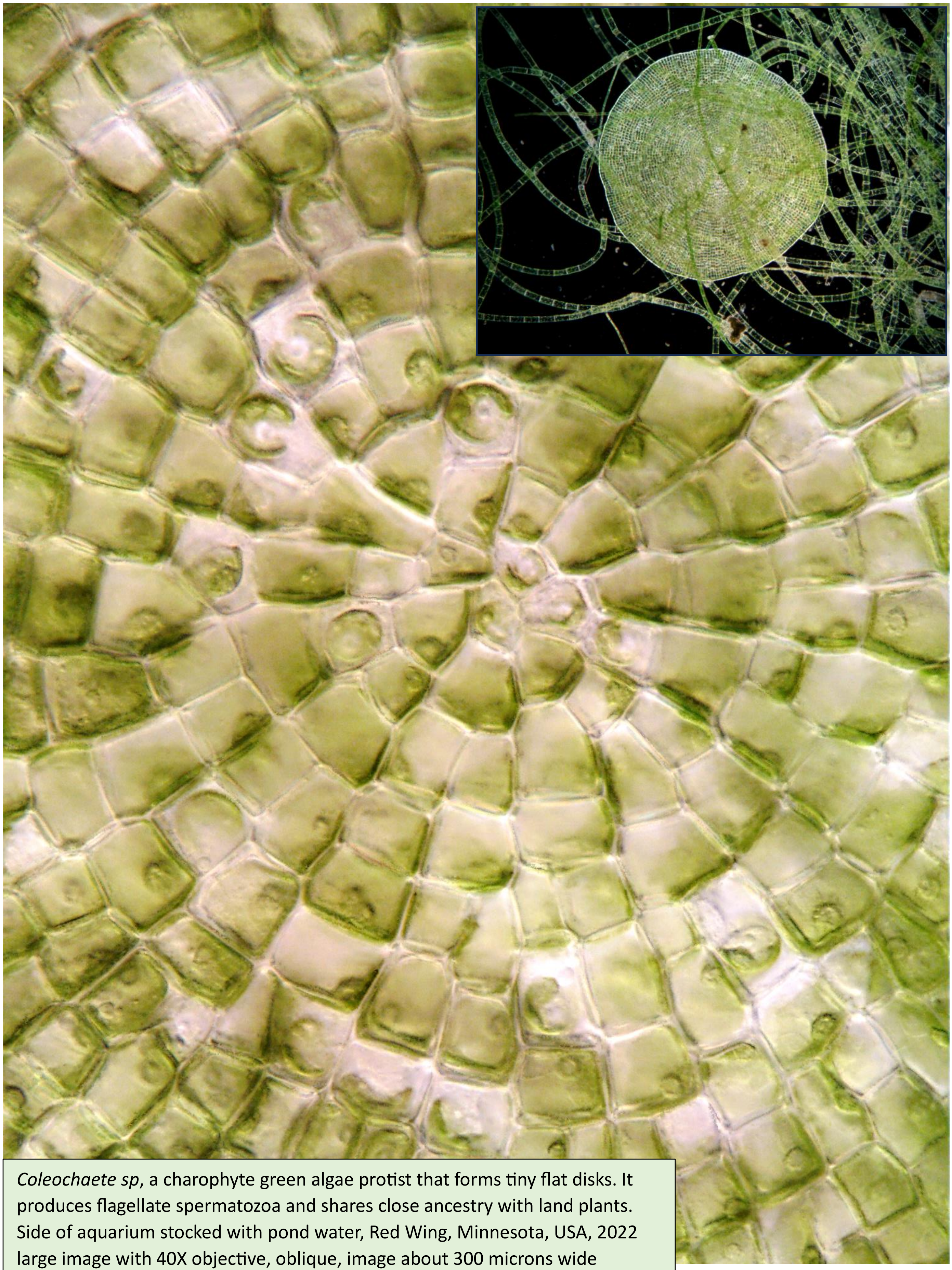
Opisthokonts include choanoflagellates (clade is ancestral to slime molds, fungi and animals)



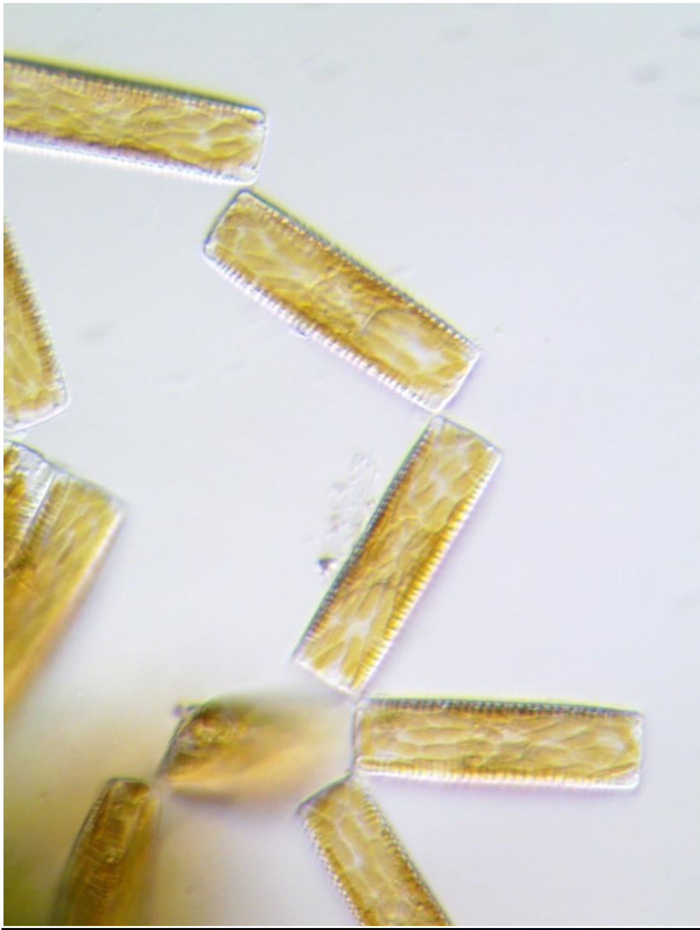
Closterium sp, a desmid, one of a group of unicellular green algae that have symmetric halves. Mississippi River backwater, Lake City, Minnesota, USA, Dec 2019
40X objective, oblique, desmid about 350 µ long

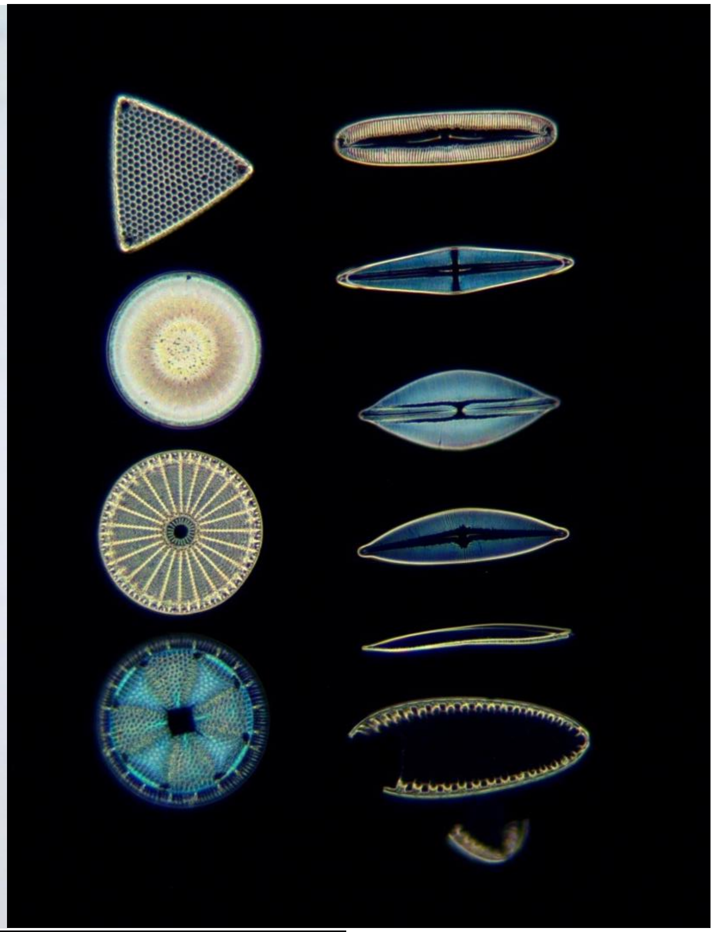


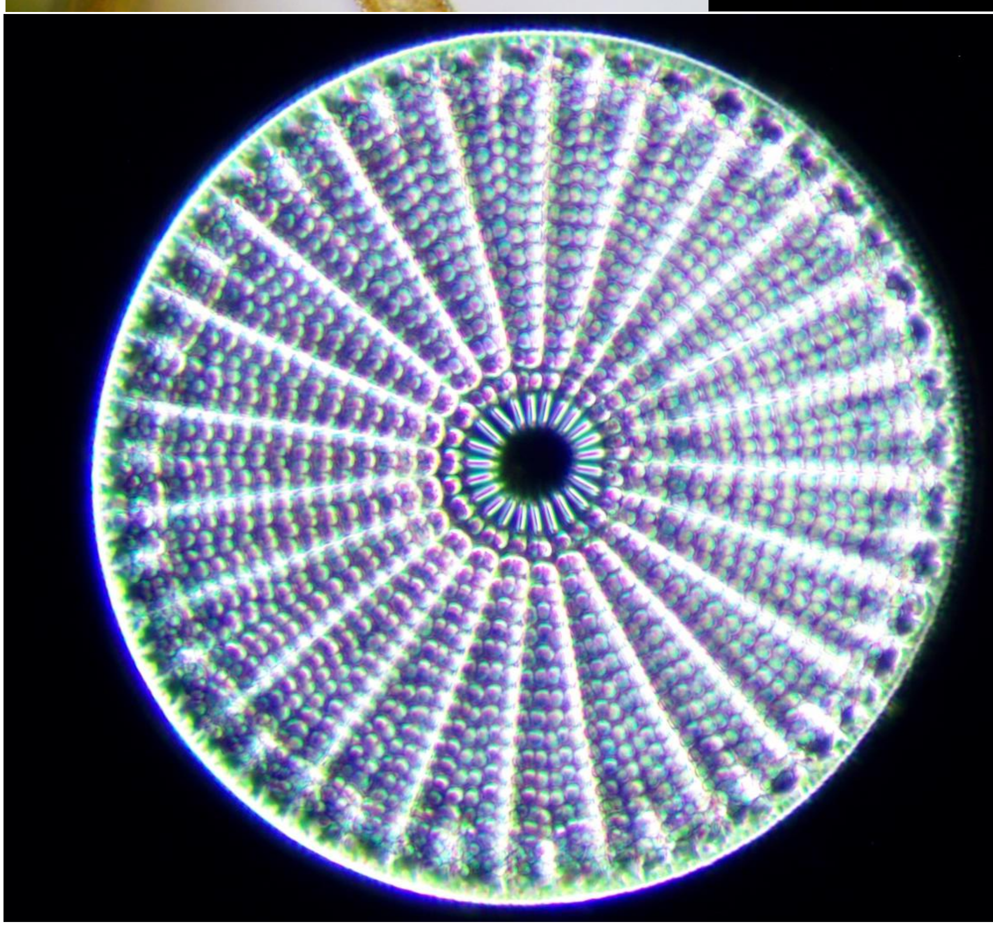
Volvox sp, a flagellated green algae protist that lives in wonderful spherical colonies. Mississippi River, Red Wing, Minnesota, USA, Dec 2019
40X objective, oblique, colony about 200 microns wide



Coleochaete sp, a charophyte green algae protist that forms tiny flat disks. It produces flagellate spermatozoa and shares close ancestry with land plants.
Side of aquarium stocked with pond water, Red Wing, Minnesota, USA, 2022
large image with 40X objective, oblique, image about 300 microns wide
inset with 4X objective, dark field, *Coleochaete* colony about 1.5 mm diameter







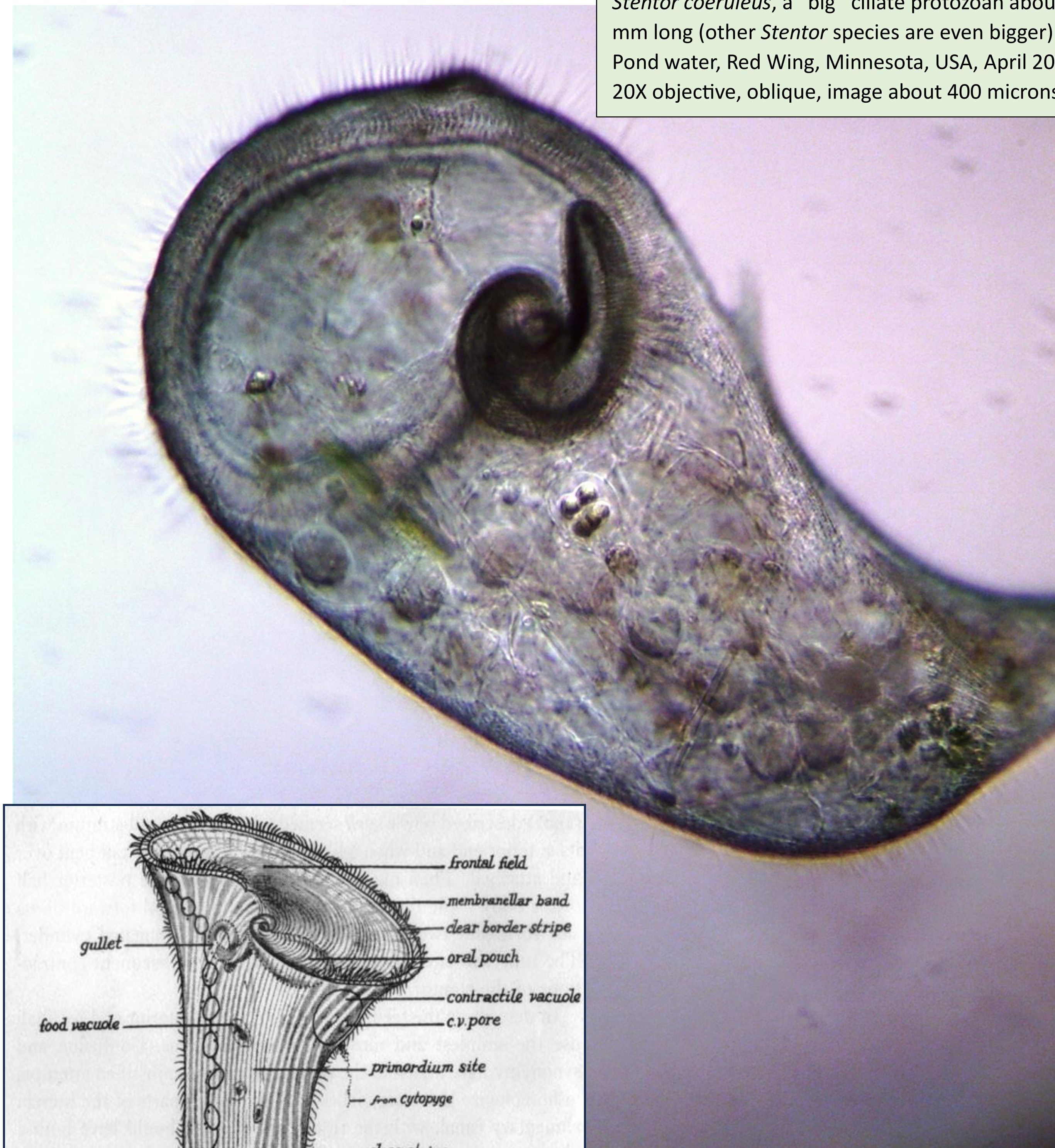
Microscopists love beautiful diatoms, often used as test objects. Diatoms are brown algae protists that live in ornate silica “jewel boxes” with a top and bottom frustule. They are marine and freshwater phytoplankton from Jurassic times until today.

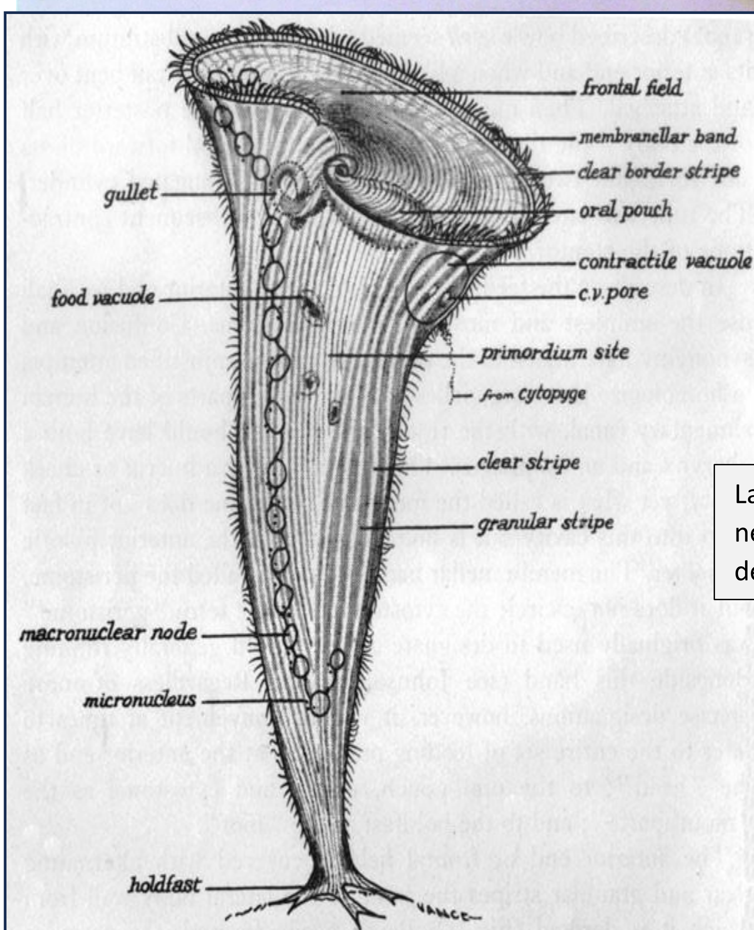
top left- live *Diatoma sp.* from Lake Byllesby, Minnesota, USA June 2022, 40X obj., cropped, individual diatoms about 50 microns long

top right- 10 arranged diatoms type slide “Harry Ross” (1950’s NY, USA seller of microscopes, telescopes) 10X obj, cropped, dark field

bottom- *Arachnoidiscus ehrenbergii*
from the Ross type slide
50X oil objective, dark field,
diatom about 150 microns diameter

Stentor coeruleus, a “big” ciliate protozoan about ½ mm long (other *Stentor* species are even bigger)
Pond water, Red Wing, Minnesota, USA, April 2019
20X objective, oblique, image about 400 microns wide





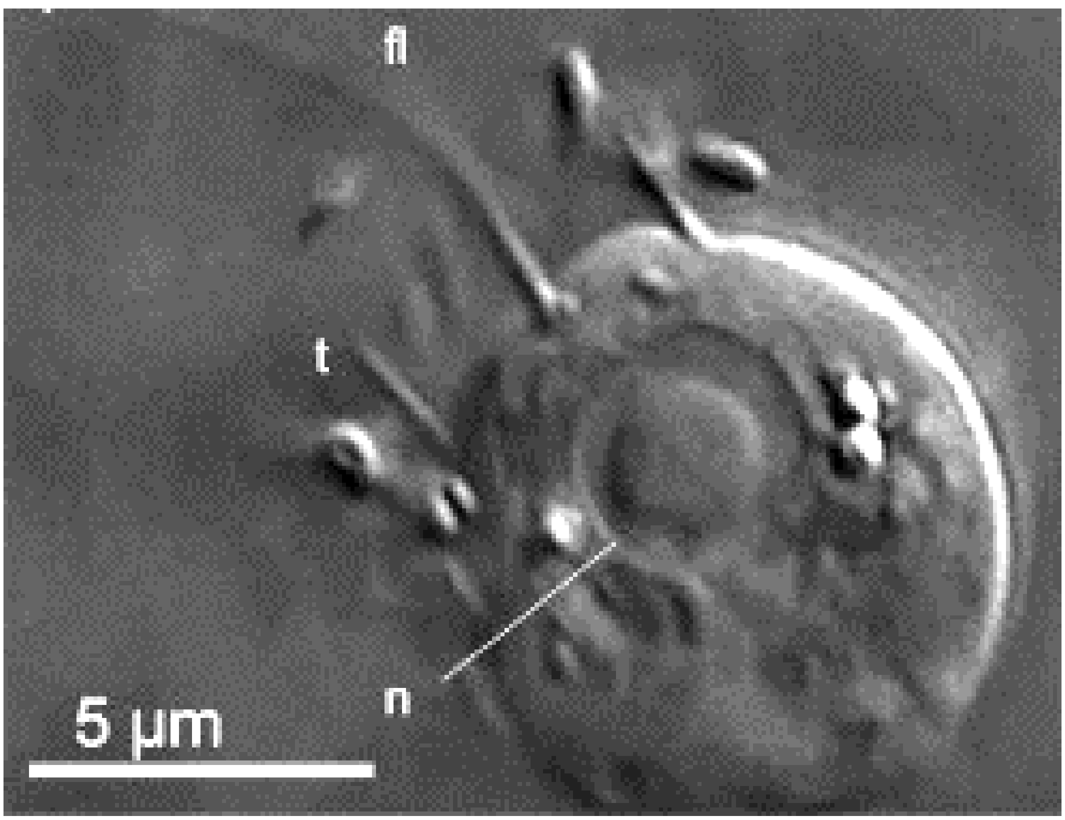
Late 19th century microscopists had microscopes nearly as good as ours today and studied *Stentor* in detail. drawing from Biodiversity Heritage Library

The origin of life is still mysterious in some aspects, but we fill in more of the picture every year. Carbon, hydrogen, oxygen and nitrogen are some of the most abundant elements in the cosmos. In the lab (Urey- Miller and other experiments) and more importantly, spontaneously in nature, they join to make some organic molecules, the building blocks of life. Soon after the earth cooled enough to form oceans, complex chemistry started brewing, likely at hot undersea vents. A greasy bag surrounded some nucleic acid (probably RNA first) and when it could replicate, it could evolve. The first cellular life formed about 3.8 billion years ago and fairly quickly the population of cells (called LUCA, the last common universal ancestor of life) evolved and split into bacteria and their archaea relatives. Neither has a nucleus or true inner compartments; they are both prokaryotic ('before kernel'). Life evolved for about 2 billion more years without producing anything bigger than a long strand of cyanobacteria (blue green algae) a few microns wide. Then about 1.7 billion years ago an archaeal cell engulfed an α-proteobacteria and created a mutually beneficial symbiosis. The bacteria evolved into mitochondria, the powerhouse of eukaryotic cells, and the new kind of cell also acquired a nucleus to store and organize more DNA information. This ancestor of all eukaryotes (protists and multicellular life) is denoted LECA, the last common eukaryotic ancestor.

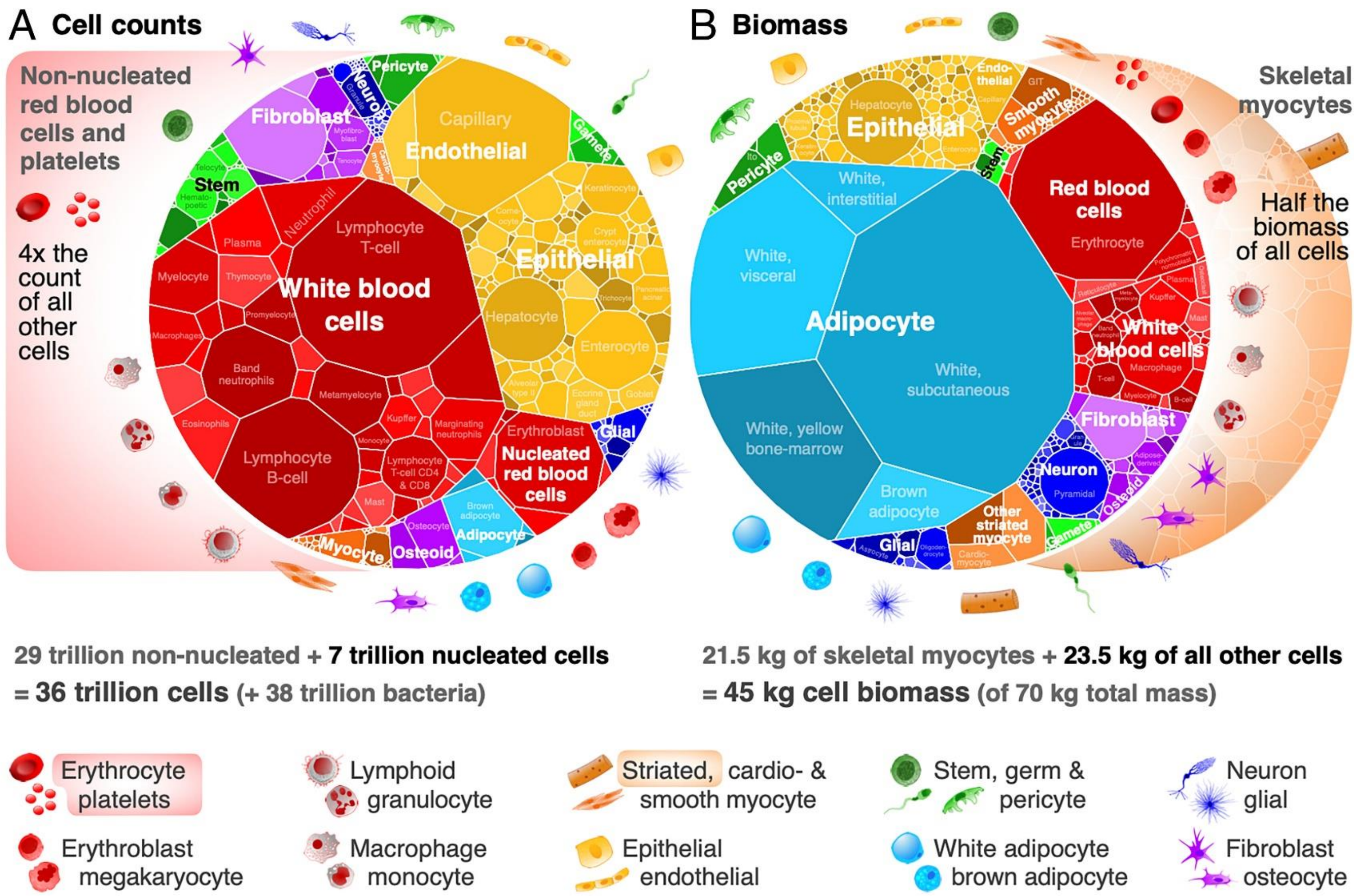
The roots of the first protist groups that diverged from LECA are obscure. For years it was thought that metamonads (including *Giardia*) and an odd amoeboid called *Breviata* were the most primitive, as they lack mitochondria. But we found nonfunctional relics of mitochondria and related genes inside these odd anaerobic protists (indicating they once had mitochondria) and we don't know which are the most primitive of surviving protist groups. Most protists have a number of specialized organelles (membrane bound particles with particular functions) in the cell, such as chloroplasts (which perform photosynthesis). The first chloroplasts were formed by a cyanobacteria engulfed by a protist becoming an endosymbiont. Some of these organelles were not inherited from ancestors but acquired by lateral transfer from other organisms (secondary and tertiary and possibly quaternary endosymbiotic events) complicating the use of these organelles to determine phylogeny (inherited relationships). Other organelles have also been looked at in detail, such whether those with cilia have one (unikont) or two (bikont) basal bodies, but those results have also lead to inconsistencies with other data. Electron microscopy and genomic data added much to the study of protist phylogeny, but taxonomy remains messy.

Fairly soon (0.2 billion years by "DNA clocks", fossils are scant) after LECA, some protists likely evolved into primitive multicellular fungal like forms (slime molds), followed by some evolving into primitive animals (sponges are oldest of extant animals) about 0.7 billion years ago. About 0.5 billion years ago green algae protists gave rise to land (true) plants. Meanwhile single celled protists kept evolving in the 1.7 billion years since their creation by the mixed marriage of two different prokaryote lines, and they therefore exhibit an extreme amount of diversity.

Many of the inventions protists came up with in their first billion years of evolutionary experimentation were passed on to the first animals and eventually to you. Fungi first, then animals later, both evolved in the opisthokont clade from choanoflagellates, amoeboid protozoans with a collar of microvilli surrounding a single flagellum. Over 150 years ago some microscopists noted how much some cells in sponges (the most primitive animals) looked like choanoflagellates, and now DNA evidence confirms the two are close relatives. Early animals (the Ediacaran fauna about 0.7 to 0.6 billion years ago) were probably mostly passive and superficially looked like plants. Over time, different kinds of animal cells evolved, arranging themselves in layers, including muscle tissues that allowed movement. Soon some wriggling worm like animals had a head, a tail, eyes and mouth and by 0.55 billion years ago some developed armor and quickly evolved into many sorts of marine animals, the "Cambrian explosion". Soon the swimming larva of some tunicates (sponge looking relatives of starfish) evolved into a fish, and by about 0.35 billion years ago the first amphibians crawled out of the water. Yesterday (in geologic time) your ancestors came out of the trees and walked upright. Since microscopes got good in the late 19 century we have known that the cell is the basic unit of life. As Rudoph Virchow said, every cell comes from another cell. Each of your body's 35 trillion cells (a 2023 estimate, downgraded from a previous 70 trillion guess) is a direct descendant of protists, over unfathomable billions of generations of cell divisions.



DIC photomicrogaph of choanoflagellate from 43,000 year old Siberian permafrost. The first 4 choanoflagellate species were described by American naturalist Henry James-Clark in 1867. At the time he noted the similarity to calcareous sponge choanocyte cells and wondered if choanoflagellates could be ancestral to animals. His amazing hunch was confirmed by molecular data about 150 years later. Image Daniel Stoupin et al Cryptic diversity within the choanoflagellate morphospecies complex *Codosiga botrytis* **European J Protistology** 2012



Eukaryotic cell composition of your human body (you also have a similar number of smaller prokaryotic bacterial and archaeal cells in your body). You are mostly tiny blood cells by cell count, but half muscle cells by weight.

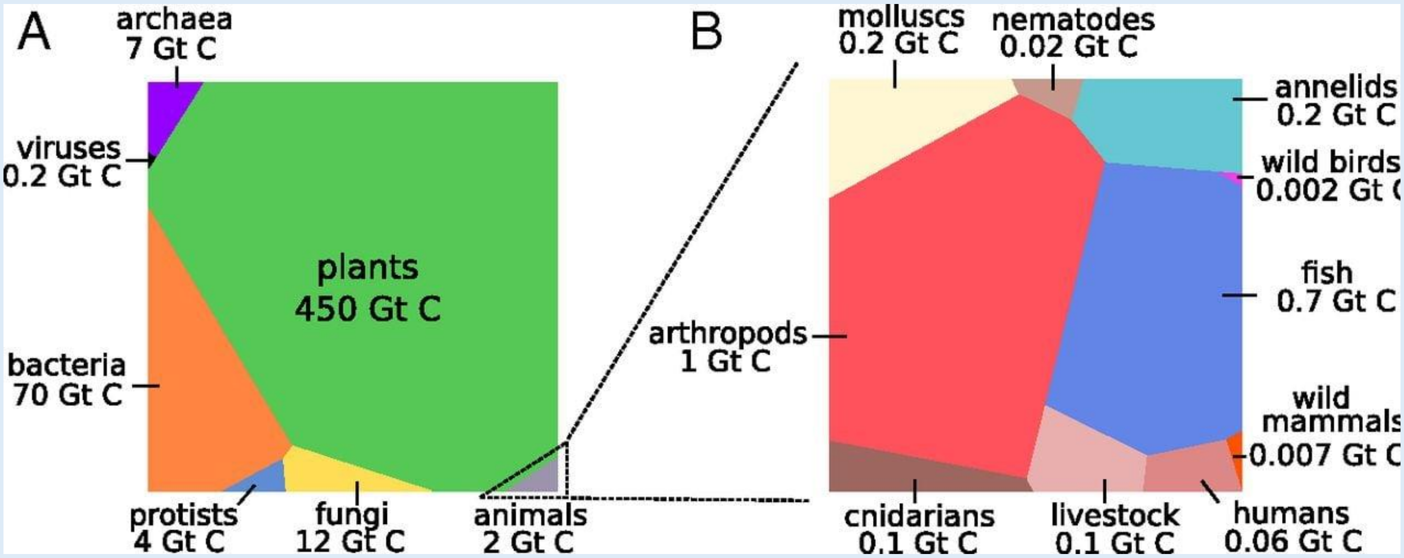
Contrasting cell count and biomass distributions by cell type. Voronoi tree maps for all 400 investigated cell types of a reference 70 kg male (area represents relative cell number or biomass). (A) Cell counts are dominated by red blood cells and platelets, which are removed from the cell count tree map. Even after removing nonnucleated blood cells (≈29 trillion), white blood cells (≈3.4 trillion) still dominate the ≈7 trillion nucleated cell count, with 98% of white blood cells as tissue resident, 1% circulating and 1% intravascular marginating. (B) Cell biomass is dominated by skeletal myocytes, comprising about half of all 45 kg of cell biomass in the body, even though they make up <0.002% of the nucleated cell count, which are removed from the biomass tree map. Most of the remaining 23.5 kg of cell biomass are white adipocytes (≈12 kg; though body fat varies widely among subjects).

Hatton, Galbraith, Merleau, Shander The human cell count and size distribution PNAS September 18, 2023 120 (39) e2303077120

What do all the microbes add up to?

You have more bacterial cells and bacterial genes than human cells and genes. But because bacteria are extra tiny prokaryotic cells thousands of times smaller than your own cells, all the bacteria in your biome (the ecosystem that is you) only add up to about 0.2 to 1.5 kg, so you are still 99% human.

Bacteria and archaea are the oldest and most widely distributed life on earth, living everywhere from hot spring vents on the ocean floor (life's point of origin?) to rocks 5 km underground to forests to you. How much do all these bacteria add up to? A fascinating 2018 review estimated the total dry carbon weight of groups of organisms on planet earth. Bacteria and archaea may be 14% of a total 550 gigatons of carbon biomass. Protozoa may add another 1%. It adds up: microbes outweigh all animals by about 40-fold!



Most living biologic carbon is in plants, particularly in trees. Animals contribute little to earth's biomass and most of that is in arthropods and fish. All the mammals add up to about half the mass of earthworms and their kin. Despite being just 1/100 of 1% of the biomass on earth, we humans have certainly left a dirty stain on the planet surface, beginning a new geologic period, the Anthropocene. Humans and livestock are about 34% and 62%, and the remaining wild mammals we did not kill are just 4% of total mammal mass.

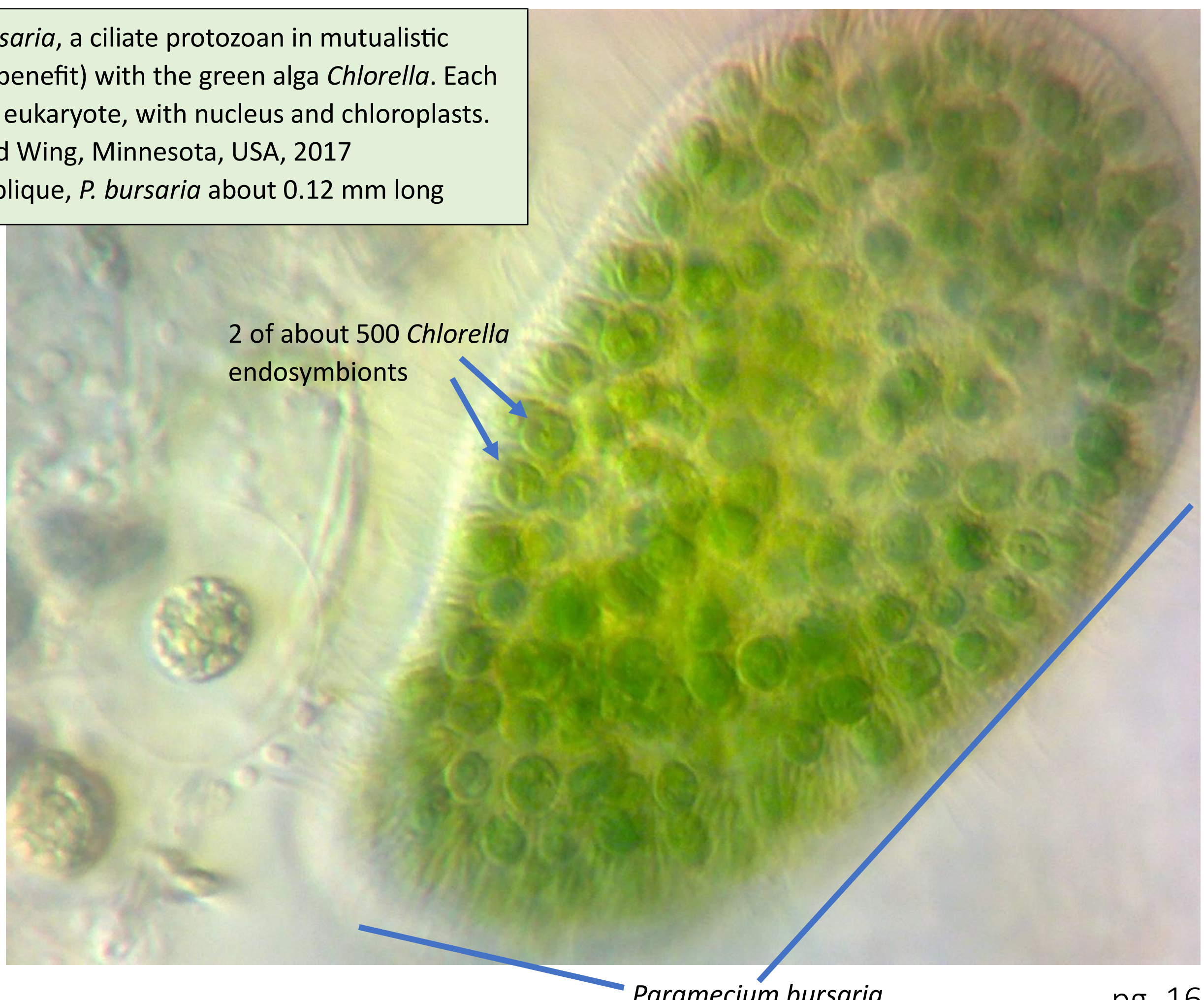
-chart from Bar-On, Phillips, Milo, Biomass distribution on earth, PNAS 2018

What is the global impact of protists?

In terms of numbers of living organisms (disregarding viruses), bacteria still rule. Protists are only about 1% of global biomass but being tiny also exist in vast numbers of individuals, more than plants and animals. Even in biomass, protists have double that of all animals combined. Protists are vital oxygen producers, decomposers, food chain links and symbionts with other life on earth. Despite being needed by all animal life, we don't know for sure how much oxygen is produced by protists. Earlier estimates were 50% but more recently we think about 25% of oxygen comes from plankton, mostly from dinoflagellates, diatoms and algae (which have a cyanobacteria component). Protozoa are everywhere water is, often eating organic debris as well as bacteria, each other and some small metazoans. Protists are vital symbionts for many animals. Many reef building corals (and some protists) have symbiotic photosynthetic green algae. Termites and some other wood and grass eaters rely on protist gut symbiotes for digestion. One of the toughest pioneering organisms on earth is lichen, a symbiotic partnership of fungi and algae that helps make soil. And of course, some protists are in a parasitic symbiosis, perhaps helping balance nature by culling animals.

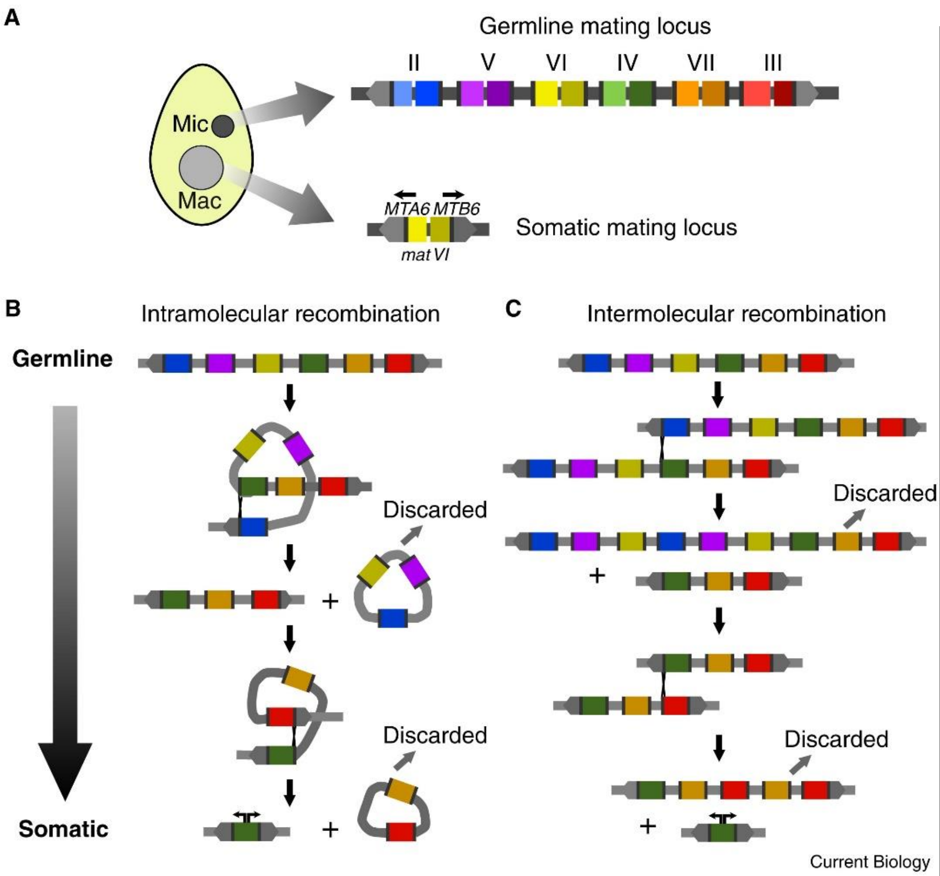
Single celled protists also continue to evolve. Those alive today show a very wide array of variations in structure**, energy metabolism**, motility, and sexual behaviors. They have no tissues or organs but can have a head and tail end, simple appendages used for locomotion, and specialized areas for ingestion of food and processing of wastes. Most are aerobic but some protists are anaerobic, processing food without oxygen. Some are photosynthetic autotrophs (make their own food) using sunlight to make sugar (using chloroplasts obtained by their ancestors from an endosymbiosis event with a cyanobacteria, or second hand from lateral transfer of chloroplasts between protist groups, or by having eukaryotic green algae symbionts within it). Some protists are heterotrophs (find food) that engulf or absorb food as chemicals, particles, dead or living bacteria or other protists. *Vorticella*, one of the first protists seen by Leeuwenhoek is sessile (immobile), using cilia to make currents to bring food particles to the part of the cell body modified to function as a mouth. Many other protists are also scavengers, performing vital ecosystem cleaning services. Others are active predators, such as *Didymium* attacking and ingesting bigger *Paramecium.* The armored ciliate *Coleps* sometimes hunts in packs then tears the prey apart communally, rather like a pack of wolves. Some protists (including many dinoflagellates) are mixotrophs and can be either photosynthetic or heterotrophic, depending on food availability.

Paramecium bursaria, a ciliate protozoan in mutualistic symbiosis (both benefit) with the green alga *Chlorella*. Each alga cell is also a eukaryote, with nucleus and chloroplasts.
Marsh water, Red Wing, Minnesota, USA, 2017
40X objective, oblique, *P. bursaria* about 0.12 mm long



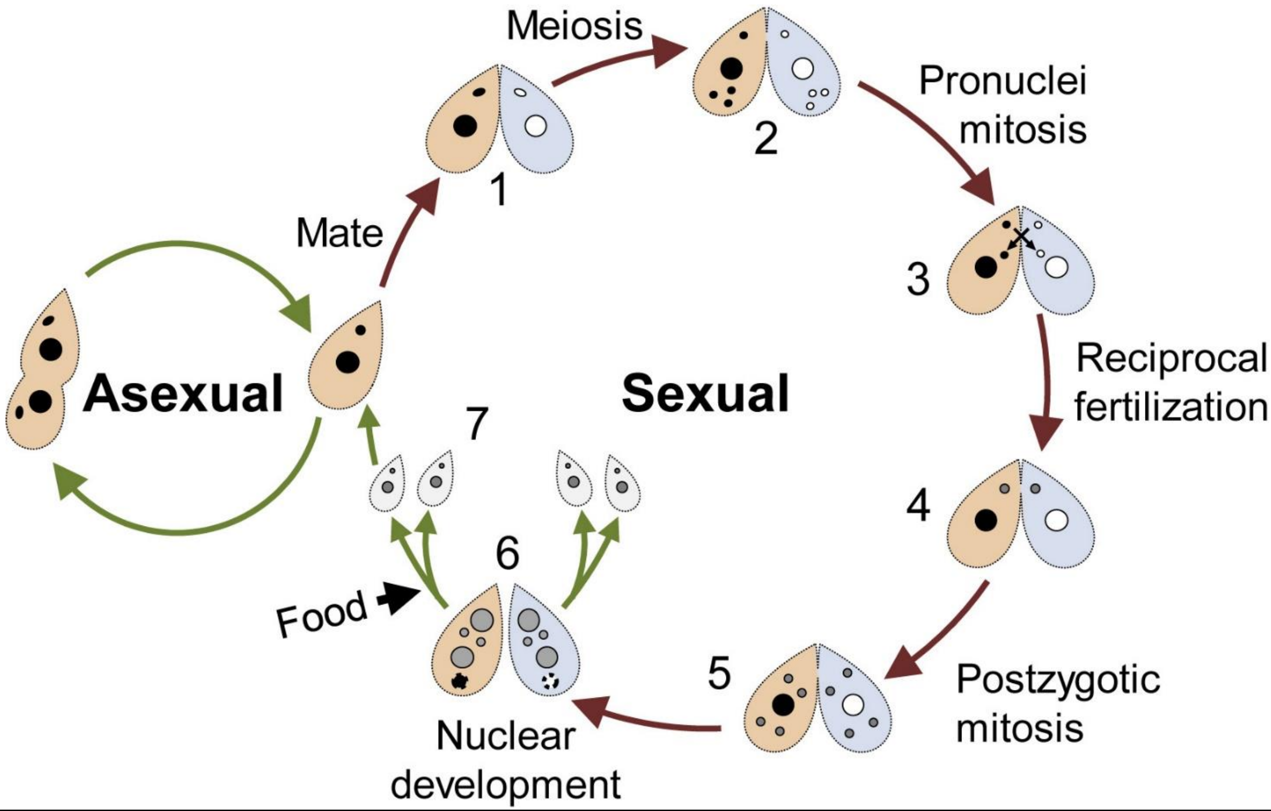
Simple fission, an effective but boring method of reproduction, in ciliate *Paramecium bursaria*. Pond water, Red Wing, MN, 2018 20X objective, phase contrast, image cropped, about 120 µ tall

Protozoa can have **great sex lives**. Protozoa invented the kinds of sex animals use, and 1.7 billion years of sexual experimentation has led to a lot of variation. Many protists reproduce by simple binary fission with one big cell becoming two identical smaller daughters. Asexual reproduction is more common in free living protozoans; most parasitic protozoa have both sexual and asexual reproductive stages. Sex can be quite complex, as I outlined for malaria, coccidia and other apicomplexans last time. Many free living protists also have asexual and sexual life cycles they can move back and forth into depending on environmental conditions. Some have conjugation whereby two of the same species temporarily join together and swap genetic material. In some cases the two identical looking protozoa have to be of opposite sexes in order to conjugate. And the ciliate *Tetrahymena thermophila* is quite a wild swinger. It has 7 different sexes (genders) and can mate with the other 6 but not with an individual of the same sex. When it does mate, the sex of the next generation is determined by random chance with jumping genes (sort of like the color of maize kernels, for which Barbara McClintock got the Nobel prize after it was found our immune system does a similar trick to make random antibodies). Cell division in *Tetrahymena* calls for some tricky gymnastics in the nucleus. You have 46 chromosomes in 2 pairs of 23, of but *T. thermophila* has 9000 tiny chromosomes in 45 sets of 200. Somewhat disappointingly, it can cheat and just pinch the nucleus in half without the complex spindle system organizing and pulling chromosome pairs apart like most eukaryotes do. Still, there is a lot going on inside these tiny (50 micron long) ciliate swingers.

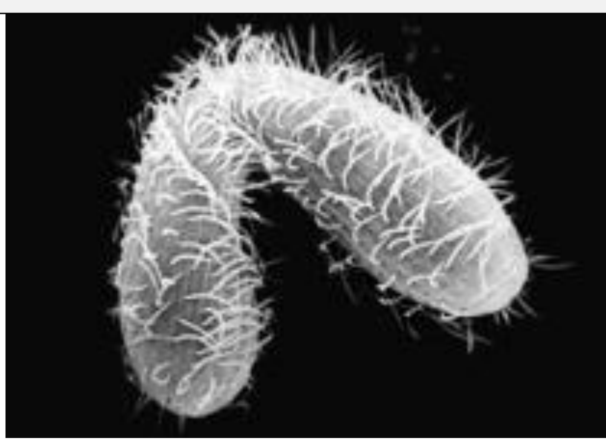


Sex selection in *Tetrahymena*
(A) *Tetrahymena* with micronucleus (Mic) and macronucleus (Mac). right diagrams depict organization of the micronuclear and macronuclear mating loci.
The germline mating locus allele of the strain depicted here contains six of the seven possible *mat* genes
(B) Elimination of alternative *mat* alleles through successive intramolecular recombination events. The *mat IV* allele was selected for macronuclear expression in this example. (C) Alternative model showing elimination of germline *mat* alleles by intermolecular recombination. Additional chromosomes used for recombination could come from the second germline allele and/or from replicated recombination intermediates.

J Umen, Swinging Ciliates' Seven Sexes **Current Biology** 2013 vol 23, iss 11

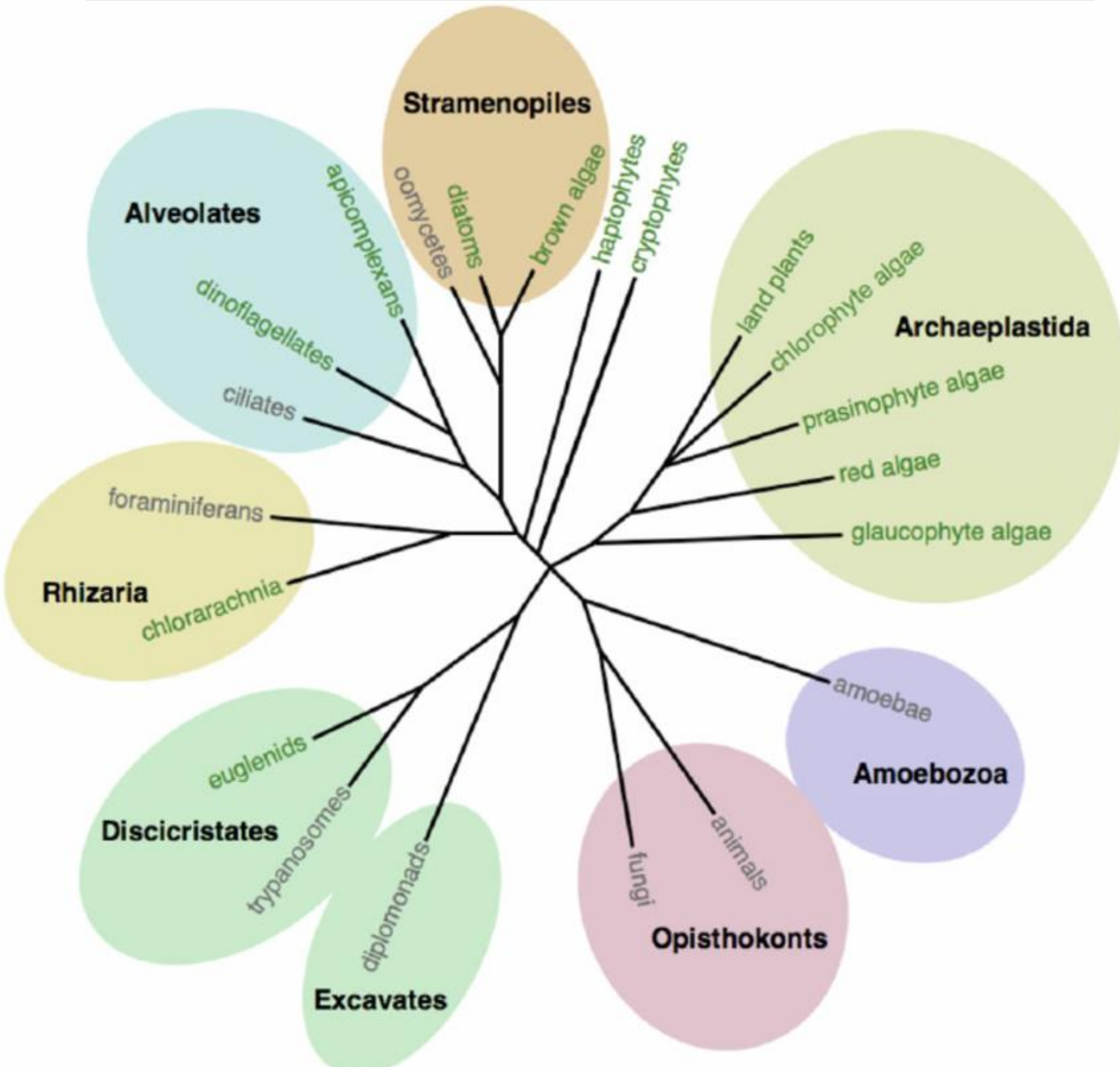


The life cycle of *Tetrahymena* consists of two stages: sexual and asexual. To start the sexual stage, two starved cells conjugate (1). The micronucleus (MIC) of each conjugant first undergoes meiosis (2). A randomly chosen meiotic product in each conjugant divides mitotically and generates two gamete pronuclei (3). Next, the two conjugants exchange one of their two gamete pronuclei, after which the pronuclei fuse to form a genetically identical diploid fertilization nucleus in each conjugant (4); thus, each conjugant gets a haploid genome from each parent. The fertilization nucleus undergoes two rounds of mitotic division, generating four nuclei in each conjugant (5). Two of these nuclei differentiate into new macronuclei (MACs) and the other two remain MICs (6). The old MAC is degraded, and the two "exconjugants" separate from one another. After feeding, the exconjugants undergo their first postzygotic fission; each of the new MACs is distributed into a different daughter cell, the "karyonide" cells (7). At this stage, the one MIC and one MAC condition has been restored. Subsequently, during the asexual part of the life cycle, the MIC divides mitotically and the MAC divides amitotically at every succeeding cell cycle. Amitotic division of the polyploid MAC results in the random segregation of parental chromosomes among daughter cells, leading to segregation of genetic diversity among individuals (allelic assortment). Ultimately, by asexual reproduction and allelic assortment, an individual MAC tends to become homozygous for its entire genome. Yan, Yang, Han, Chen, Xiong, Hamilton, Orias, Miao, Evolution of the mating type gene pair and multiple sexes in Tetrahymena, **iScience** 2021 vol 24 iss 1

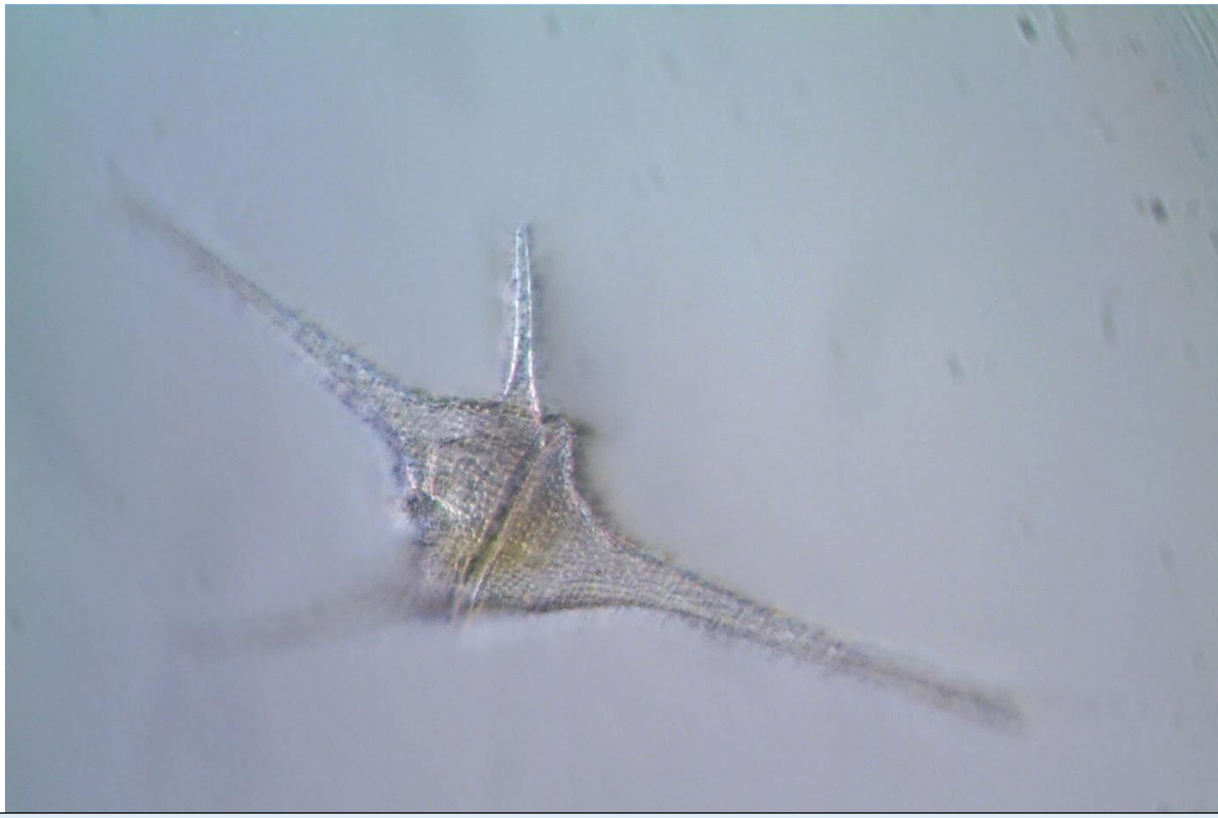


A mating pair of the ciliate *Tetrahymena*, scanning electron micrograph, each protist about 50 microns long. *Tetrahymena* is a well studied model organism and the first ciliate to have its genome read, in 2006. The first protist genome project was *Plasmodium falciparum* in 2002. image from Cornell University at nature.com

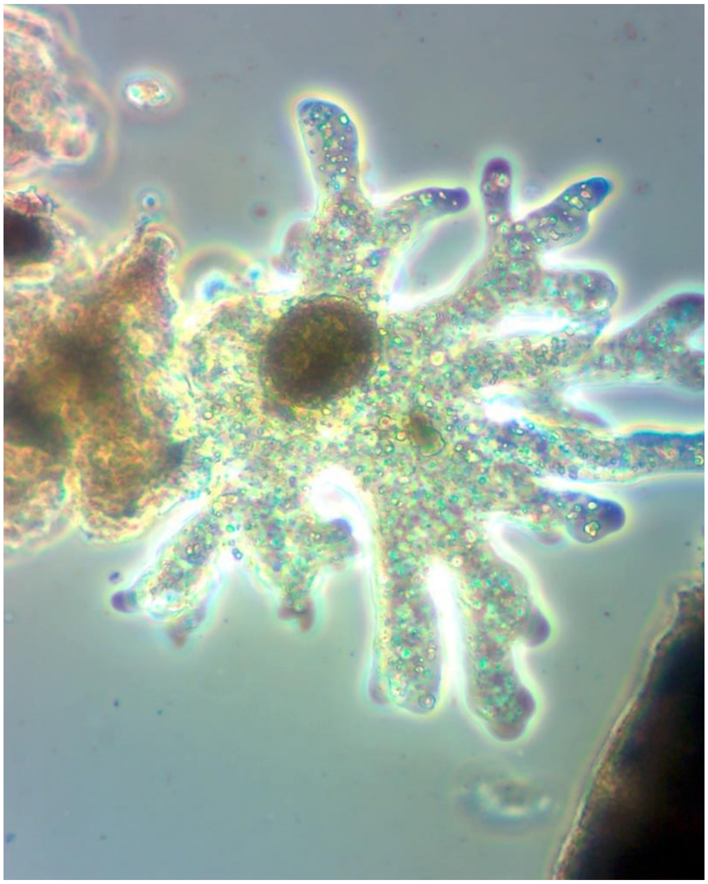
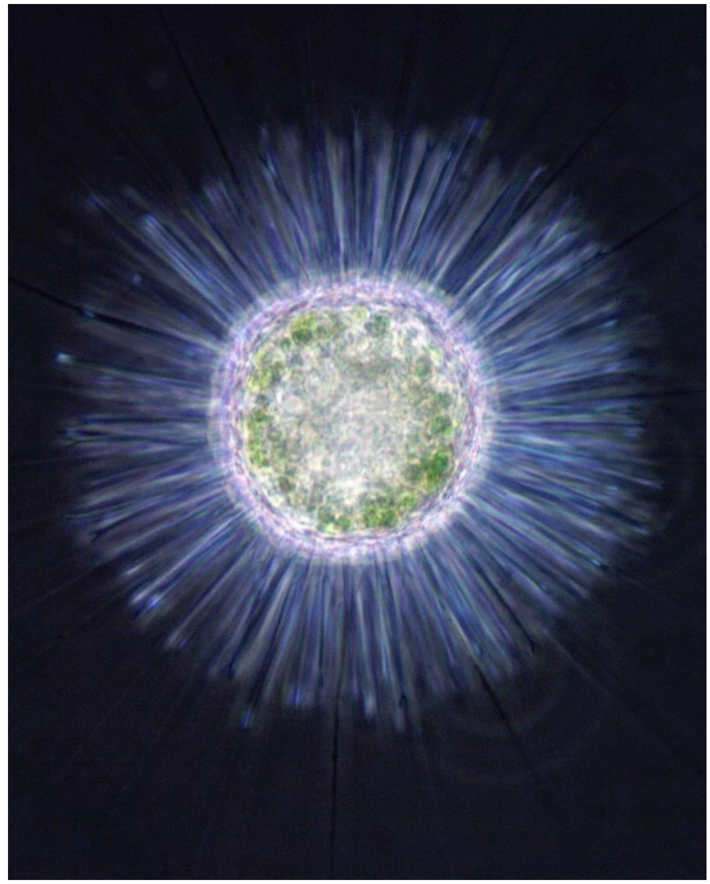
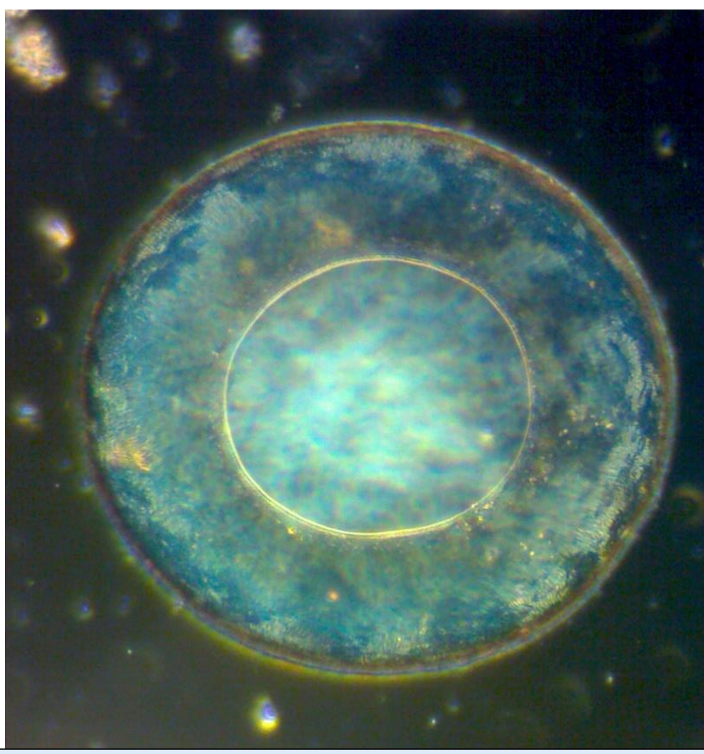
Family tree of Eukaryotes
All life descended from LUCA, the last common universal ancestor. It gave rise to bacteria and archaea. All eukaryotes are descended from LECA, the last common eukaryotic ancestor (which resulted from an endosymbiosis event, the most important mixed marriage ever.



Simple tree of eukaryotic life. Note land plants are a clade within Archaeoplastida, while fungi and animals are separate clades within Opisthokonta. True plants (aka embryophytes) arose from freshwater algae (chlorophytes). Green algae exist as single celled species and as increasingly complex strands and colonies of all sizes. The brown algae kelp forms underwater forests 60 meters tall, but lacking specialized tissues is considered is a protist. Multicellular plants are not a surprise. Tree from Cock, Coelho, Algal models in plant biology. *Journal of experimental botany* 2011

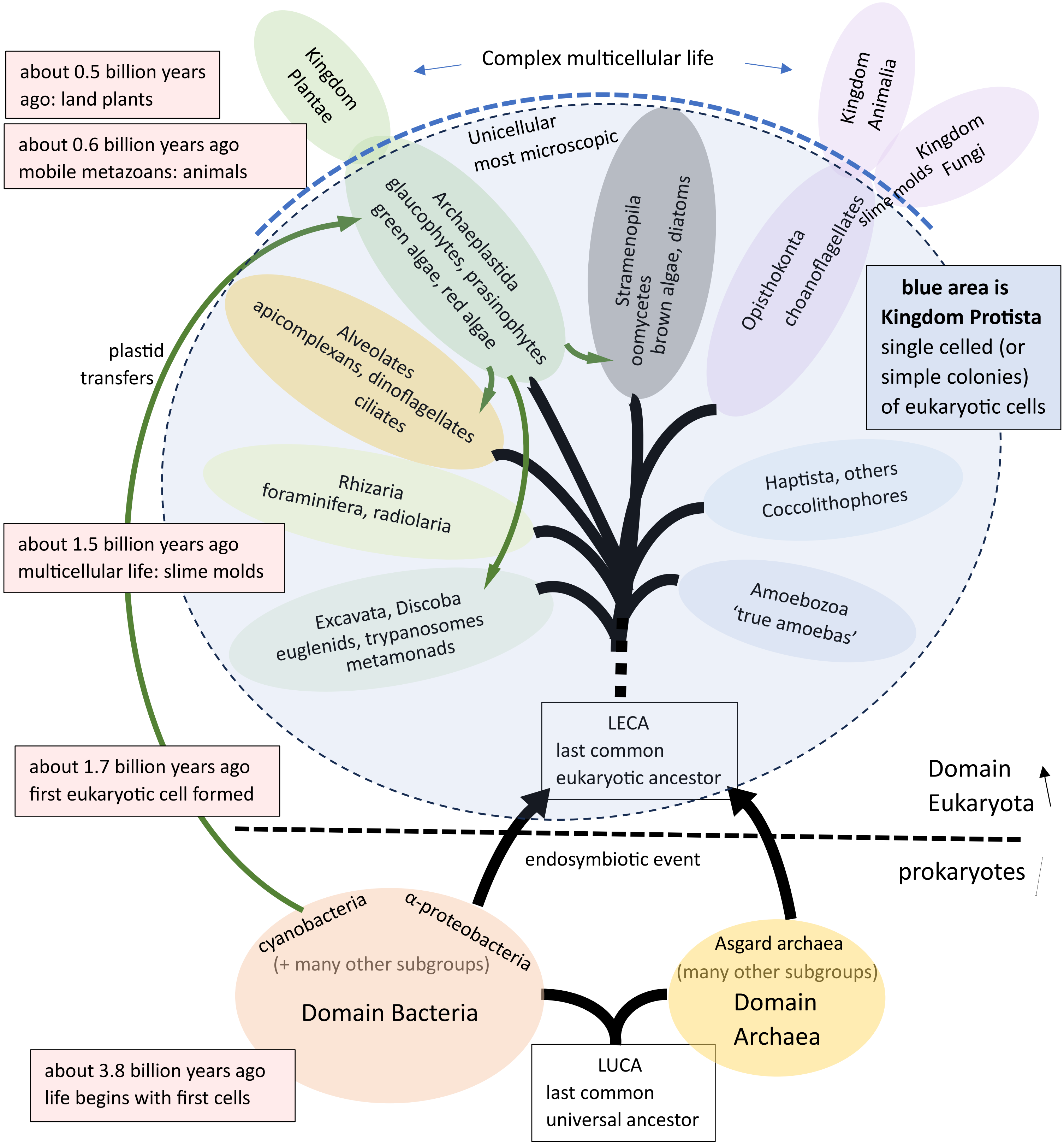


Dinoflagellates are important marine and freshwater protozoans in the Alveolate clade. Most are mixotrophs, able to gain energy from either eating food or by photosynthesis, depending on conditions. “Toxic” red tides are dinoflagellate blooms (related fish kills are now thought to be caused by depletion of nutrients and oxygen).
Above- *Ceratium sp.* from freshwater pond Red Wing, Minnesota, USA June 2022, 40X objective, stacked images
Below- “Xanthidium” fossil in flint, Victorian slide by JT Norman, also 40X objective, both images about 300 microns wide
“Desmid” fossils in Cretaceous flint were discovered by CG Ehrenberg in Germany in the 1830’s and later shown to be dinoflagellate cysts. They were soon found in England as well and a number of 19th century commercial mounts were sold.

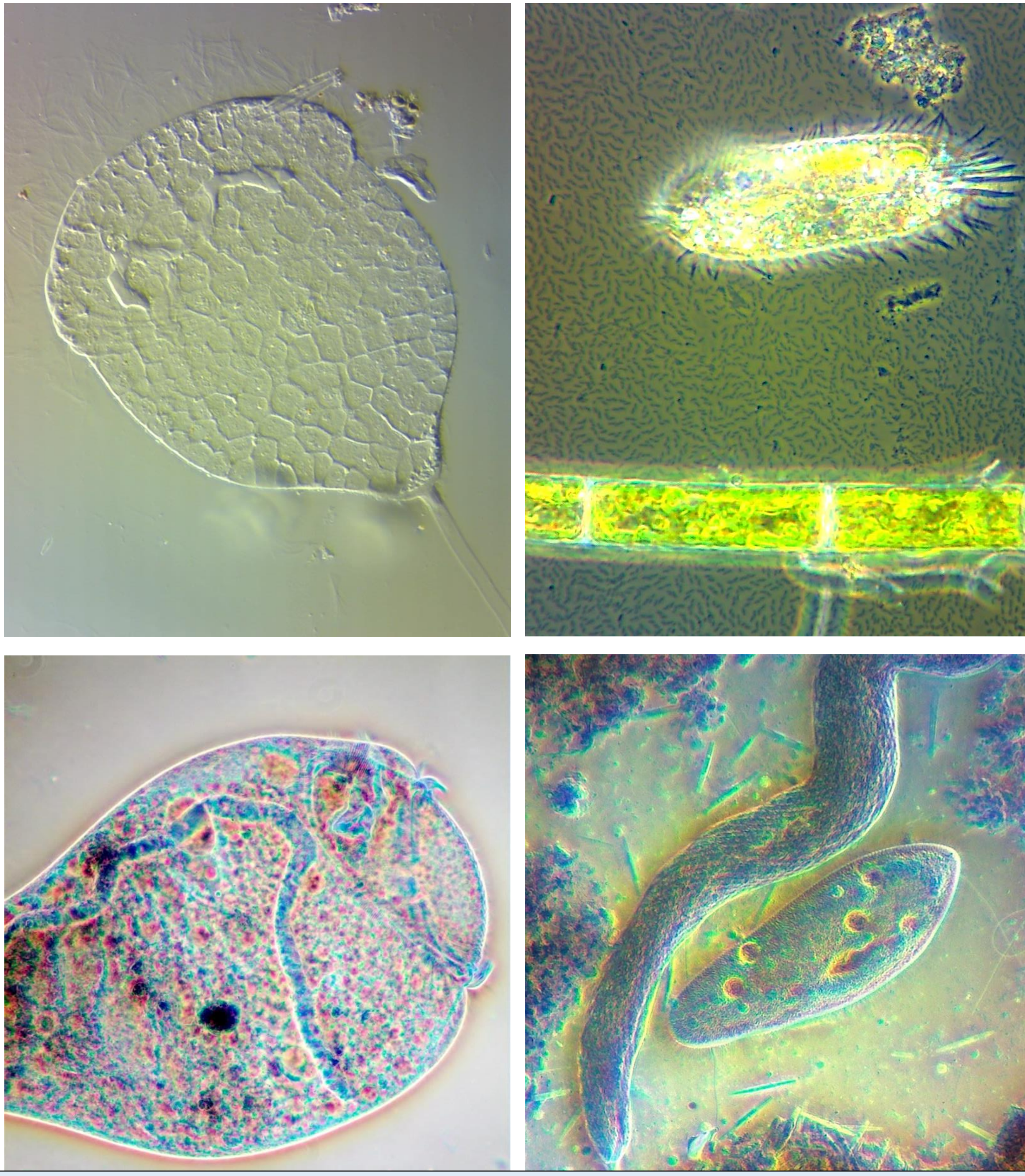





Diverse amoeba like free-living protozoans from pond water, Red Wing, MN, USA (I used 10X, 40X, 40X, 2.5 X objectives)
upper left- classic amoeba, likely *Amoeba proteus*, phylum Amoebozoa, fun to watch vacuoles tumble through cytoplasm
upper right- a heliozoan, phylum Sarcomastigophora, fresh water relative of forams & radiolarians (latter have glass tests)
lower left- *Arcella sp.* an amoeba (phylum Amoebozoa) with a finely perforated chitinous test (shell or “house”)
lower right- foraminifera (aka forams, clade SAR) are important fossils (Cambrian to recent) with mostly calcareous tests

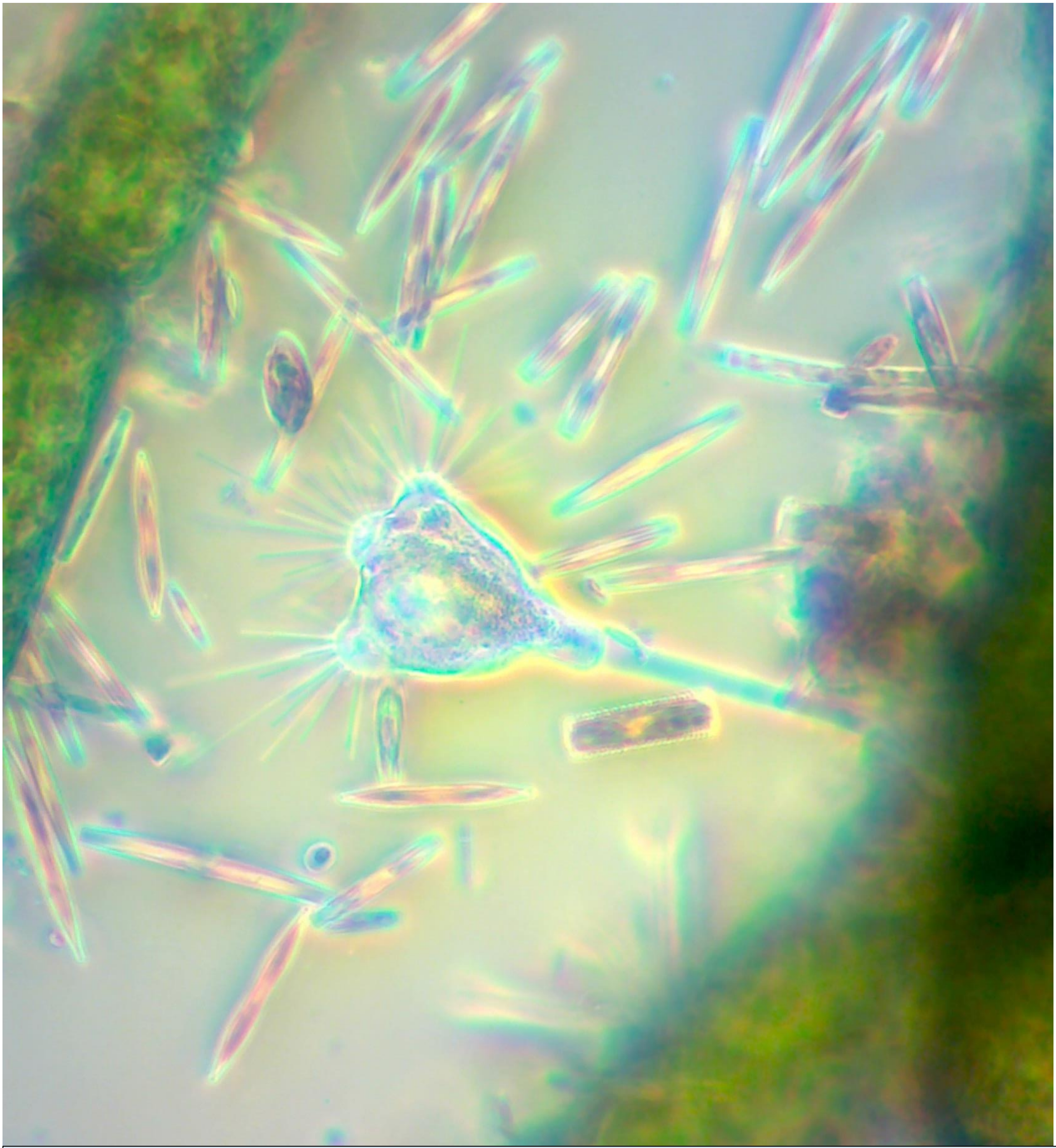
Protozoa are at the center of the tree of life



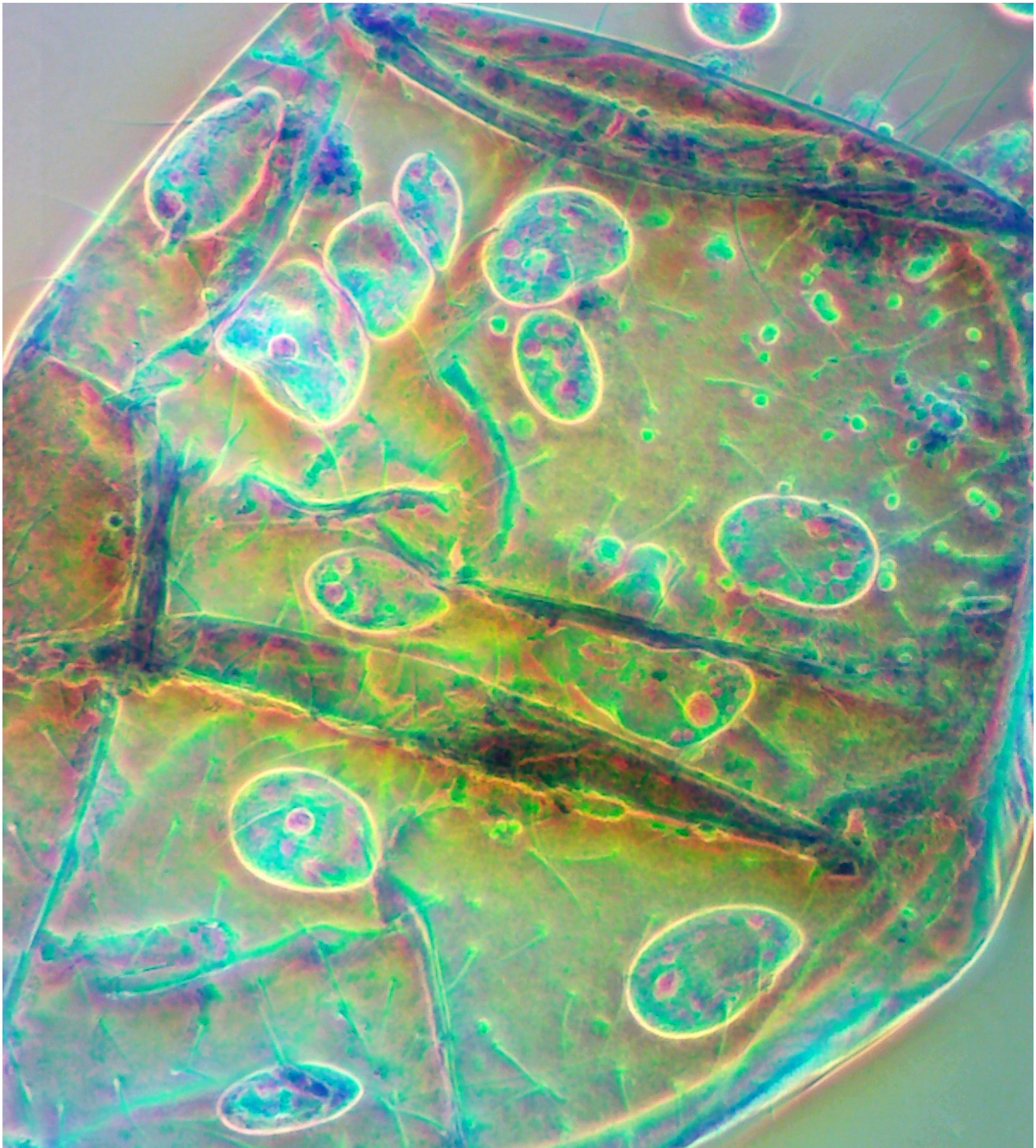
Simplified tree of life by author, Ed Ward, 2024



Ciliates from pond water, Red Wing, MN, USA (40X, 40X, 20X, 10X objectives; oblique, phase and circular oblique lighting)
Ciliates have cilia and 2 nuclei, and are Alveolates (along with dinoflagellates and parasitic apicomplexa) the A in clade SAR
upper left- *Vorticella* or related stalked peritrich, one of the first protists seen under a microscope in 1700s.
upper right- *Oxytricha* or related hypotrich grazing on coccobacilli bacteria; green alga in background.
lower left- *Stentor sp*, huge (1 mm long) ciliate visible to the naked eye; the long worm like organelle is its macronucleus.
lower right- *Spirostomum* and *Paramecium* exploring among diatoms and bacteria (latter not seen at low magnification).



Suctorian ciliate from Lake Pepin, Minnesota, USA, possibly *Acineta sp.* or related, July 2017, 20X obj., circular oblique. Image about 220 microns wide. The stalked protist (center, with straight tentacles) is seen among diatom and green algal protists. Suctorians are widespread (freshwater, marine, some commensal) ciliates that lack cilia as adults. They reproduce by budding off small ciliated larva. The predatory adults feed mostly on other ciliates, sucking out the victim's cytoplasm with their tentacles. Suctorian's taxonomic affinities with other protozoa were unknown when they were discovered around 1850. A century later they were found to be strange ciliates, as confirmed by recent genomic data.



Multiple ciliates exploring the inside of an arthropod molt, pond water, Red Wing, MN, USA in 2016
10X objective, circular oblique lighting (COL), image about 350 microns = 0.35 mm wide
Inspired by Paul James' *Micscape* articles in 2002 - 2003 I used a lot of circular oblique illumination, produced by using an oversize phase contrast annulus (in this case a 20X annulus with a 10X objective). The images can be pleasing, with detail not seen in brightfield. The chromatic aberration of COL is worsened here by using short 34 mm focal length AO objectives from a series 1 scope on a 410 Microstar, which has a telan lens designed for modern 45 mm DIN focal length objectives. Additionally, a cheap ($30) Celestron digital eyepiece oversaturated the colors. Eventually I collected a set of 45 mm DIN objectives and seldom use COL nowadays.

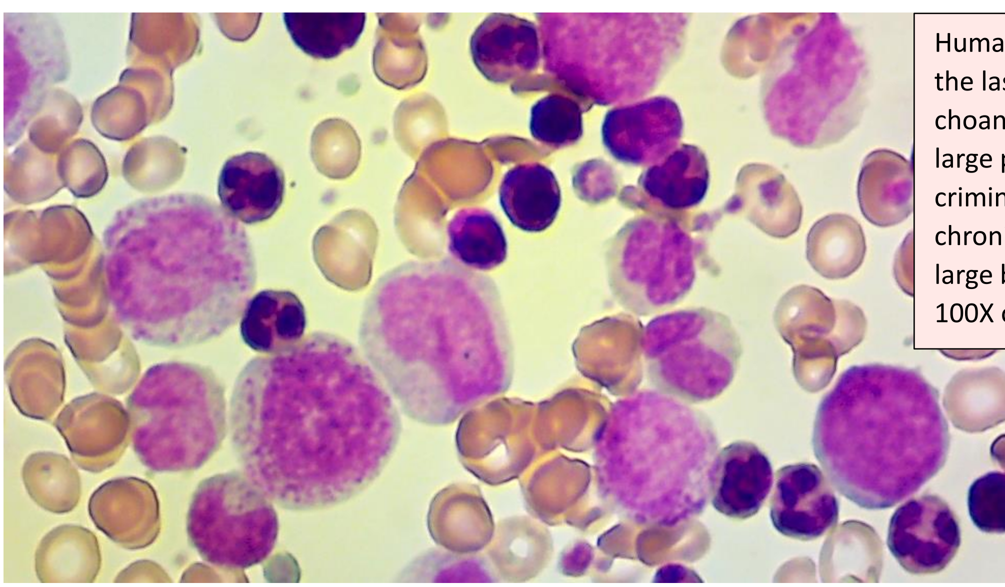
Cell Theory and the Theory of Evolution

These 2 great ideas are the foundation of modern biology and underlie agriculture and healthcare. Both theories arose in the mid19th century Europe and are reproved a million times daily in applications around the world.

As atoms make up matter, cells are the basic unit of all life. Robert Hooke saw tiny boxes in cork with his microscope in 1665, calling them “cells”. German scientists Theodor Schwann and Matthias Jakob Schleiden had better microscopes and in 1838 proposed all plants and animals are made up of cells. The talented Rudolph Virchow completed the basic cell theory in 1855 by proclaiming all cells come from preexisting cells, in Latin “omnis cellula e cellula.”

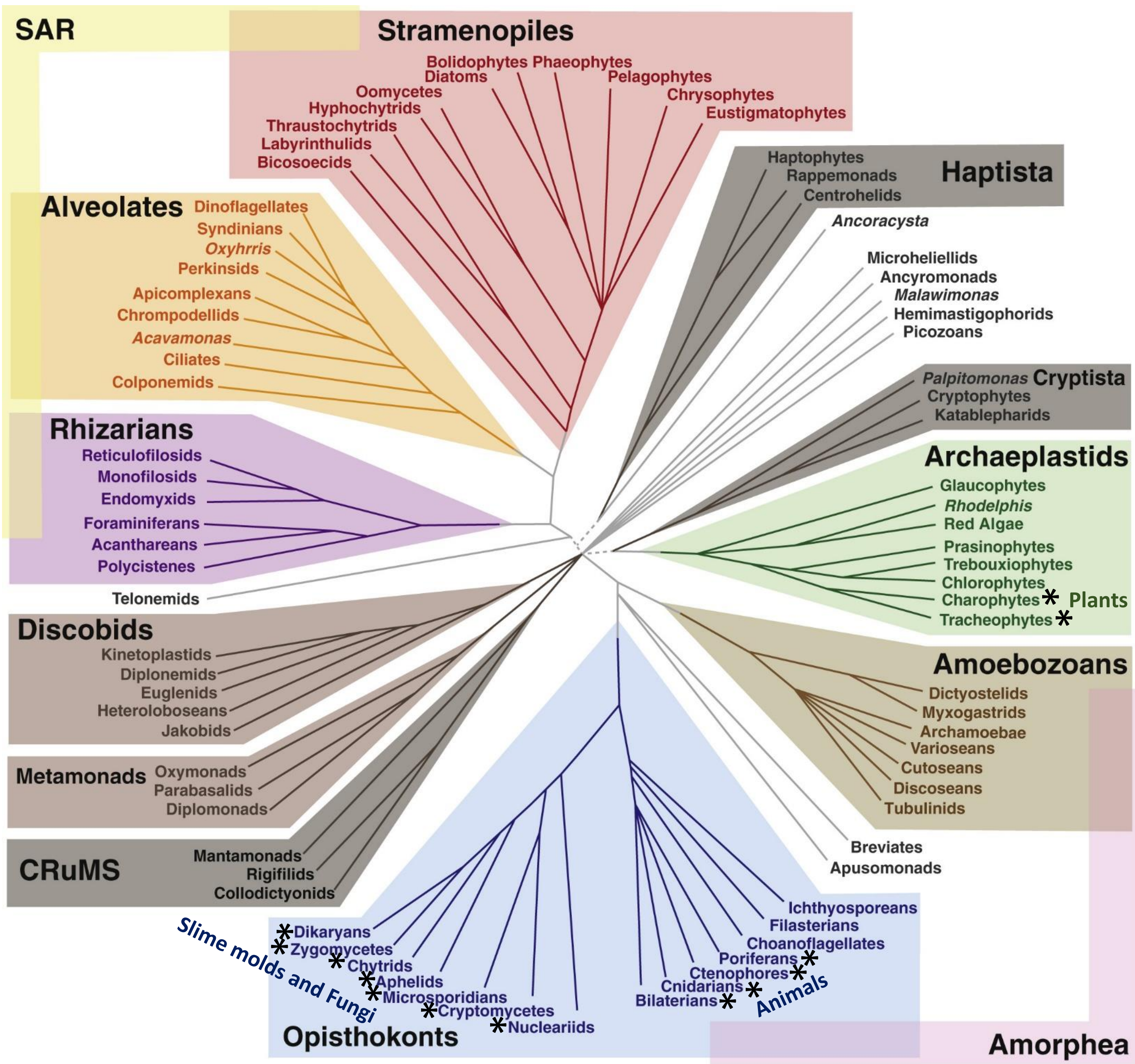
Around the same time a humble genius In England solved the mystery of why we have so many different kinds of animals and plants. In 1859 Charles Darwin published *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. Evolution is so logical it is inevitable. Living beings copy themselves, but sometimes imperfectly, making something slightly different. In the struggle to live and breed some individuals can run faster or are otherwise favored. So the next generation comes from survivors that are a little different. Some populations get split up by barriers. Over deep time (the earth is about 4.55 billion years old) a single cell became all the amazing life on the planet today, from bacteria to *Paramecium* to mushrooms to trees to you. Every living thing becomes fined tuned for its way of life, making life look like it was designed. Yet there was no designer, just the logical results of how natural life processes (explainable by chemistry and physics) worked out.

Run the evolutionary clock backwards and eventually you get to the first living cell. Every cell in every being is a direct descendant of that single cell (if life arose more than once, the others got wiped out long ago). Everything alive today uses variations of the same DNA and protein biochemistry of our common ancestor cell. That first very tiny cell grew and split into 2 daughters. They also split into 2 and so on. Thanks to exponential math they filled the oceans. Some learned to make food from sunlight, producing oxygen as a toxic waste. After billions of generations there were many different kinds of cells and one day one gobbled up another as lunch, but the lunch didn’t die. The “lunch” became mitochondria, finding a nice new place to live and paying rent with high energy molecules. Naked DNA got organized into chromosomes inside a nucleus and life was off to do bigger things. With extra stored information, daughter cells could be different than each other yet still programmed to cooperate with each other. Multicellular life was born: bodies made of hundreds or millions or eventually trillions of cells. Inside your body is a wonderland. It’s like a bustling city with skyscrapers and highways and factories populated by 35 trillion resident cells. All those cells came from 1 cell formed when your mom and dad had sex. And those 2 cells came in turn from earlier and earlier cells. You have the most massive convoluted family tree ever, all the way back to the first bacterial cell at a steaming hot, sulfurous deep sea vent 3.8 billion years ago. During all those years there were only a few great leaps; most of the changes were tiny and incremental (but added up over time). You, *Homo sapiens*, might be one of the great leaps, or not. You have the abstract reasoning required for complex language and math, leading to civilization and eventually to industrial technologies powerful enough to destroy the planet or to create a paradise on earth. Please choose wisely.



Human blood cells, distant descendants of the last universal common ancestor and of choanoflagellate protozoa. In this case the large purple cells belong to a dangerous criminal gang (1950’s hospital slide, likely chronic myelogenous leukemia with many large blasts and smaller mature neutrophils) 100X oil obj, cropped, RBCs about 7 µ wide

Recent eukaryotic tree of life. All clades (groups of related organisms) on this tree are protists, except those few I designate with asterisks* Fungi and animals arose from opisthokont protozoa, and plants arose from archaeplastid protists. Fungi diverged from protists about 1.5 billion years ago, early animals arose about 0.7 billion years ago and the first land plants were about 0.5 billion years ago.



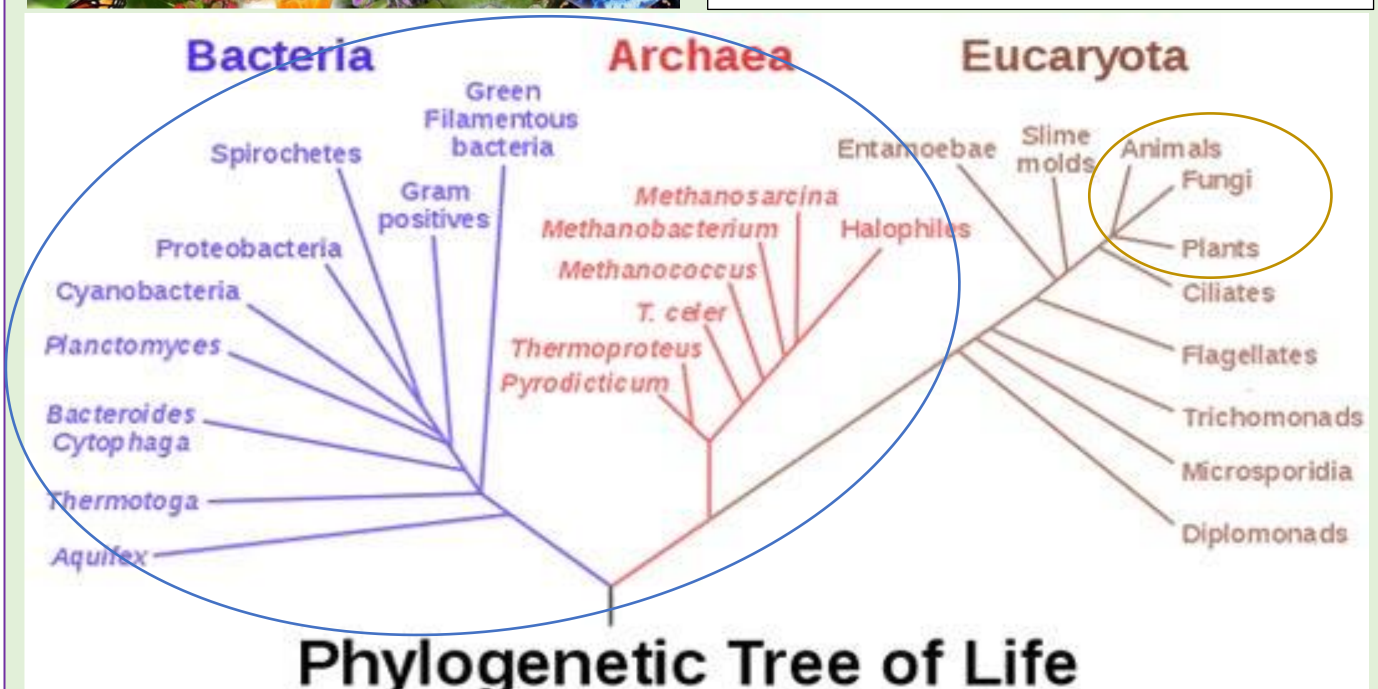
from Keeling, PJ, Burki, F Progress towards the Tree of Eukaryotes, **Current Biology** 2019

Evolution

Evolution explains the rich strangeness of life. In 1859 evolution was a new theory, laid out in clear and humble detail by Charles Darwin in his book *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. Darwin had gathered thousands of his own and others' observations about the varieties of life, but at that time known fossils were fewer and the mechanisms of inheritance were unknown (even though Czech monk Gregor Mendel discovered the laws of genetics in pea plants in the 1860's). Evolution is now a proven fact: millions of new bits of evidence show how the first cell evolved into a myriad of different forms on planet earth. Evolution remains a powerful and changing (all science is subject to modification by additional information) central theory in biology that makes sense of new evidence from biologic and medical research. I see evolution in the hospital daily as bacteria evolve to be resistant to antibiotics, and the COVID-19 virus evolves to be more docile.

Darwin's theory is amazing in that upon careful reflection it seems inevitable. People create different breeds of dogs, pigeons and other domesticated animals through selective breeding. Darwin showed nature does much the same thing. It can be observed that all organisms come from the reproduction of previous organisms, that there are differences between individuals in a group (i.e. some are faster or slower) and that parents reproduce imperfect copies of themselves (we now know genes are shuffled and sometimes mutate). Darwin noticed that there is a struggle in nature for organisms to survive and produce offspring. In real circumstances (gazelles being chased by cheetahs for example) survival is not just random but favors certain bodily abilities (i.e. running faster) so the next generation comes from selected (faster) surviving parents and so each generation becomes slightly adapted in particular ways. Over deep time (the earth is now known to be about 4.55 billion years old) a single cell became all the amazing life on the planet today, from bacteria to *Paramecium* to grass to mushrooms to worms to you. Every living thing becomes fined tuned for its way of life, making life look like it was designed. Yet there was no designer, just the logical results of how natural life processes (based on chemistry, physics and math) worked out.

Most of the tree of life is prokaryotic -extra tiny, no nucleus: bacteria and archae. All true multicellular life is in the small circle I added to the right. In between are protists, nucleated single celled life. Carl Woese used ribosomal RNA data for the tree.



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See also attributions at bottom of trees of life included in this article

Micscape is a high quality website hosted in the UK and made great by contributors from around the globe. *Micscape* Magazine always has lots of good information for amateur microscopists wanting to learn more about how to do it yourself.

For 2024 I offer *Micscape's* readers a series of articles about parasites, illustrated in part from my slide collection.

This month I took a break from describing tiny creatures that can potentially harm people. Instead, I celebrate the interesting and beautiful free living protists that occupy wet habitats everywhere. If you want to know more about parasitic disease causing protozoa, see the February 2024 edition of *Micscape* Magazine.

Plankton includes some protozoa but also many other interesting creatures as well. If you wish to learn more about freshwater plankton see the April 2023 edition of *Micscape* Magazine.

Some people are real experts and know much more than I do on these subjects. I would be pleased to have any mistakes or misunderstandings corrected.

I am Ed Ward in the state of Minnesota, USA.

Your comments are always welcomed, my email is eward1897 AT gmail DOT com

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