



Biology II

LABORATORY MANUAL

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Laboratory Manual For SCI104
(Biology II at Roxbury Community College)
Nikolaus Sucher

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¹https://en.wikipedia.org/wiki/Connective_tissue

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³https://en.wikipedia.org/wiki/Nervous_tissue

⁴<https://en.wikipedia.org/wiki/Epithelium>

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⁵https://commons.wikimedia.org/wiki/File:Haeckel_Lichenes.jpg

⁶https://en.wikipedia.org/wiki/Kunstformen_der_Natur

⁷https://commons.wikimedia.org/wiki/File:Haeckel_Musciniae.jpg

⁸https://en.wikipedia.org/wiki/Kunstformen_der_Natur

⁹https://commons.wikimedia.org/wiki/File:Lifecycle_moss_svg_diagram.svg

¹⁰https://commons.wikimedia.org/wiki/File:Haeckel_Hepaticae.jpg

¹¹https://en.wikipedia.org/wiki/Kunstformen_der_Natur

¹²https://commons.wikimedia.org/wiki/File:Liverwort_life_cycle.jpg

¹³https://commons.wikimedia.org/wiki/File:Haeckel_Filicinae_92.jpg

¹⁴https://en.wikipedia.org/wiki/Kunstformen_der_Natur

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¹⁵https://commons.wikimedia.org/wiki/File:Mature_flower_diagram.svg

¹⁶https://commons.wikimedia.org/wiki/File:Angiosperm_life_cycle_diagram-en.svg

¹⁷https://en.wikipedia.org/wiki/Chrysaora_colorata

¹⁸https://en.wikipedia.org/wiki/Anthopleura_xanthogrammica

¹⁹https://commons.wikimedia.org/wiki/File:Hydra_nematocyst_firing_01.png

²⁰[https://en.wikipedia.org/wiki/Hydra_\(genus\)#/media/File:Hydra-Foto.jpg](https://en.wikipedia.org/wiki/Hydra_(genus)#/media/File:Hydra-Foto.jpg)

²¹https://commons.wikimedia.org/wiki/File:Obelia_geniculata.jpg

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²²https://commons.wikimedia.org/wiki/File:Haeckel_Ascidiae.jpg

²³https://commons.wikimedia.org/wiki/File:Illu_epithelium.jpg

²⁴<https://commons.wikimedia.org/wiki/File:Skin.png>

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²⁵https://commons.wikimedia.org/wiki/File:Illu_compact_spongy_bone.jpg

²⁶https://commons.wikimedia.org/wiki/File:Human_skeleton_front_en.svg

²⁷https://commons.wikimedia.org/wiki/File:Biological_classification_L_Pengo_vflip.svg

²⁸<https://commons.wikimedia.org/wiki/File:CollapsedtreeLabels-simplified.svg>

²⁹<https://commons.wikimedia.org/wiki/File:Phylogenetic-Groups.svg>

³⁰https://commons.wikimedia.org/wiki/File:Geologic_Clock_with_events_and_periods.svg

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Acknowledgements

At RCC³¹, introductory (general) biology is split into two courses (SCI103, Biology I, and SCI104, Biology II) which are taught over two semesters. The two courses were developed in the 1970s by Prof. Georgia Whitman essentially representing botany (Biology I) and zoology (Biology II). Some 20 years ago, Prof. Kyrsis Rodriguez who served as the biology course coordinator until her retirement reduced the botany content and introduced molecular aspects of biology.

In 2015, the Massachusetts Department of Higher Education with its MassTransfer Pathways³² initiative prompted a statewide critical evaluation of the structure and content of foundational courses, such as introductory biology, in order to make these courses eligible for transfer across the Commonwealth amongst public higher education institutions. In 2016, we have been fortunate to receive a large grant from the Massachusetts Life Sciences Center³³ to upgrade and modernize our science labs. We have taken this opportunity to re-structure our courses along the lines of current practice of introductory biology teaching such that the Biology I course introduces the molecular and cellular basis of life, while the Biology II course is focused on the basics of organismal biology. Overall the proposed changes will expose our students to modernized (up-to-date) concepts of biology and thus make them more competitive.

This laboratory manual was inspired by “Encounters with Life³⁴” by Larry J. Scott and Hans F. E. Wachtmeister published by the Morton Publishing Company in Englewood CO. We used that manual at RCC for many years. Progress in the biological sciences is, however, relentless and so over the years, our requirements changed, and we felt that it was best to put together a new manual specifically tailored to our needs.

The creation of this manual was greatly facilitated and owes a major debt to Wikipedia³⁵ and its large number of voluntary contributors. I very liberally copied from many Wikipedia pages and then remixed, edited, adapted and added text. With your continued support and help this manual can only get better over time. I urge you to email me with your criticisms and suggestions at nsucher@rcc.mass.edu³⁶. This manual is made available as an open educational resource under Creative Commons Attribution-Share Alike 3.0 Unported³⁷ United States License for others to do as I did and improve and adapt to specific requirements.

I wish to thank Dr. Hillel Sims, Dean of STEM, for the laboratory safety and microscope videos. Last but not least, I want to thank all lab technicians, my teaching colleagues and our students who work together to translate mere words into an exciting laboratory experience for all of us at RCC.

³¹<http://www.rcc.mass.edu>

³²<http://www.mass.edu/masstransfer/>

³³<http://www.masslifesciences.com>

³⁴<https://www.morton-pub.com/catalog/biology/encounters-life-7e>

³⁵<https://www.wikipedia.org>

³⁶<mailto:nsucher@rcc.mass.edu>

³⁷<https://creativecommons.org/licenses/by-sa/3.0/deed.en>

Course Description

This course provides an introduction to the biology and classification of plants, fungi, and animals, their tissues, organ systems, development and reproduction. Four hours of lecture and a two-hour lab session are required each week.

Learning Objectives

- Understand and explain the evolutionary relationships between the major groups of fungi, plants and animals, and their relationships with their habitats, and with other groups of organisms.
- Identify the appropriate external and internal structures and their functions in representative organisms.
- Identify some of the distinguishing characteristics of the major fungi, plant and animal groups studied, and to be able to recognize and/or give examples of organisms belonging to these major groups.
- Identify (through the microscope, and in diagrams) representative examples of the major somatic tissue groups, and to demonstrate understanding of their characteristics, functions, and location in the vertebrate organism.
- Identify the basic anatomy of the different organ systems studied, and to understand the related basic physiology.
- Recognize some of the factors which influence human health, and the causes and symptoms of some common human diseases.
- Learn and demonstrate basic lab dissections.

How to do well in this class

Before the lab

- Know what's coming up. Each week, look at the schedule to know what lab is coming up.
- Be prepared. Read the chapter in the manual **before** you come to the lab.
- In your own words, explain to yourself or someone else what the upcoming lab is about.

During the lab

- Read the lab instructions carefully.
- Follow the instructions carefully.
- Make sure that you know what you are doing and why you are doing it.
- Don't be afraid to ask your instructor for help or clarifications.

After the lab

- Ask yourself: what was this lab about? What did we do? How did we do it?
- In your own words, tell yourself or someone else what you learned in the lab.

Chapter 1

Lab Safety

The laboratory classes are hands-on. Some classes require the use of hazardous chemicals and materials. Safety in the classroom is the #1 priority for students and faculty. To ensure a safe science laboratory, a list of rules must be followed at all times.

Please watch the RCC safety video¹



Prior to your participation in the science lab course, you must read this safety document and sign and return the acknowledgment and agreement page.

1.1 General rules

1. NO FOOD, BEVERAGES, GUM, in the labs. Cell phone usage is also prohibited in the lab.
2. Conduct yourself in a responsible manner at all times in the lab. Horseplay, pranks and practical jokes are prohibited and will not be tolerated. If you participate in inappropriate behavior the INSTRUCTOR HAS THE RIGHT TO ASK YOU TO LEAVE THE lab.
3. Students cannot be in the lab without an instructor present.
4. Read all lab procedures, precautions, and equipment instructions thoroughly before each lab. Follow all written and verbal instructions carefully. Perform only those experiments authorized by the instructor. If during the lab you don't understand, stop and ask the instructor before proceeding. Never do anything in the lab that is outside of your instructors directions or that is not in your lab procedure.
5. Do not begin lab activities; touch any chemicals or equipment until you are instructed to do so.
6. Work areas should be kept organized and clean at all times. Only necessary items (lab notebook, worksheets, etc.) should be on your workbench. Backpacks and purses must be stored under the benches or against the walls. CLEAN ALL OF YOUR WORK SURFACES AND EQUIPMENT AT THE END OF THE EXPERIMENT. Safely dispose of waste in its proper container and place glassware in the grey bins by the sink. DO NOT STACK GLASSWARE. If the bin is full ask the instructor for another bin.
7. Keep aisles clear. Push lab stools under the lab benches when not in use.
8. Know where the safety equipment is and how to use it. This includes the first aid kit, eye-wash station, safety shower, fire extinguisher, and fire blanket. Know the location of the fire alarm, and emergency phone. In the event of a fire drill during lab time containers must be closed, gas valves off, fume hood, and all electrical equipment must be turned off.
9. NEVER DISPOSE OF ANYTHING IN THE SINK. All materials are to be disposed of in the proper hazardous waste containers with the assistance of the instructor. All waste containers must be closed and placed inside a secondary containment bin.
10. As classes in these labs use toxic chemicals, keep your hands away from your face, eyes and mouth while working in the lab. Always wash your hands thoroughly with warm water and soap before leaving the lab to prevent injury or illness. This is part of proper lab procedure.
11. Students are not permitted in the prep room areas (between the lab rooms).
12. Handle all living organisms used for lab experiments in a respectful, humane manner.
13. Microscopes must be properly cleaned, the electrical cords properly wrapped, and returned to

¹<https://youtu.be/NxcsyTv7stQ>

their places with their protective covers.

Disposal of all hazardous waste is **ONLY** to be handled by the instructor and in a manner consistent with federal, state and local hazardous waste disposal regulations. Organic solvents are never to be disposed of down the sink; receptacles will be provided as needed for their collection. All hazardous chemical substances must be placed in the appropriate type of container and labeled with chemical, name and date, sealed and placed upright in a gray plastic bin.

1.2 Class dissections

14. Preserved biological specimens should be treated with respect and disposed of in a clear plastic bag and placed inside the hazardous waste drum located in the classroom. This container must be sealed at the end of each dissection.
15. When using sharp objects always carry the tips pointed down and away from you. When dissecting, cut away from your body. Grasp the instrument only by the handle. Never try to catch falling sharp instruments or glassware. When you are finished dissecting, wash and dry your instruments and dissecting pan before storing them in the proper location. Do not leave any instruments in the sink.

1.3 Clothing

16. Students must wear lab goggles when using chemicals, or using heat. **NO EXCEPTIONS!** Lab coats are mandatory for Anatomy and Physiology, Biology, Microbiology, Chemistry, and Biotechnology labs, except for when lecturing and working on dry lab (for example, looking at models or prepared slides) activities.
17. Gloves should be worn when handling solutions, solids, specimens, etc.
18. Proper dress should always be observed in the lab. Long hair must be tied back. Loose or baggy clothing (especially sleeves), dangling jewelry, hats, shorts, short skirts, bare midriffs, high heels, sleeveless shirts, and open toed or open heeled shoes and sandals are prohibited in the lab. Failure to comply may result in expulsion from class.

1.4 Handling chemicals

18. Always work in a well-ventilated area. Use the fume hood when working with volatile substances or poisonous vapors, or any chemical with an odor.
19. Never smell a chemical by sniffing. Use your hand to wave the chemical towards your nose.
20. **DO NOT TASTE, TOUCH** or smell anything unless instructed to do so. You should wear a lab apron and gloves at all times. Your instructor will tell you the proper gloves to wear depending on the chemical being used.
21. **CHECK EACH LABEL TWICE** before removing any of its contents. Take only what is needed of each chemical. **NEVER** return unused chemicals to their original container; put it in the waste container.
22. Do not use your fingers to transfer solid chemicals. Use a scoop or spatula.
23. Use a rubber bulb, pipette, or pi-pump when transferring liquid chemicals. **NEVER USE YOUR MOUTH TO PIPETTE!**
24. When transferring reagents hold the containers away from your body while working on the bench.
25. Acids must be handled with extreme care. You will be shown the proper method for diluting strong acids. **ALWAYS ADD ACID TO WATER**, swirl or invert the solution and be careful of the heat produced particularly with sulfuric acid.
26. Handle hazardous liquids over a pan to contain spills.
27. Never handle flammable liquids anywhere near an open flame or heat source.
28. Be careful when transporting chemicals across the lab. Hold securely and walk carefully.
29. **NEVER POUR CHEMICALS INTO SINK.** Waste should be disposed of in the proper hazardous waste container provided.

1.5 Glassware

30. Never handle broken glass with bare hands. Use a brush and a dustpan to clean up broken glass. Place uncontaminated broken glass in the white and blue broken glass receptacles. Contaminated trash goes in the biohazard bin.
31. Fill wash bottles only with distilled water and use only as intended, e.g.; rinsing glassware, adding water to a container.

32. Never use chipped, cracked or dirty glassware to avoid shattering.
33. Never immerse hot glassware in cold water. It may shatter.
34. Never place dirty glassware with the clean glassware. All dirty glassware should be placed in gray wash bins. DO NOT STACK DIRTY GLASSWARE IN BINS.
43. You must spray down your lab bench with Lysol after each lab. Do not wipe with paper towels. The bench surface must remain wet for at least five minutes for the Lysol to destroy any micro organisms.
44. Wash your hands thoroughly before and after each lab as well as before you leave the lab for any reason.
45. Dispose of contaminated broken glass in the bio-hazard bin. Please wrap the broken glass in paper towels before disposal so that the broken glass doesn't cut through the bag. Dispose of uncontaminated glass in the white and blue cardboard glass boxes.

1.6 Heating substances

35. Exercise extreme caution when using a gas burner. Be careful to keep hair, loose clothing and hands away from flames at all times. Wear safety goggles. Do not put any substances into the flame unless specifically instructed to do so. Never reach over an exposed flame. The instructor will provide a demonstration of the proper way to operate a Bunsen burner. Never leave a lighted burner or hot plate unattended. Always turn the burner or hot plate off when not in use.
36. Do not point the open end of a test tube being heated at yourself or anyone else. Never look into a container that's being heated.
37. Heated metals and glass remain hot for a very long time. They should be set aside to cool and picked up with caution. Use tongs or heat protective gloves.

1.7 Handling microbiology materials

38. Please be aware that micro labs include work with pathogenic organisms. Be alert. Conduct yourself in a responsible manner at all times.
39. If you spill anything notify your instructor immediately. There are special procedures to be followed for spills containing microorganisms.
40. A lab coat must be worn during lab activities. Lab coats/aprons may never leave the lab. If you must leave the lab during a class then your lab coat must be removed.
41. Gloves must be worn at all times when working with bacteria. Gloves need to be disposed of in the biohazard waste container.
42. All contaminated waste must be disposed of in the biohazard container. Do not overfill biohazard containers.

Chapter 2

Fungi

Fungi¹ are uni- or multicellular heterotrophic, eukaryotic organisms with external digestion. Fungi include microorganisms such as yeasts and molds and mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of protists, plants and animals. The English word fungus has been adopted from the Latin word *fungus* (mushroom) which in turn is derived from the Greek word *sphongos* meaning “sponge”.

Fungi acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment (a process referred to as external digestion) unlike animals, which ingest food and digest it internally in their digestive systems (a process referred to as internal digestion). Fungi do not photosynthesize. Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the Eumycota (true fungi or Eumycetes), which share a common ancestor (form a monophyletic group), an interpretation that is also strongly supported by molecular phylogenetics. This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology (from the Greek *mykes*, mushroom). In the past, mycology was regarded as a branch of botany, although it is now known fungi are genetically more closely related to animals than to plants.

Abundant worldwide, most fungi are inconspicuous because of the small size of their structures, and their cryptic lifestyles in soil or on dead matter. Fungi include symbionts of plants, animals, or other fungi and also parasites. They may become

noticeable when fruiting, either as mushrooms or as molds. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange in the environment. They have long been used as a direct source of human food, in the form of mushrooms and truffles; as a leavening agent for bread; and in the fermentation of various food products, such as wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases and insect pests. Many species produce bioactive compounds called mycotoxins that are toxic to animals including humans. The fruiting structures of a few species contain psychotropic compounds and are consumed recreationally or in traditional spiritual ceremonies. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals. Losses of crops due to fungal diseases (e.g., rice blast disease) or food spoilage can have a large impact on human food supplies and local economies.

The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from unicellular aquatic chytrids to large mushrooms. However, little is known of the true biodiversity of Kingdom Fungi, which has been estimated at 2.2 million to 3.8 million species, of which only 120,000 have been described. 8000 of them are detrimental to plants and 300 can be pathogenic to humans. Ever since the pioneering 18th and 19th century taxonomical works of Carl Linnaeus, Christian Hendrik Persoon, and Elias Magnus Fries, fungi have been classified according to their morphology (e.g., characteristics such as spore color or microscopic features) or physiology. Advances in molecular genetics have

¹<https://en.wikipedia.org/wiki/Fungus>

opened the way for DNA analysis to be incorporated into taxonomy, which has sometimes challenged the historical groupings based on morphology and other traits. Phylogenetic studies published in the last decade have helped reshape the classification within Kingdom Fungi, which is divided into one subkingdom, seven phyla, and ten subphyla.

2.1 Microscopic Morphology

Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 μm in diameter and up to several centimeters in length. Hyphae grow at their tips (apices); new hyphae are typically formed by emergence of new tips along existing hyphae by a process called branching, or occasionally growing hyphal tips fork, giving rise to two parallel-growing hyphae. Hyphae also sometimes fuse when they come into contact, a process called hyphal fusion (or anastomosis). These growth processes lead to the development of a mycelium, an interconnected network of hyphae. Hyphae can be either septate or coenocytic. Septate hyphae are divided into compartments separated by cross walls (internal cell walls, called septa, that are formed at right angles to the cell wall giving the hypha its shape), with each compartment containing one or more nuclei; coenocytic hyphae are not compartmentalized. Septa have pores that allow cytoplasm, organelles, and sometimes nuclei to pass through; an example is the dolipore septum in fungi of the phylum Basidiomycota. Coenocytic hyphae are in essence multinucleate supercells. Many species have developed specialized hyphal structures for nutrient uptake from living hosts; examples include haustoria in plant-parasitic species of most fungal phyla, and arbuscules of several mycorrhizal fungi, which penetrate into the host cells to consume nutrients. Although fungi are opisthokonts—a grouping of evolutionarily related organisms broadly characterized by a single posterior flagellum—all phyla except for the chytrids have lost their posterior flagella. Fungi are unusual among the eukaryotes in having a cell wall that, in addition to glucans (e.g., -1,3-glucan) and other typical components, also contains the biopolymer chitin.

2.2 Macroscopic Morphology

Fungal mycelia can become visible to the naked eye, for example, on various surfaces and substrates, such

as damp walls and spoiled food, where they are commonly called molds. Mycelia grown on solid agar media in laboratory petri dishes are usually referred to as colonies. These colonies can exhibit growth shapes and colors (due to spores or pigmentation) that can be used as diagnostic features in the identification of species or groups. Some individual fungal colonies can reach extraordinary dimensions and ages as in the case of a clonal colony of *Armillaria solidipes*, which extends over an area of more than 900 ha (3.5 square miles), with an estimated age of nearly 9,000 years. The apothecium—a specialized structure important in sexual reproduction in the ascomycetes—is a cup-shaped fruit body that is often macroscopic and holds the hymenium, a layer of tissue containing the spore-bearing cells. The fruit bodies of the basidiomycetes (basidiocarps) and some ascomycetes (ascocarps) can sometimes grow very large, and many are well known as mushrooms.

2.3 Reproduction

Fungal reproduction is complex, reflecting the differences in lifestyles and genetic makeup within this diverse kingdom of organisms. It is estimated that a third of all fungi reproduce using more than one method of propagation. Environmental conditions trigger genetically determined developmental states that lead to the creation of specialized structures for sexual or asexual reproduction. These structures aid reproduction by efficiently dispersing spores or spore-containing propagules.

Asexual reproduction occurs via vegetative spores (conidia) or through mycelial fragmentation. Mycelial fragmentation occurs when a fungal mycelium separates into pieces, and each component grows into a separate mycelium. Mycelial fragmentation and vegetative spores maintain clonal populations adapted to a specific niche, and allow more rapid dispersal than sexual reproduction. The “Fungi imperfecti” (fungi lacking the perfect or sexual stage) or Deuteromycota comprise all the species that lack an observable sexual cycle. Deuteromycota is not an accepted taxonomic clade, and is now taken to mean simply fungi that lack a known sexual stage.

2.4 *Aspergillus*

*Aspergillus*² is a genus consisting of a few hundred species of mold found in various climates worldwide. *Aspergillus* was first catalogued in 1729 by the Italian priest and biologist Pier Antonio Micheli. Viewing the fungi under a microscope, Micheli was reminded of the shape of an aspergillum (holy water sprinkler), from Latin spargere (to sprinkle), and named the genus accordingly. *Aspergillum* is an asexual spore-forming structure common to all *Aspergillus* species; around one-third of species are also known to have a sexual stage.

2.5 *Penicillium*

*Penicillium*³ ascomycetous fungi are of major importance in the natural environment as well as food and drug production. Some members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria. Other species are used in cheesemaking. The genus was first described in the scientific literature by Johann Heinrich Friedrich Link in his 1809 work *Observationes in ordines plantarum naturales*, writing “*Penicillium*. *Thallus e floccis caespitosis septatis simplicibus aut ramosis fertilibus erectis apice penicillatis*”, where *penicillatis* referred to “pencil-like” (referring to a Camel’s hair pencil brush).

2.6 *Rhizopus stolonifer*

*Rhizopus stolonifer*⁴ is commonly known as black bread mold. It is a member of Zygomycota and considered the most important species in the genus *Rhizopus*. It is one of the most common fungi in the world and has a global distribution although it is most commonly found in tropical and subtropical regions. It is a common agent of decomposition of stored foods. Like other members of the genus *Rhizopus*, *R. stolonifer* grows rapidly, mostly in indoor environments.

²<https://en.wikipedia.org/wiki/Aspergillus>

³<https://en.wikipedia.org/wiki/Penicillium>

⁴https://en.wikipedia.org/wiki/Rhizopus_stolonifer

2.7 Yeast

Yeasts⁵ are single-celled fungi. The first yeast originated hundreds of millions of years ago, and 1,500 species are currently identified. They are estimated to constitute 1% of all described fungal species. Yeasts are unicellular organisms which evolved from multicellular ancestors, with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudohyphae or false hyphae. Yeast sizes vary greatly, depending on species and environment, typically measuring 3–4 µm in diameter, although some yeasts can grow to 40 µm in size. Most yeasts reproduce asexually by mitosis, and many do so by the asymmetric division process known as budding.

Yeasts, with their single-celled growth habit, can be contrasted with molds, which grow hyphae. Fungal species that can take both forms (depending on temperature or other conditions) are called dimorphic fungi (“dimorphic” means “having two forms”).

By fermentation, the yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohols – for thousands of years the carbon dioxide has been used in baking and the alcohol in alcoholic beverages. It is also a centrally important model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms. Researchers have used it to gather information about the biology of the eukaryotic cell and ultimately human biology. Other species of yeasts, such as *Candida albicans*, are opportunistic pathogens and can cause infections in humans.

Yeasts do not form a single taxonomic or phylogenetic grouping. The term “yeast” is often taken as a synonym for *Saccharomyces cerevisiae* (baker’s or brewer’s yeast) but the phylogenetic diversity of yeasts is shown by their placement in two separate phyla: the Ascomycota and the Basidiomycota. The budding yeasts (“true yeasts”) are classified in the order Saccharomycetales, within the phylum Ascomycota.

2.8 View Prepared Slides of Fungi Lacking Sexual Stages

1. *Aspergillus* conidiophores (Figure 2.1)

⁵<https://en.wikipedia.org/wiki/Yeast>

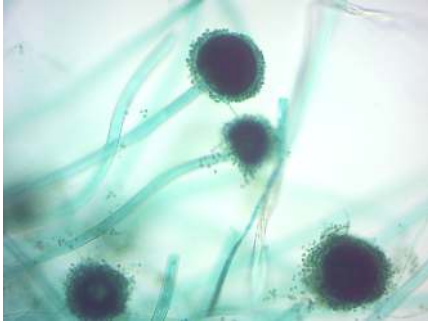


Figure 2.1: *Aspergillus* conidiophores.

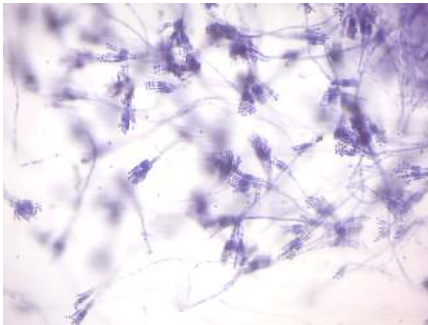


Figure 2.2: *Penicillium* conidiophores.

- Locate: conidiophores and conidiospores
2. *Penicillium* (Figure 2.2)
 - Locate: conidiophores and conidiospores (asexual spores)
 3. *Penicillium* on orange peel (Figure 2.3)

Sexual reproduction with meiosis has been directly observed in all fungal phyla except Glomeromycota (genetic analysis suggests meiosis in Glomeromycota as well). It differs in many aspects from sexual reproduction in animals or plants. Differences also exist between fungal groups and can be used to discriminate species by morphological differences in sexual structures and reproductive

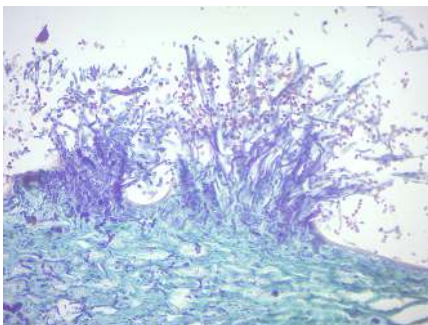


Figure 2.3: *Penicillium* growing on orange peel.

strategies. The major fungal groupings have initially been delineated based on the morphology of their sexual structures and spores; for example, the spore-containing structures, asci (sacs) and basidia (clubs), can be used in the identification of ascomycetes (sac fungi) and basidiomycetes (club fungi), respectively. Some species may allow mating only between individuals of opposite mating type, whereas others can mate and sexually reproduce with any other individual or itself. Species of the former mating system are called heterothallic, and of the latter homothallic.

Most fungi have both a haploid and a diploid stage in their life cycles. In sexually reproducing fungi, compatible individuals may combine by fusing their hyphae together into an interconnected network; this process, anastomosis, is required for the initiation of the sexual cycle. Many ascomycetes and basidiomycetes go through a dikaryotic stage, in which the nuclei inherited from the two parents do not combine immediately after cell fusion, but remain separate in the hyphal cells.

In ascomycetes, dikaryotic hyphae of the hymenium (the spore-bearing tissue layer) form a characteristic hook at the hyphal septum. During cell division, formation of the hook ensures proper distribution of the newly divided nuclei into the apical and basal hyphal compartments. An ascus (plural asci) is then formed, in which karyogamy (nuclear fusion) occurs. Asci are embedded in an ascocarp, or fruiting body. Karyogamy in the asci is followed immediately by meiosis and the production of ascospores. After dispersal, the ascospores may germinate and form a new haploid mycelium.

2.9 View Prepared Slides of Ascomycetes

1. *Peziza*⁶ (Figure 2.4) apothecium
 - Locate: Hymenium layer, ascus with 8 ascospores, and infertile hyphae
2. Yeast (Figure 2.5)

⁶<https://en.wikipedia.org/wiki/Peziza>

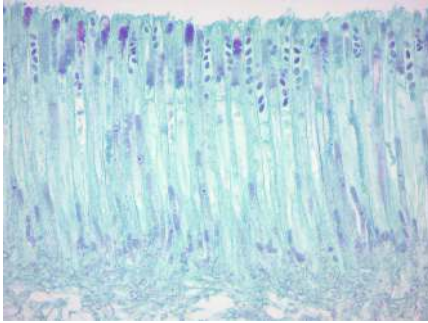
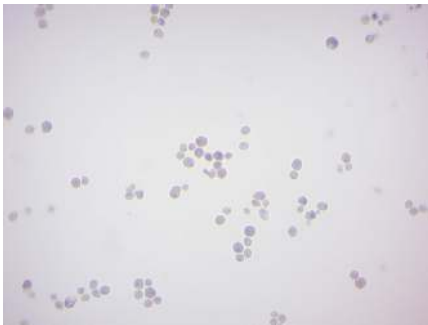
Figure 2.4: *Peziza* apothecium.Figure 2.6: *Coprinus* basidia with spores.

Figure 2.5: Yeast.

Figure 2.7: *Rhizopus* sporangia.

2.10 View Prepared Slides of Basidiomycetes

Sexual reproduction in basidiomycetes is similar to that of the ascomycetes. Compatible haploid hyphae fuse to produce a dikaryotic mycelium. However, the dikaryotic phase is more extensive in the basidiomycetes, often also present in the vegetatively growing mycelium. A specialized anatomical structure, called a clamp connection, is formed at each hyphal septum. As with the structurally similar hook in the ascomycetes, the clamp connection in the basidiomycetes is required for controlled transfer of nuclei during cell division, to maintain the dikaryotic stage with two genetically different nuclei in each hyphal compartment. A basidiocarp is formed in which club-like structures known as basidia generate haploid basidiospores after karyogamy and meiosis. The most commonly known basidiocarps are mushrooms, but they may also take other forms.

1. *Coprinus*⁷ (Figure 2.6)

- Locate: gills, basidium with 4 basidiospores, hyphae

⁷<https://en.wikipedia.org/wiki/Coprinus>

2.11 View Prepared Slides of Glomeromycetes

In glomeromycetes (formerly zygomycetes), haploid hyphae of two individuals fuse, forming a gametangium, a specialized cell structure that becomes a fertile gamete-producing cell. The gametangium develops into a zygospore, a thick-walled spore formed by the union of gametes. When the zygospore germinates, it undergoes meiosis, generating new haploid hyphae, which may then form asexual sporangiospores. These sporangiospores allow the fungus to rapidly disperse and germinate into new genetically identical haploid fungal mycelia.

1. *Rhizopus* sporangia (Figure 2.7)

- Locate: sporangium with spores, sporangiophore, and rhizoid and stolon, if possible

2. *Rhizopus* conjugation (Figure 2.8)

- Locate: gametangia (isogametes), zygospores, and various kinds of hyphae

3. *Rhizopus* combination (sporangia and zygotes)

- Locate: sporangium with spores, zygospores, gametangia, and hyphae

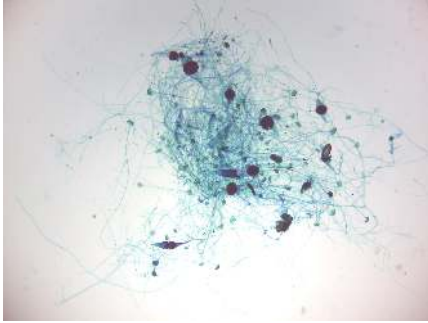


Figure 2.8: *Rhizopus* conjugation.

2.12 Lichen

A lichen⁸ (Figure 2.9) is a composite organism that arises from algae or cyanobacteria living among filaments of multiple fungi in a symbiotic relationship. The combined lichen has properties different from those of its component organisms. Lichens come in many colors, sizes, and forms. The properties are sometimes plant-like, but lichens are not plants. Lichens may have tiny, leafless branches (fruticose), flat leaf-like structures (foliose), flakes that lie on the surface like peeling paint (crustose), or other growth forms. Lichens occur from sea level to high alpine elevations, in many environmental conditions, and can grow on almost any surface. Lichens are abundant growing on bark, leaves, mosses, on other lichens, and hanging from branches “living on thin air” (epiphytes) in rain forests and in temperate woodland. They grow on rock, walls, gravestones, roofs, exposed soil surfaces, and in the soil as part of a biological soil crust. Different kinds of lichens have adapted to survive in some of the most extreme environments on Earth: arctic tundra, hot dry deserts, rocky coasts, and toxic slag heaps. They can even live inside solid rock, growing between the grains. It is estimated that 6% of Earth’s land surface is covered by lichen. There are about 20,000 known species of lichens. Lichens may be long-lived, with some considered to be among the oldest living things. They are among the first living things to grow on fresh rock exposed after an event such as a landslide. The long life-span and slow and regular growth rate of some lichens can be used to date events.

⁸<https://en.wikipedia.org/wiki/Lichen>

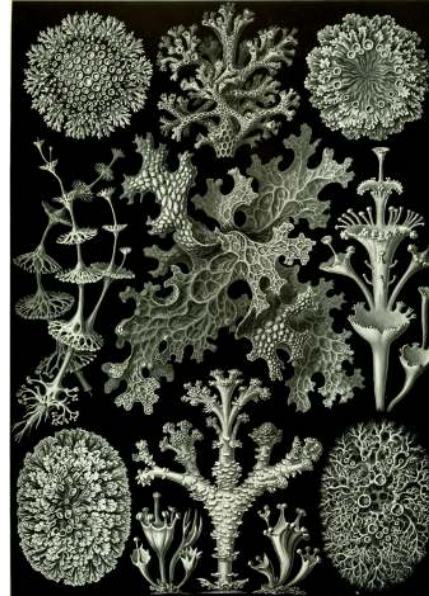


Figure 2.9: Lichens⁹ from Ernst Haeckel’s *Kunstformen der Natur*¹⁰, 1904.

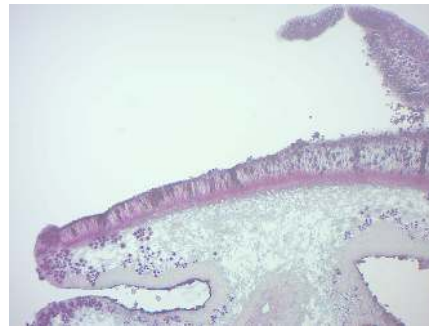


Figure 2.10: Foliose lichen thallus and apothecia.

2.13 View Prepared Slides of Lichens

1. Foliose lichen thallus and apothecia (Figure 2.10)
 - Locate: fungal hyphae, and algal cells inside

2.14 View Living Organisms

1. Yeast
2. *Rhizopus stolonifer* plate
3. *Aspergillus* plate
4. *Penicillium* plate

2.15 Review Questions

1. What are fungi?
2. How do fungi get their nutrients?
3. What are yeasts?
4. What are lichen?
5. What is the name of the spore containing structure in sac fungi?
6. In club fungi, spores are attached to _____.
7. What is a zygospore?
8. What are conidiophores?

Chapter 3

Non-vascular Plants and Plants Without Seeds

Plants¹ are multicellular, photoautotrophic eukaryotes. The term Viridiplantae (Latin for “green plants”) includes the flowering plants, conifers and other gymnosperms, ferns, clubmosses, hornworts, liverworts, mosses and the green algae, and excludes the red and brown algae. Historically, plants formed one of two kingdoms covering all living things that were not animals, and both algae and fungi were treated as plants; however, all current definitions of “plant” exclude the fungi and some algae, as well as the prokaryotes (the archaea and bacteria).

Green plants have cell walls containing cellulose and obtain most of their energy from sunlight via photosynthesis by primary chloroplasts, derived from endosymbiosis with cyanobacteria. Their chloroplasts contain chlorophylls a and b, which gives them their green color. Some plants are parasitic and have lost the ability to produce normal amounts of chlorophyll or to photosynthesize. Plants are characterized by sexual reproduction and alternation of generations, although asexual reproduction is also common.

There are over 300,000 species of plants, of which the great majority, over 260,000, are seed plants. Green plants provide a substantial proportion of the world’s molecular oxygen and are the basis of most of Earth’s ecologies, especially on land. Plants that produce grains, fruits and vegetables form humankind’s basic foodstuffs, and have been domesticated for millennia. Plants play many roles in culture. They are used as ornaments and, until recently and in great variety, they have served as the source of most medicines and drugs. The scientific study of plants is known as botany, a branch of biology.

The evolution of plants has resulted in increas-

ing levels of complexity, from the earliest algal mats, through bryophytes, lycopods, ferns to the complex gymnosperms and angiosperms of today.

3.1 Embryophytes

The plants that are likely most familiar to us are the multicellular land plants, called embryophytes. Embryophytes include the vascular plants, such as ferns, conifers and flowering plants. They also include the bryophytes, of which mosses and liverworts are the most common. All of these plants have eukaryotic cells with cell walls composed of cellulose, and most obtain their energy through photosynthesis, using light, water and carbon dioxide to synthesize food. A few plant species do not photosynthesize but are parasites on other species of photosynthetic plants. Embryophytes are believed to have evolved from green algae.

3.2 Non-vascular plants

Non-vascular plants are plants without a vascular system consisting of xylem and phloem. Although non-vascular plants lack these particular tissues, many possess simpler tissues that are specialized for internal transport of water. Non-vascular plants do not have a wide variety of specialized tissue types. Mosses and leafy liverworts have structures that look like leaves but are not true leaves because they are single sheets of cells with no stomata, no internal air spaces and have no xylem or phloem.

¹<https://en.wikipedia.org/wiki/Plant>

3.3 Bryophytes

Bryophytes² are an informal group consisting of three divisions of non-vascular land plants (embryophytes), the liverworts, hornworts and mosses. They are characteristically limited in size and prefer moist habitats although they can survive in drier environments. The bryophytes consist of about 20,000 plant species. Bryophytes produce enclosed reproductive structures (gametangia and sporangia), but they do not produce flowers or seeds. They reproduce via spores. The term “bryophyte” comes from bryon “tree-moss, oyster-green” and phyton “plant”.

The defining features of bryophytes are:

- Their life cycles are dominated by the gametophyte stage.
- Their sporophytes are unbranched.
- They do not have a true vascular tissue containing lignin (although some have specialized tissues for the transport of water).

Bryophytes first appeared during the early Paleozoic. They can only survive where moisture is available for significant periods, although some species are desiccation-tolerant. Most species of bryophytes remain small throughout their life-cycle. This involves an alternation between two generations: a haploid stage, called the gametophyte, and a diploid stage, called the sporophyte. In bryophytes, the sporophyte is always unbranched and remains nutritionally dependent on its parent gametophyte. The bryophytes have the ability to secrete a cuticle on their outer surface, a waxy layer that confers resistant to desiccation. In the mosses and hornworts, a cuticle is usually only produced on the sporophyte. Stomata are not found in liverworts, but occur on the sporangia of mosses and hornworts, allowing gas exchange while controlling water loss.

3.4 Mosses

Mosses³ (Figure 3.1)⁴ are small flowerless plants that typically grow in dense green clumps or mats, often in damp or shady locations. The individual plants are usually composed of simple leaves that are generally only one cell thick, attached to a stem that may be branched or unbranched and has only a limited role



Figure 3.1: Mosses⁵ from Ernst Haeckel’s *Kunstformen der Natur*⁶, 1904.

in conducting water and nutrients. Although some species have conducting tissues, these are generally poorly developed and structurally different from similar tissue found in vascular plants. Mosses do not have seeds and after fertilization develop sporophytes with unbranched stalks topped with single capsules containing spores. They are typically 0.2–10 cm tall, though some species are much larger. There are approximately 12,000 species. Mosses are commonly confused with lichens, hornworts, and liverworts.

3.4.1 Life cycle

The moss life-cycle (Figure 3.2) starts with a haploid spore that germinates to produce a protonema (pl. protonemata), which is either a mass of thread-like filaments or thalloid (flat and thallus-like). Massed moss protonemata typically look like a thin green felt, and may grow on damp soil, tree bark, rocks, concrete, or almost any other reasonably stable surface. This is a transitory stage in the life of a moss, but from the protonema grows the gametophyte that is structurally differentiated into stems and leaves. A single mat of protonemata may develop several gametophore shoots, resulting in a clump of moss. From the tips of the gametophyte stems or branches develop the sex organs of the mosses. The female organs are known as archegonia (sing. archegonium) and are protected by a group of modified leaves known as the perichaetum (plural, perichaeta). The archego-

²<https://en.wikipedia.org/wiki/Bryophyte>

³<https://en.wikipedia.org/wiki/Moss>

⁴https://commons.wikimedia.org/wiki/File:Haeckel_Muscinae.jpg

nia are small flask-shaped clumps of cells with an open neck (venter) down which the male sperm swim. The male organs are known as antheridia (sing. antheridium) and are enclosed by modified leaves called the perigonium (pl. perigonia). The surrounding leaves in some mosses form a splash cup, allowing the sperm contained in the cup to be splashed to neighboring stalks by falling water droplets. In the presence of water, sperm from the antheridia swim to the archegonia and fertilization occurs, leading to the production of a diploid sporophyte. The sperm of mosses is biflagellate, i.e. they have two flagellae that aid in propulsion. Since the sperm must swim to the archegonium, fertilization cannot occur without water. Some species (for example *Mnium hornum* or several species of *Polytrichum*) keep their antheridia in so called 'splash cups', bowl-like structures on the shoot tips that propel the sperm several decimeters when water droplets hit it, increasing the fertilization distance. After fertilization, the immature sporophyte pushes its way out of the archegonial venter. It takes about a quarter to half a year for the sporophyte to mature. The sporophyte body comprises a long stalk, called a seta, and a capsule capped by a cap called the operculum. The capsule and operculum are in turn sheathed by a haploid calyptra which is the remains of the archegonial venter. The calyptra usually falls off when the capsule is mature. Within the capsule, spore-producing cells undergo meiosis to form haploid spores, upon which the cycle can start again. The mouth of the capsule is usually ringed by a set of teeth called peristome. Most mosses rely on the wind to disperse the spores.

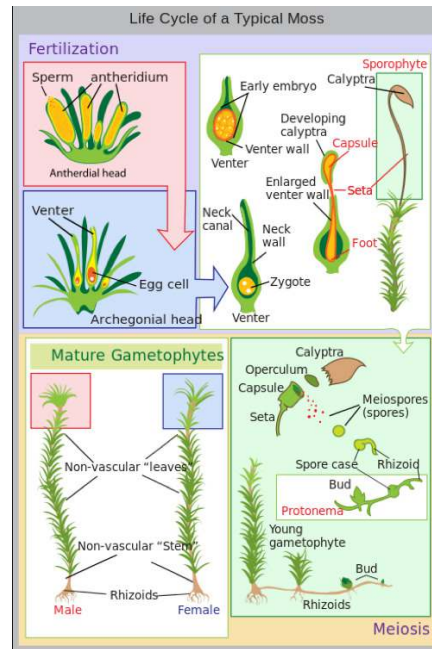


Figure 3.2: Life cycle of mosses.⁷



Figure 3.3: Moss archegonium.

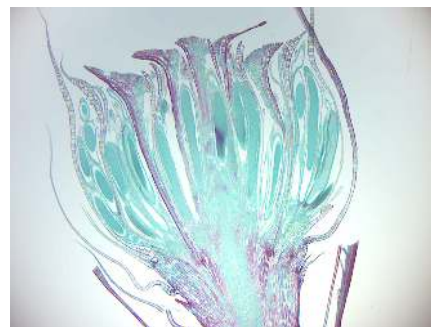


Figure 3.4: Moss antheridium.

3.5 View Prepared Slides of Mosses

1. Moss archegonium (Figure 3.3)
 - Identify: female gametophyte tissue; archegonium with egg inside the venter
2. Moss antheridium (Figure 3.4)
 - Identify: male gametophyte tissue; antheridia with sperms inside; paraphyses (sterile filaments)
3. Moss mature capsule (Figure 3.5)
 - Identify: capsule; spores; operculum (cap); seta
4. Moss protonema with bulbs w.m.
 - Identify: protonema filaments; gametophyte bulbs



Figure 3.5: Moss mature capsule.

3.6 Liverworts

The Marchantiophyta⁸ (Figure 3.6⁹) are a division of non-vascular land plants commonly referred to as hepatics or liverworts. Like mosses and hornworts, they have a gametophyte-dominant life cycle, in which cells of the plant carry only a single set of genetic information. It is estimated that there are about 9000 species of liverworts. Some of the more familiar species grow as a flattened leafless thallus, but most species are leafy with a form very much like a flattened moss. Liverworts are typically small, usually from 2–20 mm wide with individual plants less than 10 cm long, and are therefore often overlooked. However, certain species may cover large patches of ground, rocks, trees or any other reasonably firm substrate on which they occur. They are distributed globally in almost every available habitat, most often in humid locations although there are desert and Arctic species as well. Some species can be a nuisance in shady greenhouses or a weed in gardens.

3.6.1 Life cycle

The life of a liverwort starts from the germination of a haploid spore to produce a protonema, which is either a mass of thread-like filaments or else a flattened thallus. The protonema is a transitory stage in the life of a liverwort, from which will grow the mature gametophyte plant that produces the sex organs. The male organs are known as antheridia (singular: antheridium) and produce the sperm cells. Clusters of antheridia are enclosed by a protective layer of cells called the perigonia (plural: perigonia). As in other



Figure 3.6: Liverworts¹⁰ from Ernst Haeckel's *Kunstformen der Natur*¹¹, 1904.

land plants, the female organs are known as archegonia (singular: archegonium) and are protected by the thin surrounding perichaetum (plural: perichaeta). Each archegonium has a slender hollow tube, the “neck”, down which the sperm swim to reach the egg cell. Liverwort species may be either dioecious or monoecious. In dioecious liverworts, female and male sex organs are borne on different and separate gametophyte plants. In monoecious liverworts, the two kinds of reproductive structures are borne on different branches of the same plant. In either case, the sperm must move from the antheridia where they are produced to the archegonium where the eggs are held. The sperm of liverworts is biflagellate, i.e. they have two tail-like flagellae that enable them to swim short distances, provided that at least a thin film of water is present. Their journey may be assisted by the splashing of raindrops. When sperm reach the archegonia, fertilization occurs, leading to the production of a diploid sporophyte. After fertilization, the immature sporophyte within the archegonium develops three distinct regions: (1) a foot, which both anchors the sporophyte in place and receives nutrients from its “mother” plant, (2) a spherical or ellipsoidal capsule, inside which the spores will be produced for dispersing to new locations, and (3) a seta (stalk) which lies between the other two regions and connects them. When the sporophyte has developed all three regions, the seta elongates, pushing its way out of the archegonium and rupturing it. While the foot remains anchored within the parent plant, the

⁸<https://en.wikipedia.org/wiki/Marchantiophyta>

⁹https://commons.wikimedia.org/wiki/File:Haeckel_Hepaticae.jpg

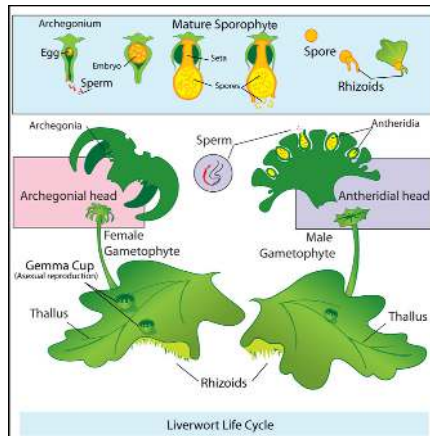


Figure 3.7: Life cycle of liverworts.¹²

capsule is forced out by the seta and is extended away from the plant and into the air. Within the capsule, cells divide to produce both elater cells and spore-producing cells. The elaters are spring-like, and will push open the wall of the capsule to scatter themselves when the capsule bursts. The spore-producing cells will undergo meiosis to form haploid spores to disperse, upon which point the life cycle can start again. Some liverworts are capable of asexual reproduction. Some thallose liverworts such as *Marchantia polymorpha* produce small disc-shaped gemmae in shallow cups. *Marchantia* gemmae can be dispersed up to 120 cm by rain splashing into the cups.

3.7 View Prepared Slides of Liverworts

1. *Marchantia* life history
2. *Marchantia* thallus (Figure 3.8)
 - Identify: pores, tissue of lamina, rhizoids
3. *Marchantia* archegonia (Figure 3.9)
 - Identify: archegonium with egg inside the venter; tissue of archegoniophore (female gametophyte)
4. *Marchantia* antheridia (Figure 3.10)
 - Identify: antheridia with sperms; tissue of antheridiophore (Male gametophyte); air chambers
5. *Marchantia* sporophyte (Figure 3.11)
 - Identify: sporophyte and its three parts: foot, seta (stalk), and capsule; spores and elaters inside the capsule; tissue of the female gametophyte
6. *Marchantia* gemma cup (Figure 3.12)
 - Identify: gemma cup and gemmae

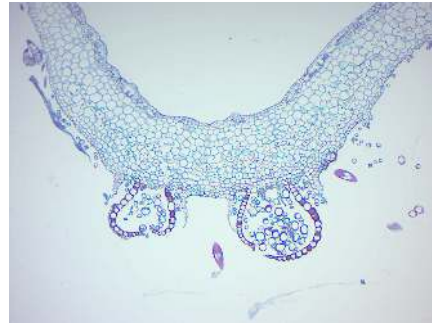


Figure 3.8: *Marchantia* thallus.

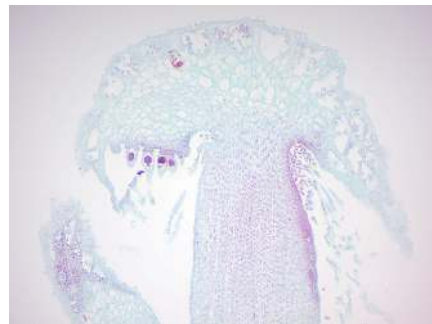


Figure 3.9: *Marchantia* archegonia.

3.8 Vascular plants

Vascular plants¹³ (from Latin vasculum: duct), also known as tracheophytes (from the equivalent Greek term trachea) and also higher plants, form a large group of plants (c. 308,312 accepted known species) that are defined as those land plants that have lignified tissues (the xylem) for conducting water and minerals throughout the plant. They also have a specialized non-lignified tissue (the phloem) to conduct products of photosynthesis. Vascular plants include the clubmosses, horsetails, ferns, gymnosperms (in-

¹³https://en.wikipedia.org/wiki/Vascular_plant

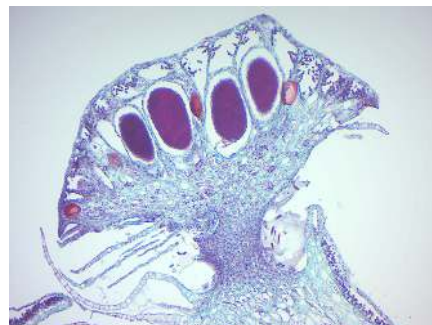


Figure 3.10: *Marchantia* antheridia.

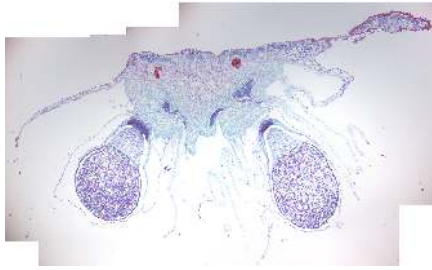


Figure 3.11: *Marchantia* sporophyte.



Figure 3.12: *Marchantia* gemma cup.

cluding conifers) and angiosperms (flowering plants). Scientific names for the group include Tracheophyta and Tracheobionta.

Vascular plants are distinguished by two primary characteristics:

1. Vascular plants have vascular tissues which distribute resources through the plant. This feature allows vascular plants to evolve to a larger size than non-vascular plants, which lack these specialized conducting tissues and are therefore restricted to relatively small sizes.
2. In vascular plants, the principal generation phase is the sporophyte, which is usually diploid with two sets of chromosomes per cell. Only the germ cells and gametophytes are haploid. By contrast, the principal generation phase in non-vascular plants is the gametophyte, which is haploid with one set of chromosomes per cell. In these plants, only the spore stalk and capsule are diploid.

Vascular plants first appeared during the Silurian period, and by the Devonian had diversified and spread into many different terrestrial environments. They developed a number of adaptations that allowed them to spread into increasingly more arid places, notably the vascular tissues xylem and phloem, that transport water and food throughout the organism. Root systems capable of obtaining soil water and nutrients also evolved during the Devonian. In modern vascular plants, the sporophyte is

typically large, branched, nutritionally independent and long-lived, but there is increasing evidence that Paleozoic gametophytes were just as complex as the sporophytes. The gametophytes of all vascular plant groups evolved to become reduced in size and prominence in the life cycle.

The first seed plants, pteridosperms (seed ferns), now extinct, appeared in the Devonian and diversified through the Carboniferous. In these the micro gametophyte is reduced to pollen and the mega gametophyte remains inside the megasporangium, attached to the parent plant. A megasporangium invested in protective layer called an integument is known as an ovule. After fertilization by means of sperm deposited by pollen grains, an embryo develops inside the ovule. The integument becomes a seed coat, and the ovule develops into a seed. Seed plants can survive and reproduce in extremely arid conditions, because they are not dependent on free water for the movement of sperm, or the development of free living gametophytes.

3.9 Pteridophyta

The pteridophytes¹⁴ are vascular plants (with xylem and phloem) that reproduce via spores, and include the ferns, horsetails, and the lycophytes (clubmosses, spike mosses, and quillworts). These are not a monophyletic group because ferns and horsetails are more closely related to seed plants than to the lycophytes. Therefore, “Pteridophyta” is now an invalid taxon, although the term pteridophyte remains in common use.

3.9.1 Life cycle

Ferns are vascular plants differing from lycophytes by having true leaves (megaphylls). They differ from seed plants (gymnosperms and angiosperms) in their mode of reproduction—lacking flowers and seeds. Like all land plants, they have a life cycle referred to as alternation of generations, characterized by alternating diploid sporophytic and haploid gametophytic phases. The diploid sporophyte has $2n$ paired chromosomes, where n varies from species to species. The haploid gametophyte has n unpaired chromosomes, i.e. half the number of the sporophyte. The gametophyte of ferns is a free-living organism,

¹⁴<https://en.wikipedia.org/wiki/Pteridophyte>



Figure 3.13: Ferns¹⁵ from Ernst Haeckel's *Kunstformen der Natur*¹⁶, 1904.

whereas the gametophyte of the gymnosperms and angiosperms is dependent on the sporophyte.

The life cycle of a typical fern proceeds as follows:

1. A diploid sporophyte phase produces haploid spores by meiosis.
2. A spore grows into a haploid gametophyte by mitosis. The gametophyte typically consists of a photosynthetic prothallus.
3. The gametophyte produces gametes (often both sperm and eggs on the same prothallus) by mitosis.
4. A mobile, flagellate sperm fertilizes an egg that remains attached to the prothallus.
5. The fertilized egg is now a diploid zygote and grows by mitosis into a diploid sporophyte (the typical “fern” plant).

3.10 Clubmosses

Lycopodiopsida¹⁷ is a class of herbaceous vascular plants known as the clubmosses and firmosses. They have dichotomously branching stems bearing simple leaves without ligules and reproduce by means of spores borne in sporangia at the bases of the leaves. Traditionally, the group also included the spikemosses (*Selaginella* and relatives) and the

¹⁷<https://en.wikipedia.org/wiki/Lycopodiopsida>



Figure 3.14: *Selaginella strobilus*.

quillworts (*Isoetes* and relatives) but because these groups have leaves with ligules and reproduce using spores of two different sizes both are now placed into another class, Isoetopsida that also includes the extinct *Lepidodendrales*. These groups, together with the horsetails are often referred to informally as fern allies.

3.11 Spikemosses

*Selaginella*¹⁸ is the sole genus of primitive vascular plants in the family Selaginellaceae, the spikemosses or lesser clubmosses. *Selaginella* occurs mostly in the tropical regions of the world, with a handful of species to be found in the arctic-alpine zones of both hemispheres.

3.12 View Prepared Slides of *Selaginella*

1. *Selaginella strobilus* (Figure 3.14)
 - Identify: micro- and megaspores

3.13 Horsetails

*Equisetum*¹⁹ (horsetail) is the only living genus in Equisetaceae, a family of vascular plants that reproduce by spores rather than seeds. *Equisetum* is a “living fossil” as it is the only living genus of the entire class

¹⁸<https://en.wikipedia.org/wiki/Selaginella>

¹⁹<https://en.wikipedia.org/wiki/Equisetum>

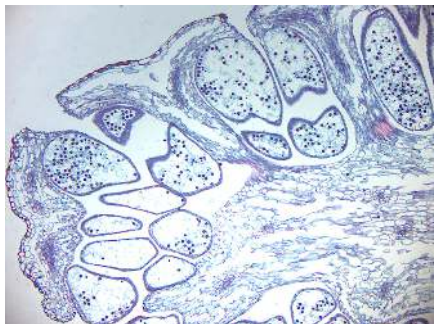


Figure 3.15: *Equisetum* strobilus.



Figure 3.16: *Psilotum* sporangium.

Equisetopsida, which for over one hundred million years was much more diverse and dominated the understory of late Paleozoic forests. Some Equisetopsida were large trees reaching to 30 meters tall.

3.14 View Prepared Slides of *Equisetum*

1. *Equisetum* strobilus (Figure 3.15)
 - Identify: sporangiphores and spores

3.15 Whisk ferns

*Psilotum*²⁰ is a genus of fern-like vascular plants, commonly known as whisk ferns. It is one of two genera in the family Psilotaceae, the other being Tmesipteris. They lack true roots and leaves, the stems being the organs containing conducting tissue.

3.16 View Prepared Slides of *Psilotum*

1. *Psilotum* sporangium (Figure 3.16)
 - Identify: Sporangium, spores.

3.17 View Prepared Slides of Ferns

1. Fern sporophyte (Figure 3.17)
2. Fern antheridia & archegonia (Figure 3.18)

- Identify: tissue of the gametophyte; antheridia; archegonia; rhizoids
3. Fern prothallus young sporophyte (Figure 3.19)
 - Identify: sporophyte, gametophyte; rhizoids

3.18 View Prepared Slides of *Cyrtomium*

*Cyrtomium*²¹ is a genus of about 15-20 species of ferns in the family Dryopteridaceae, native to Asia, Africa (including Madagascar), and the Pacific Ocean islands (Hawaii). *Cyrtomium falcatum* is a species of fern known by the common names house holly-fern and Japanese holly fern. It is native to eastern Asia. It grows from crevices, coastal cliffs, streambanks, rocky slopes, and other moist, stable areas.

1. *Cyrtomium falcatum* sorus on leaf
 - Locate sorus and identify the following: sporangia with spores inside; annular cells (annulus); lip cells; indusium (covering of the sorus).

3.19 View Living Organisms

1. Four types of Mosses
2. *Marchantia hepatica*
3. *Lycopodium lucidulum*

3.20 Review Questions

1. What are plants?
2. What are mosses?

²⁰<https://en.wikipedia.org/wiki/Psilotum>

²¹<https://en.wikipedia.org/wiki/Cyrtomium>

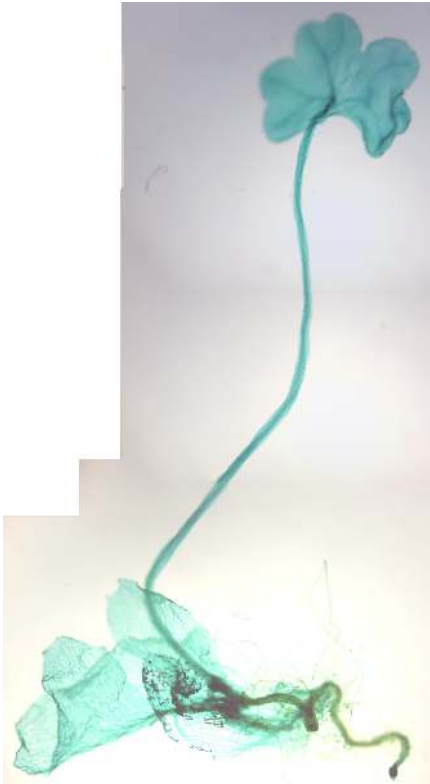


Figure 3.17: Fern sporophyte.



Figure 3.18: Fern antheridia and archegonia.

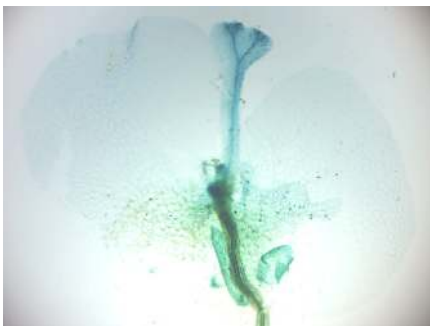


Figure 3.19: Fern prothallus young sporophyte.

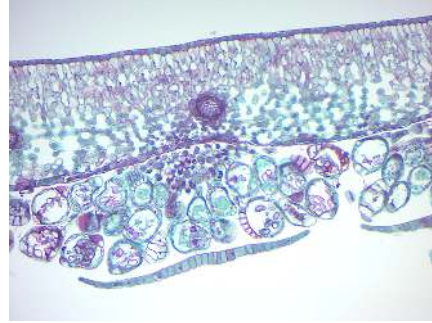


Figure 3.20: *Cyrtomium falcatum* sorus on leaf.

3. What are liverworts?
4. What are ferns?
5. What does alternation of generations mean in the life cycle of plants?
6. What is a gametophyte?
7. What is a sporophyte?

Chapter 4

Gymnosperms and Angiosperms

The gymnosperms and angiosperms together compose the spermatophytes or seed plants.

4.1 Gymnosperms

The gymnosperms¹ are a group of seed-producing plants (spermatophytes) that includes conifers (Pinophyta), cycads, Ginkgo, and gnetophytes. The term “gymnosperm” comes from the Greek composite word gymnos, “naked” and sperma, “seed”, meaning “naked seeds”. The name is based on the unenclosed condition of their seeds (called ovules in their unfertilized state). The non-encased condition of their seeds stands in contrast to the seeds and ovules of flowering plants (angiosperms), which are enclosed within an ovary. Gymnosperm seeds develop either on the surface of scales or leaves, which are often modified to form cones, or solitary as in Yew, Torreya, Ginkgo. By far the largest group of living gymnosperms are the conifers (pines, cypresses, and relatives), followed by cycads, gnetophytes (*Gnetum*, *Ephedra* and *Welwitschia*), and *Ginkgo biloba* (a single living species). Roots in some genera have fungal association with roots in the form of mycorrhiza (*Pinus*), while in some others (*Cycas*) small specialized roots called coralloid roots are associated with nitrogen-fixing cyanobacteria.

Gymnosperms, like all vascular plants, have a sporophyte-dominant life cycle, which means they spend most of their life cycle with diploid cells, while the gametophyte (gamete-bearing phase) is relatively short-lived. Two spore types, microspores and megaspores, are typically produced in pollen cones or ovulate cones, respectively. Gametophytes, as with all heterosporous plants, develop within the spore wall. Pollen grains (microgametophytes) mature

from microspores, and ultimately produce sperm cells. Megagametophytes develop from megaspores and are retained within the ovule. Gymnosperms produce multiple archegonia, which produce the female gamete. During pollination, pollen grains are physically transferred between plants from the pollen cone to the ovule. Pollen is usually moved by wind or insects. Whole grains enter each ovule through a microscopic gap in the ovule coat (integument) called the micropyle. The pollen grains mature further inside the ovule and produce sperm cells. Two main modes of fertilization are found in gymnosperms. Cycads and Ginkgo have motile sperm that swim directly to the egg inside the ovule, whereas conifers and gnetophytes have sperm with no flagella that are moved along a pollen tube to the egg. After syngamy (joining of the sperm and egg cell), the zygote develops into an embryo (young sporophyte). More than one embryo is usually initiated in each gymnosperm seed. The mature seed comprises the embryo and the remains of the female gametophyte, which serves as a food supply, and the seed coat.

4.2 View Prepared Slides of Gymnosperms

1. *Zamia* young ovule (Figure 4.1)
2. Pine ovule (Figure 4.2)
 - Identify: female gametophyte, egg, archegonia, micropyle
3. Pine young ovulate cone (Figure 4.3)
 - Identify: ovules, megasporophylls (scales)
4. Pine staminate cone (Figure 4.4)
 - Identify: microsporophyll, microsporangium, pollen grains (microspores). In pollen grains, differentiate between the cells and the “wings”
5. Pine pollen (Figure 4.5)

¹<https://en.wikipedia.org/wiki/Gymnosperm>

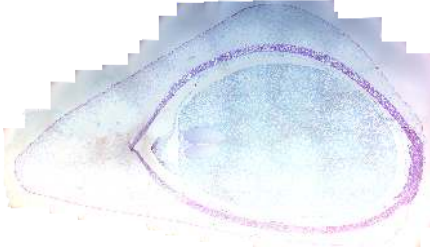


Figure 4.1: Young *Zamia* ovule.

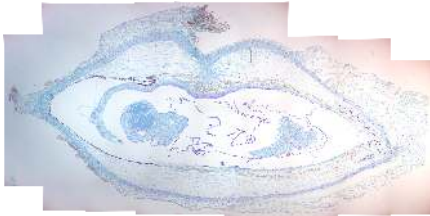


Figure 4.2: Pine ovule

- Identify: generative cell with nucleus, tube cell with nucleus, “wings”
- 6. Pine - mature embryo (Figure 4.6)
- 7. Pine needle (Figure 4.7)
 - Identify: epidermis, stomata with guard cells, hypodermis, mesophyll, resin canals, endodermis, xylem, and phloem

4.3 Angiosperms

The flowering plants, also known as angiosperms, Angiospermae or Magnoliophyta, are the most diverse group of land plants, with 416 families, approximately 13,164 known genera and c. 295,383 known species. Like gymnosperms, angiosperms are seed-producing plants. However, they are distinguished from gymnosperms by characteristics including flowers, endosperm within the seeds, and

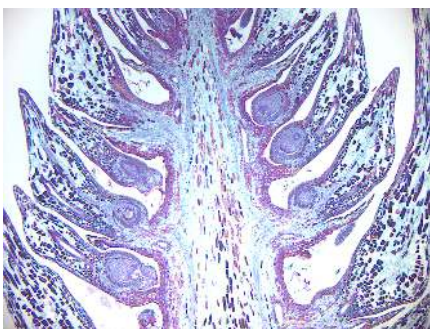


Figure 4.3: Pine young ovulate cone.

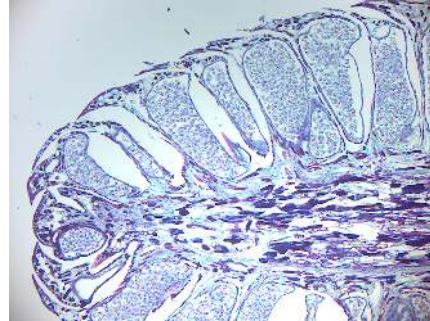


Figure 4.4: Pine staminate cone.

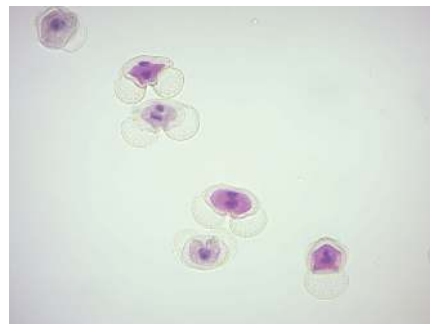


Figure 4.5: Pine pollen.

the production of fruits that contain the seeds. Etymologically, angiosperm means a plant that produces seeds within an enclosure; in other words, a fruiting plant. The term comes from the Greek words angeion (“case” or “casing”) and sperma (“seed”). The ancestors of flowering plants diverged from gymnosperms in the Triassic Period, 245 to 202 million years ago (mya), and the first flowering plants are known from 160 mya. They diversified extensively during the Lower Cretaceous, became widespread by 120 mya, and replaced conifers as the dominant trees from 100 to 60 mya.

The characteristic feature of angiosperms is the flower. Flowers show remarkable variation in form and elaboration, and provide the most trustworthy external characteristics for establishing relationships among angiosperm species. The function of the flower is to ensure fertilization of the ovule and development of fruit containing seeds. The floral apparatus may arise terminally on a shoot or from the axil of a leaf (where the petiole attaches to the stem). Occasionally, as in violets, a flower arises singly in the axil of an ordinary foliage-leaf. More typically, the flower-bearing portion of the plant is sharply distinguished from the foliage-bearing or vegetative portion, and forms a more or less elaborate branch-system called an inflorescence.



Figure 4.6: Pine embryo.

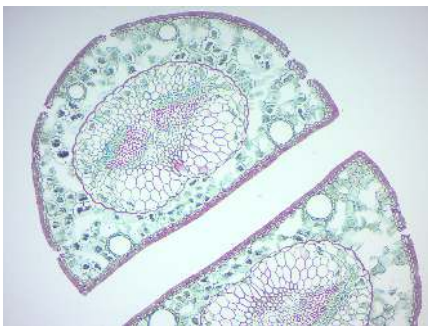


Figure 4.7: Pine needle.

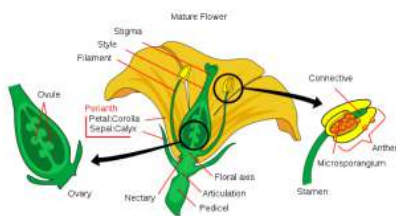


Figure 4.8: Anatomy of the flower.²

There are two kinds of reproductive cells produced by flowers. Microspores, which will divide to become pollen grains, are the “male” cells and are borne in the stamens (or microsporophylls). The “female” cells called megaspores, which will divide to become the egg cell (megagametogenesis), are contained in the ovule and enclosed in the carpel (or megasporophyll).

The flower may consist only of these parts, as in willow, where each flower comprises only a few stamens or two carpels. Usually, other structures are present and serve to protect the sporophylls and to form an envelope attractive to pollinators. The individual members of these surrounding structures are known as sepals and petals (or tepals in flowers such as *Lilium* where sepals and petals are not distinguishable from each other). The outer series (calyx of sepals) is usually green and leaf-like, and functions to protect the rest of the flower, especially the bud. The inner series (corolla of petals) is, in general, white or brightly colored, and is more delicate in structure. It functions to attract insect or bird pollinators. Attraction is effected by color, scent, and nectar, which may be secreted in some part of the flower. The characteristics that attract pollinators account for the popularity of flowers and flowering plants among humans.

While the majority of flowers are perfect or hermaphrodite (having both pollen and ovule producing parts in the same flower structure), flowering plants have developed numerous morphological and physiological mechanisms to reduce or prevent self-fertilization. Heteromorphic flowers have short carpels and long stamens, or vice versa, so animal pollinators cannot easily transfer pollen to the pistil (receptive part of the carpel). Homomorphic flowers may employ a biochemical (physiological) mechanism called self-incompatibility to discriminate between self and non-self pollen grains. In other species, the male and female parts are morphologically separated, developing on different flowers.

4.3.1 Sexual Reproduction

Double fertilization refers to a process in which two sperm cells fertilize cells in the ovary. This process begins when a pollen grain adheres to the stigma of the pistil (female reproductive structure), germinates, and grows a long pollen tube. While this pollen tube is growing, a haploid generative cell travels down the tube behind the tube nucleus. The generative cell divides by mitosis to produce two haploid (n) sperm cells. As the pollen tube grows, it makes its way from

the stigma, down the style and into the ovary. Here the pollen tube reaches the micropyle of the ovule and digests its way into one of the synergids, releasing its contents (which include the sperm cells). The synergid that the cells were released into degenerates and one sperm makes its way to fertilize the egg cell, producing a diploid ($2n$) zygote. The second sperm cell fuses with both central cell nuclei, producing a triploid ($3n$) cell. As the zygote develops into an embryo, the triploid cell develops into the endosperm, which serves as the embryo's food supply. The ovary will now develop into a fruit and the ovule will develop into a seed.

As the development of embryo and endosperm proceeds within the embryo sac, the sac wall enlarges and combines with the nucellus (which is likewise enlarging) and the integument to form the seed coat. The ovary wall develops to form the fruit or pericarp, whose form is closely associated with type of seed dispersal system.

Frequently, the influence of fertilization is felt beyond the ovary, and other parts of the flower take part in the formation of the fruit, e.g., the floral receptacle in the apple, strawberry, and others.

The character of the seed coat bears a definite relation to that of the fruit. They protect the embryo and aid in dissemination; they may also directly promote germination. Among plants with indehiscent fruits, in general, the fruit provides protection for the embryo and secures dissemination. In this case, the seed coat is only slightly developed. If the fruit is dehiscent and the seed is exposed, in general, the seed-coat is well developed, and must discharge the functions otherwise executed by the fruit.

Flowering plants generate gametes using meiosis. Meiosis takes place in the ovule (a structure within the ovary that is located within the pistil at the center of the flower). A diploid cell (megaspore mother cell) in the ovule undergoes meiosis (involving two successive cell divisions) to produce four cells (megaspores or female gametes) with haploid nuclei. One of these four cells (megaspore) then undergoes three successive mitotic divisions to produce an immature embryo sac (megagametocyte) with eight haploid nuclei. Next, these nuclei are segregated into separate cells by cytokinesis to producing 3 antipodal cells, 2 synergid cells and an egg cell. Two polar nuclei are left in the central cell of the embryo sac.

Pollen is also produced by meiosis in the male anther (microsporangium). During meiosis, a diploid microspore mother cell undergoes two successive mei-

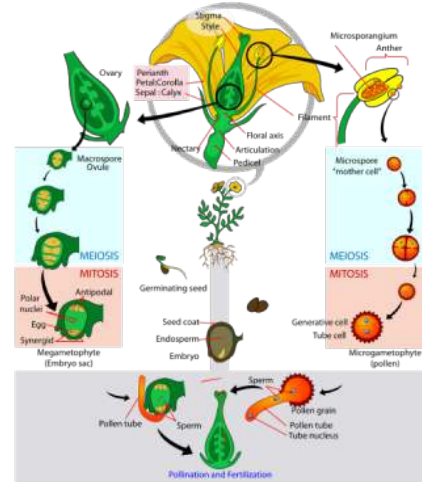


Figure 4.9: Life cycle of angiosperms.³

otic divisions to produce 4 haploid cells (microspores or male gametes). Each of these microspores, after further mitoses, becomes a pollen grain (microgametophyte) containing two haploid generative (sperm) cells and a tube nucleus. When a pollen grain makes contact with the female stigma, the pollen grain forms a pollen tube that grows down the style into the ovary. In the act of fertilization, a male sperm nucleus fuses with the female egg nucleus to form a diploid zygote that can then develop into an embryo within the newly forming seed. Upon germination of the seed, a new plant can grow and mature.

4.4 View Prepared Slides of Angiosperms

1. Lily anther (Figure 4.10)
 - Identify: anther, microsporangium, pollen grains with tube and generative nuclei. The structure in the middle of the slide is the Lily ovary. The anthers are around the ovary.
2. Lily anther with mature pollen (Figure 4.11)
 - Identify: pollen grain, tube cell with nucleus, generative cell with nucleus
3. Lily pollen tubes (Figure 4.12)
 - Identify: stigma tissue, pollen tubes
4. Lily ovary (Figure 4.13)
 - Identify: ovary, ovules, female gametophytes (embryo sac). If this slides is not available, you can observe the lily ovary in the "Lily anther x.s." slide.
5. *Tilia* 2 year old stem (Figure 4.14)

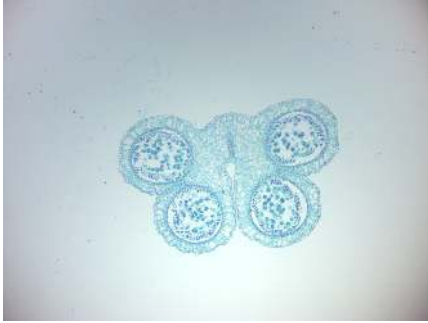


Figure 4.10: Lily anther.

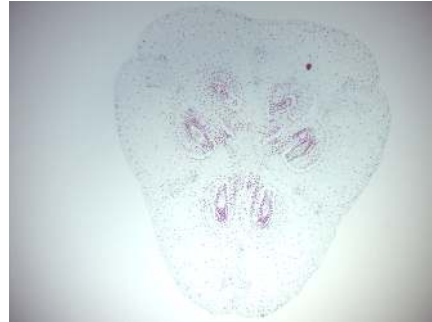


Figure 4.13: Lily ovary.

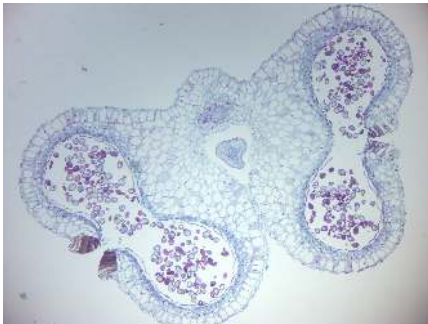


Figure 4.11: Lily anther with mature pollen.

6. *Capsella* seeds (Figure 4.15)

- Identify: embryo, cotyledons, root tip, shoot tip



Figure 4.14: *Tilia* 2 year old stem

4.5 Monocotyledons and Dicotyledons

Monocotyledons, commonly referred to as monocots are flowering plants (angiosperms) whose seeds typically contain only one embryonic leaf, or cotyledon. They constitute one of the major groups into which the flowering plants have traditionally been divided,

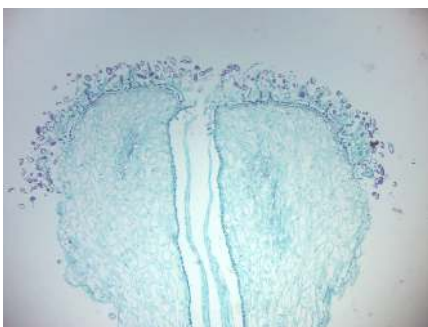


Figure 4.12: Lily pollen tubes.



Figure 4.15: *Capsella* seeds.

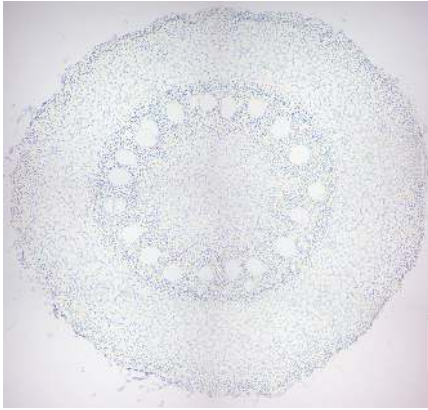


Figure 4.16: Monocot root.

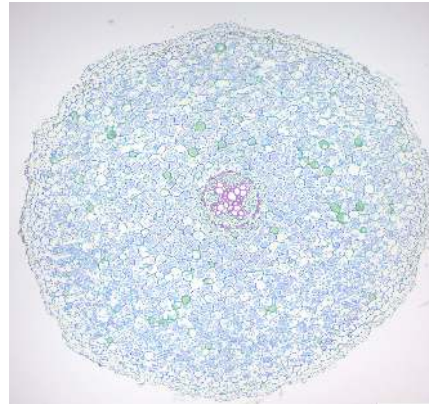


Figure 4.17: Dicot root.

the rest of the flowering plants having two cotyledons and therefore classified as dicotyledons, or dicots. However, molecular phylogenetic research has shown that while the monocots form a monophyletic group or clade (comprising all the descendants of a common ancestor), the dicots do not.

The eudicots, eudicotyledons are a monophyletic clade of flowering plants. The term means “true dicotyledons”, as it contains the majority of plants that have been considered dicots and have characteristics of the dicots. The term “eudicots” has subsequently been widely adopted in botany to refer to one of the two largest clades of angiosperms (constituting over 70% of the angiosperm species), monocots being the other.

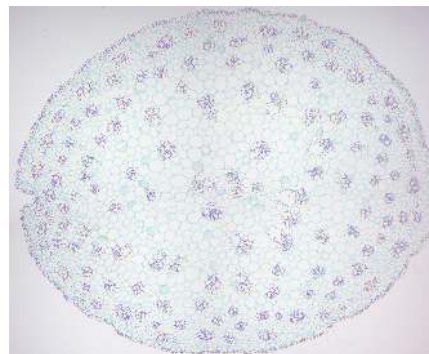


Figure 4.18: Monocot stem.

4.6 View Prepared Slides of Monocots And Eudicots

1. Monocot and dicot roots (Figures 4.16 and 4.17)
 - Identify monocot and eudicot root.
2. Monocot and dicot stems (Figures 4.18 and 4.19)
 - Identify monocot and eudicot stem.
3. Monocot and dicot leaves (Figures 4.20 and 4.21)
 - Identify monocot and eudicot leaf.
4. Monocot and dicot flower buds (Figures 4.22 and 4.23)
 - Identify monocot and eudicot flower.

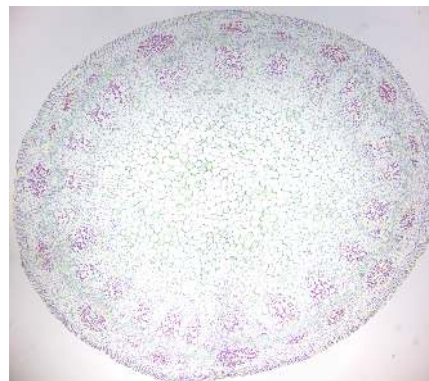


Figure 4.19: Dicot stem

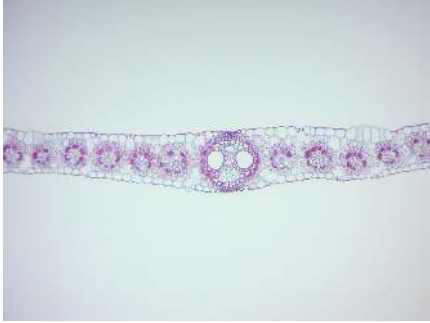


Figure 4.20: Monocot leaf.

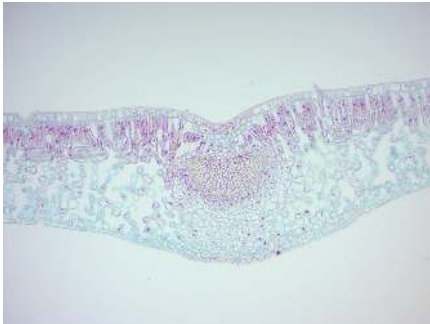


Figure 4.21: Dicot leaf.



Figure 4.22: Monocot flower bud.

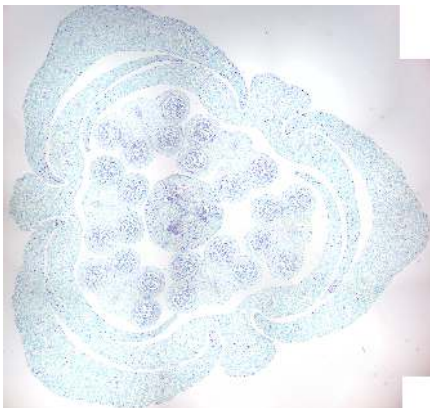


Figure 4.23: Dicot flower bud.

Table 4.1: Structural differences between monocots and dicots.

Feature	In monocots	In dicots
Leaves	Leaf shape oblong or linear, often sheathed at base, petiole seldom developed, stipules absent. Major leaf veins usually parallel.	Broad, seldom sheathed, petiole common often with stipules. Veins usually reticulate (pinnate or palmate).
Roots	Primary root of short duration, replaced by adventitial roots forming fibrous or fleshy root systems.	Develops from the radicle. Primary root often persists forming strong taproot and secondary roots.
Plant stem: Vascular bundles	Numerous scattered bundles in ground parenchyma, cambium rarely present, no differentiation between cortical and stelar regions.	Ring of primary bundles with cambium, differentiated into cortex and stele (eustelic).
Flowers	Parts in threes or multiples of three (e.g. 3, 6 or 9 petals)	Parts in fours or fives.

4.7 *Lilium*

Lilium is a genus of herbaceous flowering plants growing from bulbs, all with large prominent flowers. Lilies are a group of flowering plants which are important in culture and literature in much of the world. Most species are native to the temperate northern hemisphere, though their range extends into



Figure 4.24: Lilies

the northern subtropics. Lilies are tall perennials ranging in height from 2–6 ft.

The flowers are large, often fragrant, and come in a wide range of colors including whites, yellows, oranges, pinks, reds and purples. Markings include spots and brush strokes. The plants are late spring- or summer-flowering. Flowers are at the tip of the stem, with six tepals (sepals and petals are not distinct). The tepals are free from each other, and bear a nectary at the base of each flower. The ovary is ‘superior’, borne above the point of attachment of the anthers. The fruit is a three-celled capsule. Seeds ripen in late summer. They exhibit varying and sometimes complex germination patterns, many adapted to cool temperate climates.

4.8 Dissection of Fresh Lilies

1. The outer ring of the flower consists of sepals, and the inner ring of petals. In lilies they look nearly identical. In many other flowers, the sepals are green and the petals are colorful.
2. Peel off first the sepals and then the petals.
3. Count the number of sepals and petals. Do you notice any (orange colored) pollen on any of them?
4. After you have peeled away the sepals and petals, you can clearly see the stamens (the “male” parts of the flower). The stamens mostly consist of anthers (the long, elliptical

and brown heads on top of the filaments (the supporting stalks). Some anthers may have split open to expose the pollen grains inside.

5. Cut off the stamens and examine the anther and pollen using the stereo dissection microscope.
6. The long stalk remaining in the center of the flower is the pistil (the “female” parts) with the stigma at its top end. If the flowers are fresh, the stigma will be sticky for catching pollen. You can cut it open along the path down the style (the stalky part), to the ovule.
7. At the base of the style is the ovary. It contains the ovules that will develop into seeds if they are fertilized by sperm from the pollen.
8. Cut the ovary in half to see the ovules.
9. Examine the cut ovary using the stereo dissection microscope.
10. At the base of the ovary is the main nectary. Cut it open to see the xylem (the water conducting vessels of vascular plants) and phloem (the sugar conducting vessels).

4.9 Review Questions

1. What are gymnosperms?
2. What are angiosperms?
3. What are pollen?
4. What are cotyledons?
5. What is xylem?
6. What is phloem?

Chapter 5

Porifera, Cnidaria, Ctenophora

5.1 Animals

Animals¹ are eukaryotic, multicellular organisms that form the biological kingdom Animalia. With few exceptions, animals are motile (able to move), heterotrophic (consume organic material), reproduce sexually, and their embryonic development includes a blastula stage. The body plan of the animal derives from this blastula, differentiating specialized tissues and organs as it develops; this plan eventually becomes fixed, although some undergo metamorphosis at some stage in their lives.

Zoology is the study of animals. Currently there are over 66,000 (less than 5% of all animals) vertebrate species, and over 1.3 million (over 95% of all animals) invertebrate species in existence. Classification of animals into groups (taxonomy) is accomplished using either the hierarchical Linnaean system; or cladistics, which displays diagrams (phylogenetic trees) called cladograms to show relationships based on the evolutionary principle of the most recent common ancestor. Some recent classifications based on modern cladistics have explicitly abandoned the term “kingdom”, noting that the traditional kingdoms are not monophyletic, i.e., do not consist of all the descendants of a common ancestor.

Animals are divided by body plan into vertebrates and invertebrates. Vertebrates—fishes, amphibians, reptiles, birds, and mammals—have a vertebral column (spine); invertebrates do not. All vertebrates and most invertebrates are bilaterally symmetrical (Bilateria). Invertebrates include arthropods, molluscs, roundworms, ringed worms, flatworms, and other phyla in Ecdysozoa and Spiralia. Echinoderm larvae are initially bilaterally symmetrical, but later as adults develop radial symmetry; Cnidarians are radially symmetrical; ctenophores are biradially

symmetrical; and sponges have no symmetry.

Animal phyla appeared in the fossil record as marine species during the Cambrian explosion, about 542 million years ago. Animals emerged as a clade within Apoikozoa² as the sister group to the choanoflagellates.

5.2 Poriphora

Sponges³, the members of the phylum Porifera (meaning “pore bearer”), are multicellular organisms that have bodies full of pores and channels allowing water to circulate through them, consisting of jelly-like mesohyl sandwiched between two thin layers of cells. Sponges have unspecialized cells that can transform into other types and that often migrate between the main cell layers and the mesohyl in the process. Sponges do not have nervous, digestive or circulatory systems. Instead, most rely on maintaining a constant water flow through their bodies to obtain food and oxygen and to remove wastes. Sponges are thought to be the first to branch off the evolutionary tree from the common ancestor of all animals, making them the sister group of all other animals.

The phylum Porifera is further divided into four classes mainly according to the composition of their skeletons:

1. Hexactinellida (glass sponges) have silicate spicules, the largest of which have six rays and may be individual or fused. The main components of their bodies are syncytia in which large numbers of cell share a single external membrane.

¹<https://en.wikipedia.org/wiki/Animal>

²<https://en.wikipedia.org/wiki/Apoikozoa>

³<https://en.wikipedia.org/wiki/Sponge>

2. Calcareea have skeletons made of calcite, a form of calcium carbonate, which may form separate spicules or large masses. All the cells have a single nucleus and membrane.
3. Most Demospongiae have silicate spicules or spongin fibers or both within their soft tissues. However a few also have massive external skeletons made of aragonite, another form of calcium carbonate. All the cells have a single nucleus and membrane.
4. Archeocyatha are known only as fossils from the Cambrian period.

Sponges are similar to other animals in that they are multicellular, heterotrophic, lack cell walls and produce sperm cells. Unlike other animals, they lack true tissues and organs, and have no body symmetry. The shapes of their bodies are adapted for maximal efficiency of water flow through the central cavity, where it deposits nutrients, and leaves through a hole called the osculum. Many sponges have internal skeletons of spongin and/or spicules of calcium carbonate or silicon dioxide. All sponges are sessile aquatic animals. Although there are freshwater species, the great majority are marine (salt water) species, ranging from tidal zones to depths exceeding 8,800 m (5.5 mi).

While most of the approximately 5,000–10,000 known species feed on bacteria and other food particles in the water, some host photosynthesizing micro-organisms as endosymbionts and these alliances often produce more food and oxygen than they consume. A few species of sponge that live in food-poor environments have become carnivores that prey mainly on small crustaceans.

Sponges in temperate regions live for at most a few years, but some tropical species and perhaps some deep-ocean ones may live for 200 years or more. Some calcified demosponges grow by only 0.2 mm (0.0079 in) per year and, if that rate is constant, specimens 1 m (3.3 ft) wide must be about 5,000 years old. Some sponges start sexual reproduction when only a few weeks old, while others wait until they are several years old.

Most species use sexual reproduction, releasing sperm cells into the water to fertilize ova that in some species are released and in others are retained by the “mother”. The fertilized eggs form larvae which swim off in search of places to settle. Sponges are known for regenerating from fragments that are broken off, although this only works if the fragments include the right types of cells. A few species reproduce by budding. When conditions deteriorate, for example as



Figure 5.1: Sponges and corals.

temperatures drop, many freshwater species and a few marine ones produce gemmules, “survival pods” of unspecialized cells that remain dormant until conditions improve and then either form completely new sponges or recolonize the skeletons of their parents.

The mesohyl functions as an endoskeleton in most sponges, and is the only skeleton in soft sponges that encrust hard surfaces such as rocks. More commonly, the mesohyl is stiffened by mineral spicules, by spongin fibers or both. Demosponges use spongin, and in many species, silica spicules and in some species, calcium carbonate exoskeletons. Demosponges constitute about 90% of all known sponge species, including all freshwater ones, and have the widest range of habitats. Calcareous sponges, which have calcium carbonate spicules and, in some species, calcium carbonate exoskeletons, are restricted to relatively shallow marine waters where production of calcium carbonate is easiest. The fragile glass sponges, with “scaffolding” of silica spicules, are restricted to polar regions and the ocean depths where predators are rare. Fossils of all of these types have been found in rocks dated from 580 million years ago.

The few species of demosponge that have entirely soft fibrous skeletons with no hard elements have been used by humans over thousands of years for several purposes, including as padding and as cleaning tools. By the 1950s, though, these had been overfished so heavily that the industry almost collapsed, and most sponge-like materials are now synthetic. Sponges and their microscopic endosymbionts are now being re-

searched as possible sources of medicines for treating a wide range of diseases. Dolphins have been observed using sponges as tools while foraging.

A sponge's body is hollow and is held in shape by the mesohyl, a jelly-like substance made mainly of collagen and reinforced by a dense network of fibers also made of collagen. The inner surface is covered with choanocytes, cells with cylindrical or conical collars surrounding one flagellum per choanocyte. The wave-like motion of the whip-like flagella drives water through the sponge's body. All sponges have ostia, channels leading to the interior through the mesohyl, and in most sponges these are controlled by tube-like porocytes that form closable inlet valves. Pinacocytes, plate-like cells, form a single-layered external skin over all other parts of the mesohyl that are not covered by choanocytes, and the pinacocytes also digest food particles that are too large to enter the ostia, while those at the base of the animal are responsible for anchoring it.

The single-celled choanoflagellates resemble the choanocyte cells of sponges which are used to drive their water flow systems and capture most of their food. This along with phylogenetic studies of ribosomal molecules have been used as morphological evidence to suggest sponges are the sister group to the rest of animals. Some studies have shown that sponges do not form a monophyletic group, in other words do not include all and only the descendants of a common ancestor. Recent phylogenetic analyses suggest that comb jellies rather than sponges are the sister group to the rest of animals.

Most sponges work rather like chimneys: they take in water at the bottom and eject it from the osculum ("little mouth") at the top. Since ambient currents are faster at the top, the suction effect that they produce by Bernoulli's principle does some of the work for free. Sponges can control the water flow by various combinations of wholly or partially closing the osculum and ostia (the intake pores) and varying the beat of the flagella, and may shut it down if there is a lot of sand or silt in the water.

Although the layers of pinacocytes and choanocytes resemble the epithelia of more complex animals, they are not bound tightly by cell-to-cell connections or a basal lamina (thin fibrous sheet underneath). The flexibility of these layers and re-modeling of the mesohyl by lophocytes allow the animals to adjust their shapes throughout their lives to take maximum advantage of local water currents.

5.2.1 Reproduction

Sponges have three asexual methods of reproduction: after fragmentation; by budding; and by producing gemmules. Fragments of sponges may be detached by currents or waves. They use the mobility of their pinacocytes and choanocytes and reshaping of the mesohyl to re-attach themselves to a suitable surface and then rebuild themselves as small but functional sponges over the course of several days. The same capabilities enable sponges that have been squeezed through a fine cloth to regenerate. A sponge fragment can only regenerate if it contains both collencytes to produce mesohyl and archeocytes to produce all the other cell types. A very few species reproduce by budding. Gemmules are "survival pods" which a few marine sponges and many freshwater species produce by the thousands when dying and which some, mainly freshwater species, regularly produce in autumn. Spongocytes make gemmules by wrapping shells of spongin, often reinforced with spicules, round clusters of archeocytes that are full of nutrients.

Most sponges are hermaphrodites (function as both sexes simultaneously), although sponges have no gonads (reproductive organs). Sperm are produced by choanocytes or entire choanocyte chambers that sink into the mesohyl and form spermatocysts while eggs are formed by transformation of archeocytes, or of choanocytes in some species. Each egg generally acquires a yolk by consuming "nurse cells". During spawning, sperm burst out of their cysts and are expelled via the osculum. If they contact another sponge of the same species, the water flow carries them to choanocytes that engulf them but, instead of digesting them, metamorphose to an ameboid form and carry the sperm through the mesohyl to eggs, which in most cases engulf the carrier and its cargo.

A few species release fertilized eggs into the water, but most retain the eggs until they hatch. There are four types of larvae, but all are balls of cells with an outer layer of cells whose flagellae or cilia enable the larvae to move. After swimming for a few days the larvae sink and crawl until they find a place to settle. Most of the cells transform into archeocytes and then into the types appropriate for their locations in a miniature adult sponge.

Glass sponge embryos start by dividing into separate cells, but once 32 cells have formed they rapidly transform into larvae that externally are ovoid with a band of cilia round the middle that they

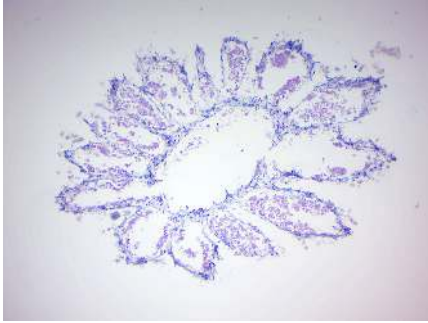


Figure 5.2: *Grantia* cross section.

use for movement, but internally have the typical glass sponge structure of spicules with a cobweb-like main syncytium draped around and between them and choanosyncytia with multiple collar bodies in the center. The larvae then leave their parents' bodies.

5.3 *Grantia*

*Grantia*⁴ is a genus of calcareous sponges belonging to the family Grantiidae. Grantias contain spicules and spongin fibers.

5.4 View Prepared Slides of *Grantia*

1. *Grantia* c.s. l.s. (Figures 5.2 and 5.3)
 - Identify spongocoel, radial canals, ostium, incurrent canals, collar cells (choanocytes).
2. *Grantia* thick x.s.
 - Identify spongocoel, incurrent canals, ostium, radial canals, collar cells (choanocytes).
3. *Grantia* spicules x.s. (Figure 5.4)
 - Notice shape.

5.5 Cnidaria

Cnidaria⁵ is a phylum containing over 10,000 species of animals found exclusively in aquatic (freshwater and marine) environments: they are predominantly

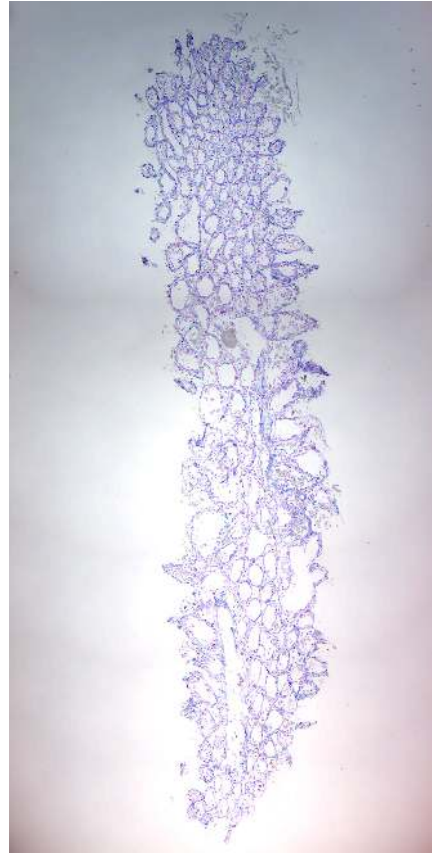


Figure 5.3: *Grantia* longitudinal section.

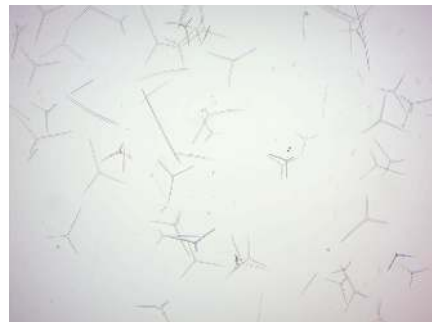


Figure 5.4: Spicules.

⁴<https://en.wikipedia.org/wiki/Grantia>

⁵<https://en.wikipedia.org/wiki/Cnidaria>

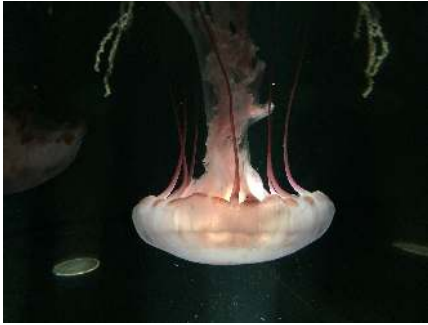


Figure 5.5: Purple striped jellyfish *Chrysaora colorata*.⁶

marine species. Their distinguishing feature is the presence of cnidocytes, specialized cells that they use mainly for capturing prey. Their bodies consist of mesoglea, a non-living jelly-like substance, sandwiched between two layers of epithelium that are mostly one cell thick. They have two basic body forms: swimming medusae (singular: medusa) and sessile polyps, both of which are radially symmetrical with mouths surrounded by tentacles that bear cnidocytes. Both forms have a single orifice and body cavity that are used for digestion and respiration. Many cnidarian species produce colonies that are single organisms composed of medusa-like or polyp-like zooids, or both (hence they are trimorphic). Cnidarians' activities are coordinated by a decentralized nerve net and simple receptors. Several free-swimming species of Cubozoa and Scyphozoa possess balance-sensing statocysts, and some have simple eyes. Statocysts are sac-like structure sensory structures containing a mineralized mass (statolith) and numerous innervated sensory hairs (setae). The statolith's inertia causes it to push against the setae when the animal accelerates. Deflection of setae by the statolith in response to gravity activates neurons, providing feedback to the animal on change in orientation and allowing balance to be maintained.

Not all cnidarians reproduce sexually, with many species having complex life cycles of asexual polyp stages and sexual medusae. Some, however, omit either the polyp or the medusa stage.

Cnidarians are classified into five groups:

1. Anthozoa (sea anemones, corals, sea pens)
2. Scyphozoa (jellyfish)
3. Cubozoa (box jellies)
4. Hydrozoa (a diverse group that includes all the freshwater cnidarians, such as *Hydra*, as well as many marine forms, and has both sessile members and colonial swimmers, such as the Por-



Figure 5.6: Giant green giant green anemone *Anthopleura xanthogrammica*.⁷

tuguese Man o' War)

5. Staurozoa (stalked jellyfish that do not have an alternation of polyp and medusa life cycle phases but are instead interpreted as an attached medusa stage, with a life style more resembling that of polypoid forms)

Most cnidarians prey on organisms ranging in size from plankton to animals several times larger than themselves, but many obtain much of their nutrition from dinoflagellates, and a few are parasites. Many are preyed on by other animals including starfish, sea slugs, fish, turtles, and even other cnidarians. Many scleractinian corals—which form the structural foundation for coral reefs—possess polyps that are filled with symbiotic photo-synthetic zooxanthellae. While reef-forming corals are almost entirely restricted to warm and shallow marine waters, other cnidarians can be found at great depths, in polar regions, and in freshwater.

Recent phylogenetic analyses support monophyly of cnidarians, as well as the position of cnidarians as the sister group of bilaterians. Fossil cnidarians have been found in rocks formed about 580 million years ago, and other fossils show that corals may have been present shortly before 490 million years ago and diversified a few million years later. However, molecular clock analysis of mitochondrial genes suggests a much older age for the crown group of cnidarians, estimated around 741 million years ago, almost 200 million years before the Cambrian period as well as

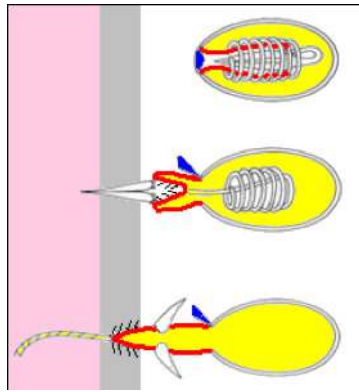


Figure 5.7: A firing *Hydra* nematocyst.⁸

any fossils.

5.5.1 Reproduction

Cnidarian sexual reproduction often involves a complex life cycle with both polyp and medusa stages. For example, in Scyphozoa (jellyfish) and Cubozoa (box jellies) larvae swim until they settle down and become polyps. Polyps grow normally but then absorb their tentacles and split horizontally into a series of disks that become juvenile medusae, a process called strobilation. The juveniles swim off and slowly grow to maturity, while the polyps re-grow and may continue strobilating periodically. The adults have gonads in the gastroderm, and these release ova and sperm into the water in the breeding season.

This phenomenon of succession of differently organized generations (one asexually reproducing, sessile polyp, followed by a free-swimming medusa or a sessile polyp that reproduces sexually) is sometimes called “alternation of asexual and sexual phases” or “metagenesis”, but should not be confused with the alternation of generations as found in plants.

Shortened forms of this life cycle are common, for example some oceanic scyphozoans omit the polyp stage completely, and cubozoan polyps produce only one medusa. Hydrozoa have a variety of life cycles. Some have no polyp stages and some (e.g. *Hydra*) have no medusae. In some species, the medusae remain attached to the polyp and are responsible for sexual reproduction; in extreme cases these reproductive zooids may not look much like medusae. Meanwhile, life cycle reversal, in which polyps are formed directly from medusae without the involvement of sexual reproduction process, was observed in both Hydrozoa (*Turritopsis dohrnii* and *Laodicea undulata*) and Scyphozoa (e. g. *Aurelia*). Anthozoa have no

medusa stage at all and the polyps are responsible for sexual reproduction.

Spawning is generally driven by environmental factors such as changes in the water temperature, and their release is triggered by lighting conditions such as sunrise, sunset or the phase of the moon. Many species of Cnidaria may spawn simultaneously in the same location, so that there are too many ova and sperm for predators to eat more than a tiny percentage — one famous example is the Great Barrier Reef, where at least 110 corals and a few non-cnidarian invertebrates produce enough gametes to turn the water cloudy. These mass spawnings may produce hybrids, some of which can settle and form polyps, but it is not known how long these can survive. In some species the ova release chemicals that attract sperm of the same species.

The fertilized eggs develop into larvae by dividing until there are enough cells to form a hollow sphere (blastula) and then a depression forms at one end (gastrulation) and eventually becomes the digestive cavity. However, in cnidarians the depression forms at the end further from the yolk (at the animal pole), while in bilaterians it forms at the other end (vegetal pole). The larvae, called planulae, swim or crawl by means of cilia. They are cigar-shaped but slightly broader at the “front” end, which is the aboral, vegetal-pole end and eventually attaches to a substrate if the species has a polyp stage.

Anthozoan larvae either have large yolks or are capable of feeding on plankton, and some already have endosymbiotic algae that help to feed them. Since the parents are immobile, these feeding capabilities extend the larvae’s range and avoid overcrowding of sites. Scyphozoan and hydrozoan larvae have little yolk and most lack endosymbiotic algae, and therefore have to settle quickly and metamorphose into polyps. Instead, these species rely on their medusae to extend their ranges.

All known cnidaria can reproduce asexually by various means, in addition to regenerating after being fragmented. Hydrozoan polyps only bud, while the medusae of some hydrozoans can divide down the middle. Scyphozoan polyps can both bud and split down the middle. In addition to both of these methods, Anthozoa can split horizontally just above the base. Asexual reproduction makes the daughter cnidarian a clone of the adult.

5.6 *Hydra*

*Hydra*⁹ is a genus of small, fresh-water organisms of the phylum Cnidaria and class Hydrozoa. They are native to the temperate and tropical regions. Biologists are especially interested in *Hydra* because of their regenerative ability — they do not appear to die of old age, or indeed to age at all. *Hydra* has a tubular, radially symmetric body up to 10 mm long when extended, secured by a simple adhesive foot called the basal disc. Gland cells in the basal disc secrete a sticky fluid that accounts for its adhesive properties.

At the free end of the body is a mouth opening surrounded by one to twelve thin, mobile tentacles. Each tentacle, or cnida (plural: cnidae), is clothed with highly specialized stinging cells called cnidocytes. Cnidocytes contain specialized structures called nematocysts, which look like miniature light bulbs with a coiled thread inside. At the narrow outer edge of the cnidocyte is a short trigger hair called a cnidocil. Upon contact with prey, the contents of the nematocyst are explosively discharged, firing a dart-like thread containing neurotoxins into whatever triggered the release which can paralyse the prey, especially if many hundreds of nematocysts are fired.

Hydra has two main body layers, which makes it “diploblastic”. The layers are separated by mesoglea, a gel-like substance. The outer layer is the epidermis, and the inner layer is called the gastrodermis, because it lines the stomach. The cells making up these two body layers are relatively simple. Hydracinin is a bactericide recently discovered in *Hydra*; it protects the outer layer against infection.

The nervous system of *Hydra* is a nerve net, which is structurally simple compared to more derived animal nervous systems. *Hydra* does not have a recognizable brain or true muscles. Nerve nets connect sensory photoreceptors and touch-sensitive nerve cells located in the body wall and tentacles.

Respiration and excretion occur by diffusion everywhere through the epidermis.

If *Hydra* are alarmed or attacked, the tentacles can be retracted to small buds, and the body column itself can be retracted to a small gelatinous sphere. *Hydra* generally react in the same way regardless of the direction of the stimulus, and this may be due to the simplicity of the nerve nets.

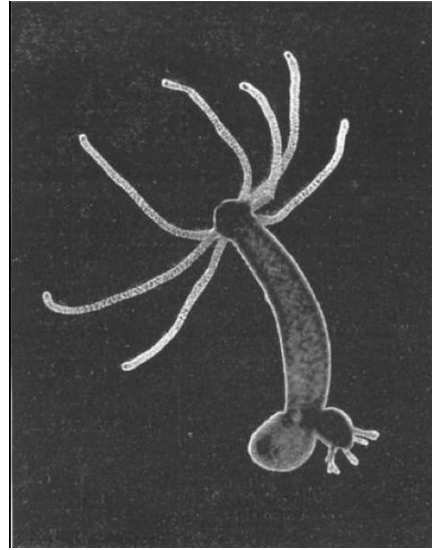


Figure 5.8: A budding *Hydra*.¹⁰

Hydra are generally sedentary or sessile, but do occasionally move quite readily, especially when hunting.

5.6.1 Reproduction and life cycle

When food is plentiful, many *Hydra* reproduce asexually by producing buds in the body wall, which grow to be miniature adults and break away when they are mature. When a hydra is well fed, a new bud can form every two days. When conditions are harsh, often before winter or in poor feeding conditions, sexual reproduction occurs in some *Hydra*. Swellings in the body wall develop into either an ovary or testes. The testes release free-swimming gametes into the water, and these can fertilize the egg in the ovary of another individual. The fertilized eggs secrete a tough outer coating, and, as the adult dies (due to starvation and/or cold), these resting eggs fall to the bottom of the lake or pond to await better conditions, whereupon they hatch into nymph *Hydra*. Some, like *Hydra circumcincta* and *Hydra viridissima*, are hermaphrodites and may produce both testes and an ovary at the same time.

Many members of the Hydrozoa go through a body change from a polyp to an adult form called a medusa. However, all *Hydra*, despite being hydrozoans, remain as polyps throughout their lives.

⁹[https://en.wikipedia.org/wiki/Hydra_\(genus\)](https://en.wikipedia.org/wiki/Hydra_(genus))

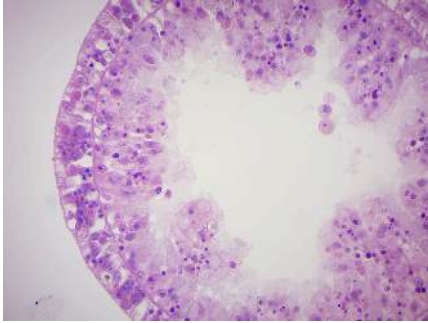


Figure 5.9: *Hydra* cross section.



Figure 5.11: *Aurelia* planula larva

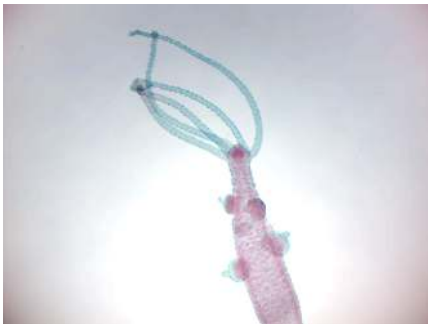


Figure 5.10: *Aurelia* spermary



Figure 5.12: *Aurelia* scyphistoma

5.7 View Prepared Slides of *Hydra*

1. *Hydra* x.s. (Figure 5.9)
 - Identify: epidermis (ectoderm), gastrodermis (endoderm), gastrovascular cavity.
2. *Hydra* spermary (Figure 5.10)
 - Identify: testis, sperms, epidermis, mesoglea, gastrodermis, gastrovascular cavity.

5.8 *Aurelia*

*Aurelia*¹¹ is a genus of scyphozoan jellyfish, commonly called moon jellies. Species of *Aurelia* can be found in the Atlantic Ocean, the Arctic Ocean and the Pacific Ocean, and are common to the waters off California, northern China, Japan, Korea, Australia, New Zealand, the Black Sea, Indonesia, the East Coast of the United States as well as Europe. *Aurelia* undergoes alternation of generations, whereby the sexually-reproducing pelagic medusa stage is either male or

female, and the benthic polyp stage reproduces asexually.

5.9 View Prepared Slides of *Aurelia*

1. *Aurelia* Planula (Figure 5.11)
2. *Aurelia* Scyphistoma (Figure 5.12)
 - Identify: tentacles, and base.
3. *Aurelia* Strobilus (Figure 5.13)
 - Identify: tentacles, base, developing medusae (ephyrae).

5.10 *Obelia*

*Obelia*¹² is a genus in the class Hydrozoa, which consists of mainly marine and some freshwater animal species and have both the polyp and medusa stages in their life cycle. The genus belongs to the phylum Cnidaria, which are all aquatic and mainly marine organisms that are relatively simple in structure. It

¹¹[https://en.wikipedia.org/wiki/Aurelia_\(name\)](https://en.wikipedia.org/wiki/Aurelia_(name))

¹²<https://en.wikipedia.org/wiki/Obelia>



Figure 5.13: *Aurelia strobilus*

is also called sea fur. *Obelia* has a worldwide distribution except the high-arctic and Antarctic seas. The medusa stage of *Obelia* species are common in coastal and offshore plankton around the world. *Obelia* are usually found no deeper than 200 meters from the water's surface, growing in intertidal rock pools and at the extreme low water of spring tides.

Through its life cycle, *Obelia* take two forms: polyp and medusa. They are diploblastic, with two true tissue layers – an epidermis (ectodermis) and a gastrodermis (endodermis), with a jelly-like mesoglea filling the area between the two true tissue layers. They carry a nerve net with no brain or ganglia. A gastrovascular cavity is present where the digestion starts and later becomes intracellular. They have incomplete digestive tracts where the food enters, is digested, and expelled through the same opening. During the polyp stage, the mouth is situated at the top of the body, surrounded by tentacles, whereas during the medusa stage, the mouth is situated at the distal end of the main body structure. Four gonads lie in this main body structure, or manubrium. When food is taken in through the mouth, it enters the manubrium. The food is then distributed through a canal system, consisting of four radial canals and an outer ring. Defense and the capture of prey are helped by unique stinging cells called cnidocytes that contain nematocysts, which are triggered by the cnidocil. It has a ridge-like structure on the inner margin, called velum. If the velum is present, it is named as craspedote medusa.

The polyp colony reproduces asexually. During this stage of life, *Obelia* are confined to substrate surfaces. On this mature colony there are individual hydranths called gastrozooids, which can be found expanded or contracted, to aid in the growth of this organism by feeding; the reproductive polyp gonozooids has medusa buds. Other hydranths are specialized for defense. The main stalky body of the colony is com-

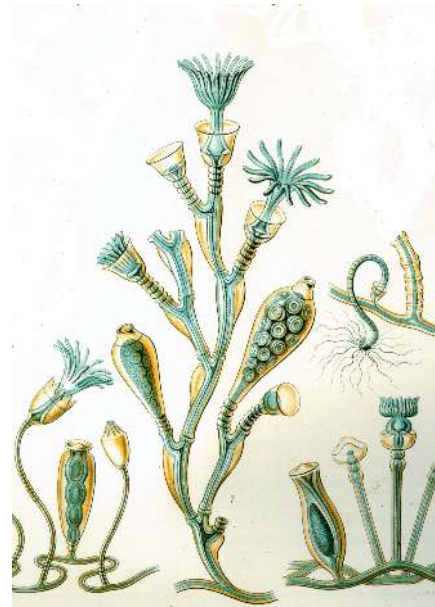


Figure 5.14: *Obelia geniculata* colony with 3 hydranths and 2 gonophores.¹³

posed of a coenosarc, which is covered by a protective perisarc.

The next generation of the life cycle begins when the medusae are released from these gonozooids, producing free swimming only male medusae velum with gonads, a mouth, and tentacles. The physical appearance of the male and female medusae velum, including their gonads, are indistinguishable, and the sex can only be determined by observing the inside of the gonads, which will either contain sperm or eggs. The medusae reproduce sexually, releasing sperm and eggs that fertilize to form a zygote, which later morphs into a blastula, then a ciliated swimming larva called a planula.

The planulae live free-swimming for a while but eventually attach themselves to some solid surface, where they begin their reproductive phase of life. Once attached to a substrate, a planula quickly develops into one feeding polyp. As the polyp grows, it begins developing branches of other feeding individuals, thus forming a new generation of polyps by asexual budding.

5.11 View Prepared Slides of *Obelia*

1. *Obelia* medusa w.m. (Figure 5.15)

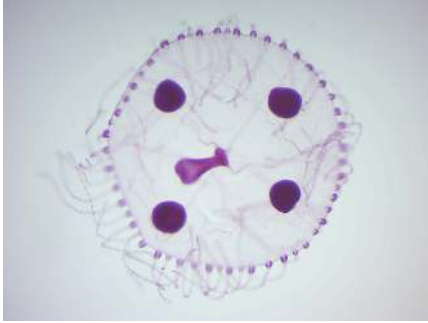


Figure 5.15: *Obelia* medusa.



Figure 5.16: *Obelia* colony.

- Identify: tentacles, manubrium, mouth, gonads.
2. *Obelia* w.m. (Figure 5.16)
 - Identify: hydranth, tentacles, mouth, hydrotheca, gonangium, gonotheca, gonopore, medusa buds, blastostyle.

5.12 *Metridium*

Members of the genus *Metridium*¹⁴, also known as plumose anemones, are sea anemones found mostly in the cooler waters of the northern Pacific and Atlantic oceans. They are characterized by their numerous threadlike tentacles extending from atop a smooth cylindrical column, and can vary from a few centimeters in height up to one meter or more. In larger specimens, the oral disk becomes densely curved and

¹⁴<https://en.wikipedia.org/wiki/Metridium>

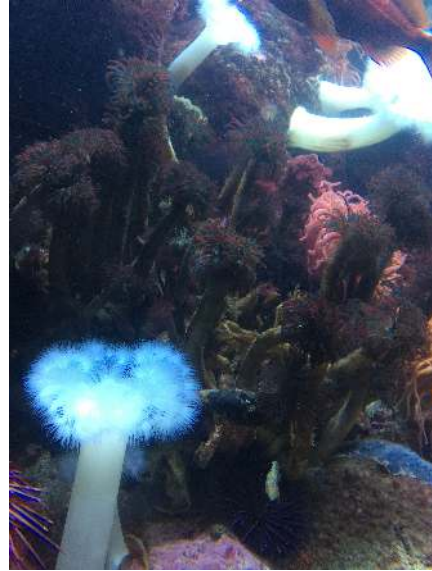


Figure 5.17: *Metridium*.

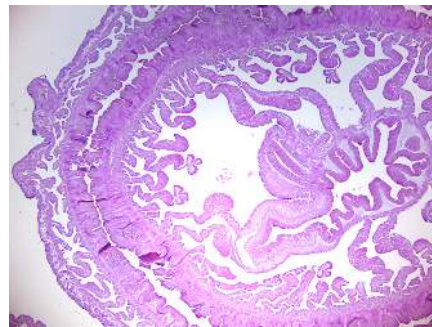


Figure 5.18: *Metridium*.

frilly.

5.13 View Prepared Slides of *Metridium*

1. *Metridium* x.s. (Figure 5.18)
 - Identify: tentacles, mouth, type of symmetry.

5.14 View Living Organisms

1. *Hydra*

5.15 *Ctenophora*

Ctenophora¹⁵ (singular ctenophore; from the Greek kteis ‘comb’ and phērō ‘carry’; commonly known as comb jellies) is a phylum of invertebrate animals that live in marine waters worldwide. They are notable for the groups of cilia they use for swimming (commonly referred to as “combs”), and they are the largest animals that swim by means of cilia. Depending on the species, adult ctenophores range from a few millimeters to 1.5 m in size. Only 100–150 species have been validated, and possibly another 25 have not been fully described and named. The textbook examples are cydippids with egg-shaped bodies and a pair of retractable tentacles fringed with tentilla (“little tentacles”) that are covered with colloblasts, sticky cells that capture prey.

The phylum has a wide range of body forms, including the flattened, deep-sea platyctenids, in which the adults of most species lack combs, and the coastal beroids, which lack tentacles and prey on other ctenophores by using huge mouths armed with groups of large, stiffened cilia that act as teeth. Almost all ctenophores are predators, taking prey ranging from microscopic larvae and rotifers to the adults of small crustaceans; the exceptions are juveniles of two species, which live as parasites on the salps on which adults of their species feed. Most species are hermaphrodites, and juveniles of at least some species are capable of reproduction before reaching the adult size and shape. This combination of hermaphroditism and early reproduction enables small populations to grow at an explosive rate.

Early writers combined ctenophores with cnidarians into a single phylum called Coelenterata on account of morphological similarities between the two groups. Like cnidarians, the bodies of ctenophores consist of a mass of jelly, with one layer of cells on the outside and another lining the internal cavity. In ctenophores, however, these layers are two cells deep, while those in cnidarians are only a single cell deep. Ctenophores also resemble cnidarians in relying on water flow through the body cavity for both digestion and respiration, as well as in having a decentralized nerve net rather than a brain. However, genomic studies have suggested that the neurons of Ctenophora, which differ in many ways from other animal neurons, evolved independently from those of the other animals, and increasing awareness of the differences between the groups has persuaded more recent authors to classify the two as

separate phyla. The position of the ctenophores in the evolutionary family tree of animals has long been debated, and the majority view at present, based on molecular phylogenetics, is that cnidarians and bilaterians are more closely related to each other than either is to ctenophores.

The traditional classification divides ctenophores into two classes, those with tentacles (Tentaculata) and those without (Nuda). The Nuda contains only one order (Beroida) and family (Beroidae), and two genera, *Beroë* (several species) and *Neis* (one species).

The *Tentaculata* are divided into the following eight orders:

1. Cydippida, egg-shaped animals with long tentacles
2. Lobata, with paired thick lobes
3. Platyctenida, flattened animals that live on or near the sea-bed; most lack combs as adults, and use their pharynges as suckers to attach themselves to surfaces
4. Ganeshida, with a pair of small lobes round the mouth, but an extended pharynx like that of platyctenids
5. Cambojiida
6. Cryptolobiferida
7. Thalassocalycida, with short tentacles and a jellyfish-like “umbrella”
8. Cestida, ribbon-shaped and the largest ctenophores

Despite their soft, gelatinous bodies, fossils thought to represent ctenophores, apparently with no tentacles but many more comb-rows than modern forms, have been found as far back as the early Cambrian, about 515 million years ago.

Adults of most species can regenerate tissues that are damaged or removed, although only platyctenids reproduce by cloning, splitting off from the edges of their flat bodies fragments that develop into new individuals.

Almost all species are hermaphrodites. Some are simultaneous hermaphrodites, which can produce both eggs and sperm at the same time, while others are sequential hermaphrodites, in which the eggs and sperm mature at different times. The gonads are located in the parts of the internal canal network under the comb rows, and eggs and sperm are released via pores in the epidermis. Fertilization is generally external, but some use internal fertilization and keep the eggs in brood chambers until they hatch. Self-fertilization has occasionally been seen in some species and it is thought that most of the

¹⁵<https://en.wikipedia.org/wiki/Ctenophora>

hermaphroditic species are self-fertile.

Development of the fertilized eggs is direct, in other words there is no distinctive larval form. Juveniles of all groups are generally planktonic, and in most species, resemble miniature adult cydippids, gradually developing their adult body forms as they grow.

5.16 Review Questions

1. What are animals?
2. What are porifera?
3. What are choanocytes?
4. What are cnidaria?
5. What are cnidocytes?
6. What is a statocyst?
7. What are ctenophora?
8. Ctenophora move by means of _____.

Chapter 6

Rotifera, Platyhelminthes, Molluska and Annelida

6.1 Rotifers

The rotifers¹ (Rotifera, commonly called wheel animals) make up a phylum of microscopic and near-microscopic pseudocoelomate animals. The word “rotifer” is derived from a Latin word meaning “wheel-bearer”, due to the corona around the mouth that in concerted sequential motion resembles a wheel (though the organ does not actually rotate). They were first described by Rev. John Harris in 1696, and other forms were described by Antonie van Leeuwenhoek² in 1703. Most rotifers are around 0.1–0.5 mm long (although their size can range from 50 μ m to over 2 mm), and are common in freshwater environments throughout the world with a few saltwater species; for example, those of genus *Synchaeta*. Some rotifers are free swimming and truly planktonic, others move by inchworming along a substrate, and some are sessile, living inside tubes or gelatinous holdfasts that are attached to a substrate. About 25 species are colonial (e.g., *Sinantharina semibullata*), either sessile or planktonic. Rotifers are an important part of the freshwater zooplankton, being a major foodsource and with many species also contributing to the decomposition of soil organic matter.

Rotifers have bilateral symmetry and a variety of different shapes. The body of a rotifer is divided into a head, trunk, and foot, and is typically somewhat cylindrical. There is a well-developed cuticle, which may be thick and rigid, giving the animal a box-like shape, or flexible, giving the animal a worm-like shape; such rotifers are respectively called lori-

cate and illoricate. Rigid cuticles are often composed of multiple plates, and may bear spines, ridges, or other ornamentation. Their cuticle is non-chitinous and is formed from sclerotized proteins.

The most distinctive feature of rotifers is the presence of a ciliated structure, called the corona, on the head. In the more primitive species, this forms a simple ring of cilia around the mouth from which an additional band of cilia stretches over the back of the head. In the great majority of rotifers, however, this has evolved into a more complex structure. The trunk forms the major part of the body, and encloses most of the internal organs. The foot projects from the rear of the trunk, and is usually much narrower, giving the appearance of a tail. The cuticle over the foot often forms rings, making it appear segmented, although the internal structure is uniform. Many rotifers can retract the foot partially or wholly into the trunk. The foot ends in from one to four toes, which, in sessile and crawling species, contain adhesive glands to attach the animal to the substratum. In many free-swimming species, the foot as a whole is reduced in size, and may even be absent.

Rotifers are dioecious and reproduce sexually or parthenogenetically. They are sexually dimorphic, with the females always being larger than the males. In some species, this is relatively mild, but in others the female may be up to ten times the size of the male. In parthenogenetic species, males may be present only at certain times of the year, or absent altogether. The female reproductive system consists of one or two ovaries, each with a vitellarium gland that supplies the eggs with yolk. Together, each ovary and vitellarium form a single syncytial structure in the anterior part of the animal, opening through an oviduct into the cloaca. Males do not usually have a

¹<https://en.wikipedia.org/wiki/Rotifer>

²https://en.wikipedia.org/wiki/Antonie_van_Leeuwenhoek

functional digestive system, and are therefore short-lived, often being sexually fertile at birth. They have a single testicle and sperm duct, associated with a pair of glandular structures referred to as prostates (unrelated to the vertebrate prostate). The sperm duct opens into a gonopore at the posterior end of the animal, which is usually modified to form a penis. The gonopore is homologous to the cloaca of females, but in most species has no connection to the vestigial digestive system, which lacks an anus. Fertilization is internal. The male either inserts his penis into the female's cloaca or uses it to penetrate her skin, injecting the sperm into the body cavity. The egg secretes a shell, and is attached either to the substratum, nearby plants, or the female's own body. A few species, such as Rotaria, are ovoviviparous, retaining the eggs inside their body until they hatch. Most species hatch as miniature versions of the adult. Sessile species, however, are born as free-swimming larvae, which closely resemble the adults of related free-swimming species. Females grow rapidly, reaching their adult size within a few days, while males typically do not grow in size at all.

6.2 Platyhelminthes

The flatworms³, flat worms, Platyhelminthes, Plathelminthes, or platyhelminths (from the Greek platy, meaning “flat” and helminth-, meaning “worm”) are a phylum of relatively simple bilaterian, unsegmented, soft-bodied invertebrates. Unlike other bilaterians, they are acoelomates (having no body cavity), and have no specialized circulatory and respiratory organs, which restricts them to having flattened shapes that allow oxygen and nutrients to pass through their bodies by diffusion. The digestive cavity has only one opening for both ingestion (intake of nutrients) and egestion (removal of undigested wastes); as a result, the food cannot be processed continuously.

Free-living flatworms are mostly predators, and live in water or in shaded, humid terrestrial environments, such as leaf litter. Cestodes (tapeworms) and trematodes (flukes) have complex life-cycles, with mature stages that live as parasites in the digestive systems of fish or land vertebrates, and intermediate stages that infest secondary hosts. The eggs of trematodes are excreted from their main hosts, whereas adult cestodes generate vast numbers of hermaphroditic, segment-like proglottids that detach

when mature, are excreted, and then release eggs. Unlike the other parasitic groups, the monogeneans are external parasites infesting aquatic animals, and their larvae metamorphose into the adult form after attaching to a suitable host.

Over half of all known flatworm species are parasitic, and some do enormous harm to humans and their livestock. Schistosomiasis, caused by one genus of trematodes, is the second-most devastating of all human diseases caused by parasites, surpassed only by malaria. Neurocysticercosis, which arises when larvae of the pork tapeworm *Taenia solium* penetrate the central nervous system, is the major cause of acquired epilepsy worldwide. The threat of flatworm parasites to humans in developed countries is rising because of the popularity of raw or lightly cooked foods, and imports of food from high-risk areas. In less developed countries, people often cannot afford the fuel required to cook food thoroughly, and poorly designed water-supply and irrigation projects increase the dangers presented by poor sanitation and unhygienic farming.

In traditional medicinal texts, Platyhelminthes are divided into

- Turbellaria
- Cestoda
- Trematoda
- Monogenea

6.3 Turbellaria

Turbellaria⁴ have about 4,500 species, are mostly free-living, and range from 1 mm (0.039 in) to 600 mm in length. Most are predators or scavengers, and terrestrial species are mostly nocturnal and live in shaded, humid locations, such as leaf litter or rotting wood. However, some are symbiotes of other animals, such as crustaceans, and some are parasites. Free-living turbellarians are mostly black, brown or gray, but some larger ones are brightly colored.

6.4 Dugesia

*Dugesia*⁵ is a representative of the class Turbellaria. These common flatworms are found in freshwater habitats of Africa, Europe, Middle East, Asia and Australia. *Dugesia* species have an elongated body

³<https://en.wikipedia.org/wiki/Flatworm>

⁴<https://en.wikipedia.org/wiki/Turbellaria>

⁵<https://en.wikipedia.org/wiki/Dugesia>

with a slightly triangle-shaped head. Usually they have grey, brown or black colors on the dorsal body surface, the ventral surface uses to be paler. These animals have a couple of eyes constituted by a multicellular pigmented cup with many retinal cells to detect the amount of light in the nearby environment. Sometimes they present supernumerary eyes. At the anterior part of the body, behind the eyes level, they have two of structures called auricles that give the triangle look to the 'head' and that allow them to detect the intensity of water current. These auricles are free of pigment and rhabdites. Each side of the anterior margin of the head have between 5 and 10 shallow sensory fossae, their number depends on the species or the individual. The sensory fossae and the auricle grooves are supplied with many nerve endings.

Dugesia digestion tract consists of a central non-pigmented tubular pharynx. Like the other triclad *Dugesia* gut consists in three ramified branches. Each branch consists of ceca, which delivers the nutrients to the body. This worm has a sac digestive plan, that is, it does not have a separate opening for waste excretion.

In *Dugesia* the ovaries are ventrally situated, they start just behind the brain, usually at the level of the fourth intestinal branch. The bursal canal runs on the right side of the copulatory apparatus and above the atrium.

They are hermaphrodites. Many species can reproduce both sexually and asexually (by parthenogenesis or by fission).

6.5 View Prepared Slides of *Dugesia*

1. *Dugesia* w.m. (Figure 6.1)
 - Identify: highly branched gastrovascular cavity (GVC), pharynx, pharyngeal pouch, eyespots, auricles. Be sure to differentiate between anterior and posterior ends.
2. *Dugesia* x.s. 3 regions (Figure 6.2)
 - Identify: Anterior region: gastrovascular cavity (GVC) surrounded by endoderm; pharynx region: pharynx, GVC surrounded by endoderm, epidermis, testis, dorsoventral muscles; posterior end: epidermis, GVC surrounded by endoderm, dorsoventral muscles, ventral nerve cord



Figure 6.1: *Dugesia*.

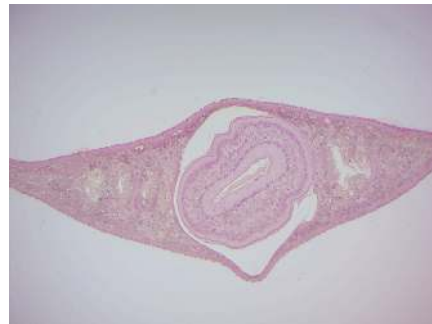


Figure 6.2: *Dugesia* cross section through pharynx region.

6.6 Cestoda

*Cestoda*⁶ are commonly called tapeworms. All cestodes are parasitic and their life histories vary, but typically they live in the digestive tracts of vertebrates as adults, and often in the bodies of other species of animals as juveniles. Over a thousand species have been described, and all vertebrate species may be parasitized by at least one species of tapeworm.

Humans are subject to infection by several species of tapeworms if they eat undercooked meat such as pork (*Taenia solium*⁷), beef (*T. saginata*⁸), and fish (*Diphyllobothrium* spp.), or if they live in, or eat food prepared in, conditions of poor hygiene (*Hymenolepis* or *Echinococcus* species).

T. saginata, the beef tapeworm, can grow up to 20 m. Species using small vertebrates as hosts, though, tend to be small.

Tapeworm parasites of vertebrates have a long history: recognizable clusters of cestode eggs, one with

⁶<https://en.wikipedia.org/wiki/Cestoda>

⁷https://en.wikipedia.org/wiki/Taenia_solium

⁸https://en.wikipedia.org/wiki/Taenia_saginata

a developing larva, have been discovered in fossil feces (coprolites) of a shark dating to the mid- to late Permian, some 270 million years ago.

The worm's head, known as a scolex, attaches to the intestine of the definitive host. In some species, the scolex is dominated by bothria, or "sucking grooves" that function like suction cups. Other species have hooks and suckers that aid in attachment.

The main nerve centre of a cestode is a cerebral ganglion in its scolex. Motor and sensory innervation depends on the number of nerves in and complexity of the scolex. Smaller nerves emanate from the ganglion to supply the general body muscular and sensory ending. The cirrus and vagina are innervated, and sensory endings around the genital pore are more plentiful than other areas. Sensory function includes both tactoreception (touch) and chemoreception (smell or taste). Some nerves are only temporary.

The body is composed of successive segments called proglottids. The sum of the proglottids is called a strobila, which is thin, and resembles a strip of tape. From this is derived the common name "tapeworm". Proglottids are continually produced by the neck region of the scolex, as long as the scolex is attached and alive. Like some other flatworms, cestodes use flame cells (protonephridia), located in the proglottids, for excretion. Mature proglottids are released from the tapeworm's end segment and leave the host in feces or migrate as independent motile proglottids. The proglottids farthest away from the scolex are the mature ones containing eggs. Mature proglottids are essentially bags of eggs, each of which is infective to the proper intermediate host.

Once anchored to the host's intestinal wall, the tapeworm absorbs nutrients through its skin as the food being digested by the host flows over and around it. Soon, it begins to grow a tail composed of a series of segments, with each segment containing an independent digestive system and reproductive tract. Older segments are pushed toward the tip of the tail as new segments are produced by the neckpiece. By the time a segment has reached the end of the worm's tail, only the reproductive tract is left. The segment then separates, carrying the tapeworm eggs out of the definitive host as what is basically a sack of eggs.

True tapeworms are exclusively hermaphrodites, with both male and female reproductive systems in their bodies. The reproductive system includes one or more testes, cirri, vas deferens, and seminal vesicles as male organs, and a single lobed or unlobed

ovary with the connecting oviduct and uterus as female organs. The common external opening for both male and female reproductive systems is known as the genital pore, which is situated at the surface opening of the cup-shaped atrium. Though they are sexually hermaphroditic, self-fertilization is a rare phenomenon. To permit hybridization, cross-fertilization between two individuals is often practiced for reproduction. During copulation, the cirri of one individual connect with those of the other through the genital pore, and then spermatozoa are exchanged.

The lifecycle of tapeworms is simple in the sense that no asexual phases occur as in other flatworms, but complicated in that at least one intermediate host is required as well as the definitive host. Many tapeworms have a two-phase lifecycle with two types of hosts. The adult *Taenia saginata* lives in the gut of a primate such as a human, but more alarming is *Taenia solium*, which can form cysts in the human brain. Proglottids leave the body through the anus and fall onto the ground, where they may be eaten with grass by an animal such as a cow. If the tapeworm is compatible with the eating animal, this animal becomes an intermediate host. The juvenile form of the worm enters through the mouth, but then migrates and establishes as a cyst in the intermediate host's body tissues such as muscles, rather than the gut. This can cause more damage to the intermediate host than it does to its definitive host. The parasite completes its lifecycle when the intermediate host passes on the parasite to the definitive host. This is usually done by the definitive host eating a suitably infected intermediate host, e.g., a human eating raw or undercooked meat.

6.7 View Prepared Slides of *Taenia*

1. *Taenia pisiformis*⁹. Identify
 - Scolex: rostellum with hooks, suckers (Figure 6.3)
 - Mature proglottid: testes, sperm duct, excretory duct, uterus, vagina, ovary, genital pore, yolk gland (Figure 6.4)
 - Gravid proglottid: zygotes in branched uterus (Figure 6.5)
2. *Taenia solium* cysticercus w.m. (Figure 6.6).
 - Identify: scolex, neck, bladder

⁹https://en.wikipedia.org/wiki/Taenia_pisiformis



Figure 6.3: *Taenia* scolex.

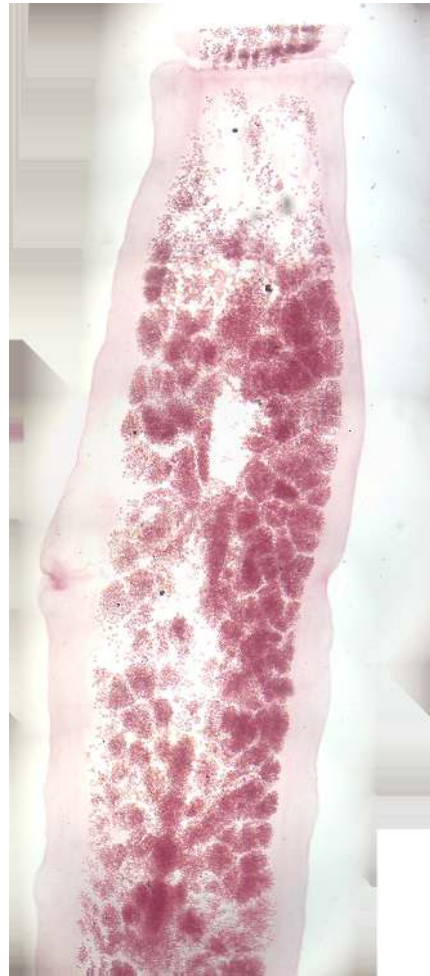


Figure 6.5: *Taenia* gravid proglottid.



Figure 6.4: *Taenia* proglottid.

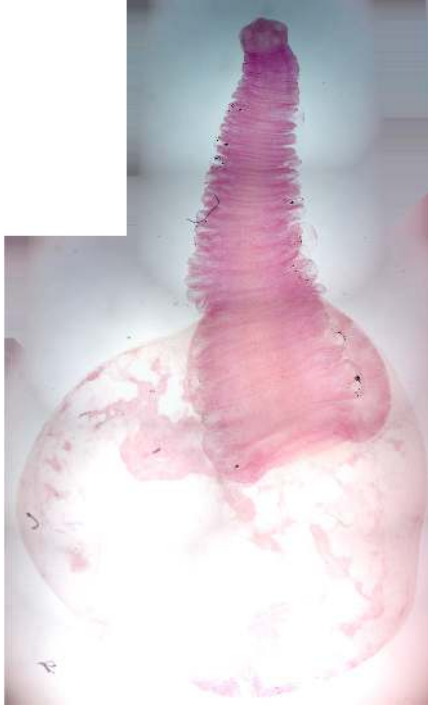


Figure 6.6: *Taenia solium* cysticercus.

6.8 Trematoda

The trematodes¹⁰ or flukes include 18,000 to 24,000 species, divided into two subclasses. Nearly all trematodes are parasites of mollusks and vertebrates. Most trematodes have a complex life cycle with at least two hosts. The primary host, where the flukes sexually reproduce, is a vertebrate. The intermediate host, in which asexual reproduction occurs, is usually a snail.

Trematodes are flattened oval or worm-like animals, usually no more than a few centimeters in length, although species as small as 1 millimeter) are known. Their most distinctive external feature is the presence of two suckers, one close to the mouth, and the other on the underside of the animal.

The body surface of trematodes comprises a tough syncytial tegument, which helps protect against digestive enzymes in those species that inhabit the gut of larger animals. It is also the surface of gas exchange; there are no respiratory organs. The mouth is located at the forward end of the animal, and opens into a muscular, pumping pharynx. The pharynx connects, via a short oesophagus, to one or two blind-ending caeca, which occupy most of the length of the body. In some species, the caeca are themselves branched.

As in other flatworms, there is no anus, and waste material must be egested through the mouth.

Although the excretion of nitrogenous waste occurs mostly through the tegument, trematodes do possess an excretory system, which is instead mainly concerned with osmoregulation. This consists of two or more protonephridia, with those on each side of the body opening into a collecting duct. The two collecting ducts typically meet up at a single bladder, opening to the exterior through one or two pores near the posterior end of the animal.

The brain consists of a pair of ganglia in the head region, from which two or three pairs of nerve cords run down the length of the body. Trematodes generally lack any specialized sense organs, although some ectoparasitic species do possess one or two pairs of simple ocelli.

Most trematodes are simultaneous hermaphrodites, having both male and female organs. There are usually two testes, with sperm ducts that join together on the underside of the front half of the animal. This final part of the male system varies considerably in structure between species, but may include sperm storage sacs and accessory glands, in addition to the copulatory organ, which is either eversible, and termed a cirrus, or non-eversible, and termed a penis.

There is usually only a single ovary. Eggs pass from it into an oviduct. The distal part of the oviduct, called ootype, is dilated. The ootype is connected to an elongated uterus that opens to the exterior in the genital pore, close to the male opening. In most trematodes, sperm cells travel through the uterus to reach the ootype, where fertilization occurs.

Almost all trematodes infect mollusks as the first host in the life cycle, and most have a complex life cycle¹¹ involving other hosts. Most trematodes are monoecious and alternately reproduce sexually and asexually. Schistosomes are dioecious.

In the definitive host, in which sexual reproduction occurs, eggs are commonly shed along with host feces. Eggs shed in water release free-swimming larval forms that are infective to the intermediate host, in which asexual reproduction occurs.

Human infections are most common in Asia, Africa, Latin and South America and the Middle East. However, trematodes can be found anywhere where untreated human waste is used as fertilizer.

¹⁰<https://en.wikipedia.org/wiki/Trematoda>

¹¹https://en.wikipedia.org/wiki/Trematode_life_cycle_stages

Schistosomiasis (also known as bilharzia, bilharziosis or snail fever) is an example of a parasitic disease caused by one of the species of trematodes (platyhelminth infection, or “flukes”), a parasitic worm of the genus *Schistosoma*. *Clonorchis*, *Opisthorchis*, *Fasciola* and *Paragonimus* species, the foodborne trematodes, are another. Other diseases are caused by members of the *Choledocystus* genus.

*Clonorchis sinensis*¹², the Chinese liver fluke, is a human liver fluke belonging to the class Trematoda, phylum Platyhelminthes. This parasite lives in the liver of humans, and is found mainly in the common bile duct and gall bladder, feeding on bile. These animals, which are believed to be the third most prevalent worm parasite in the world, are endemic to Japan, China, Taiwan, and Southeast Asia, currently infecting an estimated 30,000,000 humans. 85% of cases are found in China. The infection called clonorchiasis generally appears as jaundice, indigestion, biliary inflammation, bile duct obstruction, even liver cirrhosis, cholangiocarcinoma (CCA), and hepatic carcinoma.

An adult *C. sinensis* is a flattened (dorso-ventrally flat) and leaf-shaped fluke. The body is slightly elongated and slender, measuring 15–20 mm in length and 3–4 mm in width. It narrows down at the anterior region into a small opening called oral sucker, which act as the mouth. From the mouth run two tubes called caeca throughout the length of body. They are the digestive and excretory tracts. The posterior end is broad and blunt. A poorly developed ventral sucker lies behind the oral sucker, at about one-fourth of the body length from the anterior end. A common genital pore opens just in front of it. As a hermaphrodite, it has both male and female reproductive organs. A single rounded ovary is at the center of the body, and two testes are towards the posterior end. The uterus from the ovary, and seminal ducts from the testes meet and opens at the genital pore. The testes are highly branched. Another highly branched organs called vitellaria (or vitelline glands) are distributed on either side of the body.

The eggs are similar to those of other related flukes are often confused during diagnosis. They small and oval in shape, measuring about 30 x 15 µm in diameter. They are sharply curved and with a clear convex operculum towards the narrower end. At the broader end is a stem-shaped knob. The larva called miracidium can be seen inside the fertilized egg.

6.8.1 Life cycle

The eggs of a *C. sinensis* are released through the biliary tract, and excreted out along with the faeces. The eggs are embryonated and contain the larvae called miracidia. Unlike most other flukes in which the miracidia undergo development and swim in water to infect suitable host, the eggs of *C. sinensis* are simply deposited in water. The eggs are then eaten up by snails. Once inside of the snail body, the embryonic membrane is dissolved by the snail's digestive enzymes so that the miracidium hatches from the egg. The ciliated miracidium can move about, penetrating the intestine, enters the haemocoel and digestive gland. Here it undergoes metamorphosis into a sporocyst. The sporocyst gives rise to small larvae called rediae. The rediae burst out from the sporocyst to become the next-stage larvae called cercaria. This system of asexual reproduction allows for an exponential multiplication of cercaria individuals from one miracidium. This aids the *Clonorchis* in reproduction, because it enables the miracidium to capitalize on one chance occasion of passively being eaten by a snail before the egg dies. The mature cercariae bore out of the snail body into the freshwater environment. However, they are non-feeding and must find a fish host within 2–3 days, otherwise they die. The cercariae of *C. sinensis* are different from those of other flukes in that they do not swim. Instead, they initially hang upside-down in the water, and then sink to the bottom. They rise to the water surface to resume their initial position, and the movement is repeated again. They attack fish when they feel any disturbance in their life-style. When they detect fish, they attached themselves on the scales using their suckers. Boring their way into the fish's body, they penetrate into the fish muscle within 6 to 13 minutes. Within an hour of penetration they develop hard coverings called cysts, and become metacercariae. This protective cyst is useful when the fish muscle is consumed. The metacercariae gradually develop and become infective to their next hosts after 3 to 4 weeks. The common second intermediate hosts are freshwater fish. The metacercariae are eaten along with raw or undercooked fish. The cysts of the metacercariae are gradually digested by the human gastric acids, and upon reaching the small intestines, the entire cyst is lost. The free metacercariae penetrate the intestinal mucosa and enters the bile ducts. It takes 1–2 day for migration into the bile ducts. They start feeding on the bile secreted from the liver, and gradually grow. They become adult in about a month, and start laying eggs. The average lifespan of an adult fluke is 30 years. An individual

¹²https://en.wikipedia.org/wiki/Clonorchis_sinensis

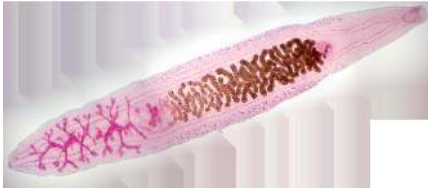


Figure 6.7: *Clonorchis sinensis*.



Figure 6.8: *Fasciola hepatica*.

fluke can produce 4,000 eggs in a day. The definitive hosts are fish-eating mammals such as dogs, cats, rats, pigs, badgers, weasels, camels, buffaloes and humans.

6.9 View Prepared Slides of Trematoda



Notice that these slides are very thick, since they contain whole mounts (w.m.) of the flukes. Use only the 4x (low power) objective of your microscope to avoid crushing these slides.

1. *Clonorchis sinensis* w.m. (Figure 6.7).
 - Identify: oral and ventral suckers, pharynx, esophagus, dead-end intestine, reproductive structures, excretory pore
2. *Fasciola hepatica* w.m. (Figure 6.8).
 - Identify: mouth, oral and ventral suckers, pharynx, uterus, intestine

6.10 *Schistosoma*

*Schistosoma mansoni*¹³ is a water-borne parasite of humans, and belongs to the group of blood flukes (*Schistosoma*). The adult lives in the blood vessels (mesenteric veins) near the human intestine. It causes intestinal schistosomiasis. Clinical symptoms are caused by the eggs. As the leading cause of schistosomiasis in the world, it is one of the most

prevalent parasite in humans. It is classified as a neglected tropical disease. As of 2016, 206.5 million people have schistosomiasis and *S. mansoni* is the major parasite. It is found in Africa, the Middle East, the Caribbean, Brazil, Venezuela and Suriname.

Unlike other flukes (trematodes) in which sexes are not separate (monoecious), schistosomes are unique in that adults are divided into males and females, thus, (dioecious). However, the two adults live in permanent partnership, a condition called in copula; for this, they are considered as hermaphrodites. The life cycle of schistosomes includes two hosts: humans as definitive hosts, where the parasite undergoes sexual reproduction, and snails as intermediate hosts, where a series of asexual reproductive takes place. *S. mansoni* is transmitted through water, where freshwater snails of the genus *Biomphalaria* act as intermediate hosts. The larvae are able to live in water and infect the hosts by directly penetrating the skin. Prevention of infection is done by improved sanitation and killing the snails. Infection is treated with praziquantel. *S. mansoni* was first noted by Theodor Maximillian Bilharz in Egypt in 1851, while discovering *S. haematobium*. Sir Patrick Manson identified it as unique species in 1902.

After the eggs of the human-dwelling parasite are emitted in the faeces and into the water, the ripe miracidium hatches out of the egg. The hatching happens in response to temperature, light and dilution of faeces with water. The miracidium searches for a suitable freshwater snail belonging to the genus *Biomphalaria*. Miracidia directly penetrate the soft tissue of snail. Inside the snail, they lose their cilia and develop into mother sporocysts. The sporocysts rapidly multiply by asexual reproduction, each forming numerous daughter sporocysts. The daughter sporocysts move to the liver and gonads of the snail, where they undergo further growth. Within 2–4 weeks, they undergo metamorphosis and give rise to fork-tailed cercariae. Stimulated by light, hundreds of cercariae penetrate out of the snail into water.

The cercaria emerge from the snail during daylight and they propel themselves in water with the aid of their bifurcated tail, actively seeking out their final host. In water, they can live for up to 12 hours, and their maximum infectivity is between 1 and 9 hours after emergence. When they recognise human skin, they penetrate it within a very short time. This occurs in three stages, an initial attachment to the skin, followed by the creeping over the skin searching for a suitable penetration site, often a hair folli-

¹³https://en.wikipedia.org/wiki/Schistosoma_mansoni

cle, and finally penetration of the skin into the epidermis using cytolytic secretions from the cercarial post-acetabular, then pre-acetabular glands. On penetration, the head of the cercaria transforms into an endoparasitic larva, the schistosomule. Each schistosomule spends a few days in the skin and then enters the circulation starting at the dermal lymphatics and venules. Here, they feed on blood, regurgitating the haem as hemozoin. The schistosomule migrates to the lungs (5–7 days post-penetration) and then moves via circulation through the left side of the heart to the hepatoportal circulation (>15 days) where, if it meets a partner of the opposite sex, it develops into a sexually mature adult and the pair migrate to the mesenteric veins. Such pairings are monogamous.

Male schistosomes undergo normal maturation and morphological development in the presence or absence of a female, although behavioural, physiological and antigenic differences between males from single-sex, as opposed to bisex, infections have been reported. On the other hand, female schistosomes do not mature without a male. Female schistosomes from single-sex infections are underdeveloped and exhibit an immature reproductive system. Although the maturation of the female worm seems to be dependent on the presence of the mature male, the stimuli for female growth and for reproductive development seem to be independent from each other.

The adult female worm resides within the adult male worm's gynaecophoric canal, which is a modification of the ventral surface of the male, forming a groove. The paired worms move against the flow of blood to their final niche in the mesenteric circulation, where they begin egg production (>32 days). The *S. mansoni* parasites are found predominantly in the small inferior mesenteric blood vessels surrounding the large intestine and caecal region of the host. Each female lays approximately 300 eggs a day (one egg every 4.8 minutes), which are deposited on the endothelial lining of the venous capillary walls. Most of the body mass of female schistosomes is devoted to the reproductive system. The female converts the equivalent of almost her own body dry weight into eggs each day. The eggs move into the lumen of the host's intestines and are released into the environment with the faeces.



Figure 6.9: *Schistosoma cercaria*.

6.11 View Prepared Slides of *Schistosoma*

1. Cercariae w.m. (Figure 6.9).

6.12 Monogenea

Monogeneans are a group of ectoparasite which are commonly found on the skin, gills, or fins of fish. They have a direct life cycle and do not require an intermediate host. Adults are hermaphrodites, meaning they have both male and female reproductive structures.

6.13 Molluska

Molluska¹⁴ are a large phylum of invertebrate animals whose members are known as mollusks. Around 85,000 extant species of mollusks are recognized. Mollusks are the largest marine phylum, comprising about 23% of all the named marine organisms. Numerous mollusks also live in freshwater and terrestrial habitats. They are highly diverse, not just in size and in anatomical structure, but also in behavior and in habitat. The phylum is typically divided into 9 or 10 taxonomic classes, of which two are entirely extinct. Cephalopod mollusks, such as squid, cuttlefish and octopus, are among the most neurologically advanced of all invertebrates—and either the giant squid or the colossal squid is the largest known invertebrate species. The gastropods (snails and slugs) are by far the most numerous mollusks in terms of classified species, and account for 80% of the total.

¹⁴<https://en.wikipedia.org/wiki/Mollusca>

The three most universal features defining modern mollusks are a mantle with a significant cavity used for breathing and excretion, the presence of a radula (except for bivalves), and the structure of the nervous system. Other than these things, mollusks express great morphological diversity, so many textbooks base their descriptions on a “hypothetical ancestral mollusk” (see image below). This has a single, “limpet-like” shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface. The underside of the animal consists of a single muscular “foot”. Although mollusks are coelomates, the coelom tends to be small. The main body cavity is a hemocoel through which blood circulates; their circulatory systems are mainly open. The “generalized” mollusk’s feeding system consists of a rasping “tongue”, the radula, and a complex digestive system in which exuded mucus and microscopic, muscle-powered “hairs” called cilia play various important roles. The generalized mollusk has two paired nerve cords, or three in bivalves. The brain, in species that have one, encircles the esophagus. Most mollusks have eyes, and all have sensors to detect chemicals, vibrations, and touch. The simplest type of molluskan reproductive system relies on external fertilization, but more complex variations occur. All produce eggs, from which may emerge trochophore larvae, more complex veliger larvae, or miniature adults.

Good evidence exists for the appearance of gastropods, cephalopods and bivalves in the Cambrian period 541 to 485.4 million years ago.

Mollusks have been and still are an important food source for anatomically modern humans. However there is a risk of food poisoning from toxins which can accumulate in certain mollusks under specific conditions, and because of this, many countries have regulations to reduce this risk. Mollusks have, for centuries, also been the source of important luxury goods, notably pearls, mother of pearl, Tyrian purple dye, and sea silk. Their shells have also been used as money in some preindustrial societies.

Mollusk species can also represent hazards or pests for human activities. Schistosomiasis (also known as bilharzia, bilharziosis or snail fever) is transmitted to humans via water snail hosts, and affects about 200 million people. Snails and slugs can also be serious agricultural pests, and accidental or deliberate introduction of some snail species into new environments has seriously damaged some ecosystems.

6.14 Bivalvia

Bivalves¹⁵ are a class of marine and freshwater mollusks that have laterally compressed bodies enclosed by a shell consisting of two hinged parts. Bivalves as a group have no head and they lack some usual molluskan organs like the radula and the odontophore (the cartilage which underlies and supports the radula and forms a ribbon of “teeth”). They include the clams, oysters, cockles, mussels, scallops, and numerous other families that live in saltwater, as well as a number of families that live in freshwater. The majority are filter feeders. The gills evolved into a specialized respiratory organ for feeding and breathing called ctenidium. Most bivalves bury themselves in sediment where they are relatively safe from predation. Others lie on the sea floor or attach themselves to rocks or other hard surfaces. Some bivalves, such as the scallops and file shells, can swim. The shipworms bore into wood, clay, or stone and live inside these substances.

The shell of a bivalve is composed of calcium carbonate, and consists of two, usually similar, parts called valves. These are joined together along one edge (the hinge line) by a flexible ligament that, usually in conjunction with interlocking “teeth” on each of the valves, forms the hinge. This arrangement allows the shell to be opened and closed without the two halves detaching. The shell is typically bilaterally symmetrical, with the hinge lying in the sagittal plane. Adult shell sizes of bivalves vary from fractions of a millimeter to over a meter in length, but the majority of species do not exceed 10 cm.

Bivalves appear in the fossil record first in the early Cambrian more than 500 million years ago. The total number of living species is about 9,200.

The sexes are usually separate in bivalves but some hermaphroditism is known. The gonads are located close to the intestines, and either open into the nephridia, or through a separate pore into the mantle cavity. The ripe gonads of male and females release sperm and eggs into the water column. Spawning may take place continually or be triggered by environmental factors such as day length, water temperature, or the presence of sperm in the water.

Fertilization is usually external. Typically, a short stage lasts a few hours or days before the eggs hatch into trochophore larvae. These later develop into veliger larvae which settle on the seabed and undergo metamorphosis into juveniles. In some species, such

¹⁵<https://en.wikipedia.org/wiki/Bivalvia>

as those in the genus *Lasaea*, females draw water containing sperm in through their inhalant siphons and fertilization takes place inside the female. These species then brood the young inside their mantle cavity, eventually releasing them into the water column as veliger larvae or as crawl-away juveniles.

Most of the bivalve larvae that hatch from eggs in the water column feed on diatoms or other phytoplankton. In temperate regions, about 25% of species are lecithotrophic, depending on nutrients stored in the yolk of the egg where the main energy source is lipids. The larvae hatching out of these rely on the energy reserves and do not feed.

6.15 Clams

Clams¹⁶ have two shells of equal size connected by two adductor muscles and have a powerful burrowing foot. Some clams have life cycles of only one year, while at least one may be over 500 years old. All clams have two calcareous shells or valves joined near a hinge with a flexible ligament, and all are filter feeders. A clam's shell consists of two (usually equal) valves, which are connected by a hinge joint and a ligament that can be external or internal. The ligament provides tension to bring the valves apart, while one or two adductor muscles can contract to close the valves. Clams also have kidneys, a heart, a mouth, a stomach, a nervous system and an anus. Many have a siphon.

Clams are protandrous hermaphrodites¹⁷ which means that they begin life as males but by the end of the first year approximately half of the population will change to females. Male clams release sperm into the water which stimulates females to expel eggs. A female may spawn several times each year, producing millions of eggs. Within 12 to 14 hours, the fertilized egg hatches into a microscopic creature called a trochophore larva (from the ancient Greek *trókhos*, meaning “wheel”, and *phoréō*, meaning “to bear, to carry” because the larva is bearing a wheel-shaped band of cilia). In less than a day, it transforms into a veliger larva (from Latin *velum* “sail, curtain, covering, veil” and *-ger* “bearing”), a free-swimming animal that has tiny wing-like lobes that propel it through the water. The foot, shell and body organs begin to form during the veliger stage, which lasts approximately 6 to 10 days. As the tiny

shell develops, the veliger drops to the sea floor and sheds its lobes. When it touches bottom, it sends out thin filaments to hold it in place. As the clam matures, a muscular foot will replace these filaments, allowing the clam to bury itself in the sediments with only its siphons protruding.

6.16 Gastropoda

Gastropods¹⁸ (previously known as univalves) are a major part of the phylum Mollusca, and are the most highly diversified class in the phylum, with 65,000 to 80,000 living snail and slug species.

In the older classification of the gastropods, there were four subclasses:

1. Opisthobranchia (gills to the right and behind the heart).
2. Gymnomorpha (no shell)
3. Prosobranchia (gills in front of the heart).
4. Pulmonata (with a lung instead of gills)

The class Gastropoda has an extraordinary diversification of habitats. Representatives live in gardens, woodland, deserts, and on mountains; in small ditches, great rivers and lakes; in estuaries, mudflats, the rocky intertidal, the sandy subtidal, in the abyssal depths of the oceans including the hydrothermal vents, and numerous other ecological niches, including parasitic ones.

Although the name “snail” can be, and often is, applied to all the members of this class, commonly this word means only those species with an external shell big enough that the soft parts can withdraw completely into it. Those gastropods without a shell, and those with only a very reduced or internal shell, are usually known as slugs; those with a shell into which they cannot withdraw are termed limpets.

The marine shelled species of gastropod include species such as abalone, conches, periwinkles, whelks, and numerous other sea snails that produce seashells that are coiled in the adult stage—though in some, the coiling may not be very visible, for example in cowries. In a number of families of species, such as all the various limpets, the shell is coiled only in the larval stage, and is a simple conical structure after that

Gastropods typically have a well-defined head with two or four sensory tentacles with eyes, and a ventral foot, which gives them their name (Greek

¹⁶<https://en.wikipedia.org/wiki/Clam>

¹⁷https://en.wikipedia.org/wiki/Sequential_hermaphroditism

¹⁸<https://en.wikipedia.org/wiki/Gastropoda>

gaster, stomach, and poda, feet). The upper pair of tentacles on the head of *Helix pomatia* have eye spots, but the main sensory organs of the snail are sensory receptors for olfaction, situated in the epithelium of the tentacles.

Sensory organs of gastropods include olfactory organs, eyes, statocysts and mechanoreceptors. Gastropods have no hearing. In terrestrial gastropods (land snails and slugs), the olfactory organs, located on the tips of the four tentacles, are the most important sensory organ. The majority of gastropods have simple visual organs, eye spots either at the tip or base of the tentacles. However, “eyes” in gastropods range from simple ocelli that only distinguish light and dark, to more complex pit eyes, and even to lens eyes. In land snails and slugs, vision is not the most important sense, because they are mainly nocturnal animals. The nervous system of gastropods includes the peripheral nervous system and the central nervous system. The central nervous system consists of ganglia connected by nerve cells.

The radula of a gastropod is usually adapted to the food that a species eats. The simplest gastropods are the limpets and abalones, herbivores that use their hard radula to rasp at seaweeds on rocks. Many marine gastropods are burrowers, and have a siphon that extends out from the mantle edge. Sometimes the shell has a siphonal canal to accommodate this structure. A siphon enables the animal to draw water into their mantle cavity and over the gill. They use the siphon primarily to “taste” the water to detect prey from a distance. Gastropods with siphons tend to be either predators or scavengers.

Almost all marine gastropods breathe with a gill, but many freshwater species, and the majority of terrestrial species, have a pallial lung.

Gastropods have open circulatory system and the transport fluid is hemolymph. Hemocyanin is present in the hemolymph as the respiratory pigment.

The primary organs of excretion in gastropods are nephridia, which produce either ammonia or uric acid as a waste product. The nephridium also plays an important role in maintaining water balance in freshwater and terrestrial species. Additional organs of excretion, at least in some species, include pericardial glands in the body cavity, and digestive glands opening into the stomach. In many marine gastropods other than the opisthobranchs, there are separate sexes; most land gastropods, however, are hermaphrodites.

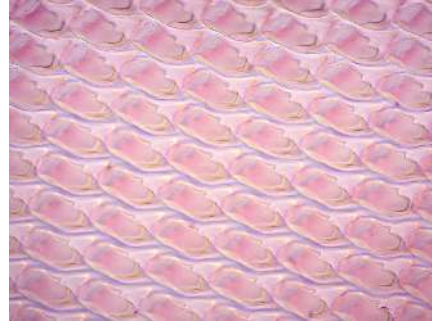


Figure 6.10: Snail radula.

6.17 View Prepared Slides of Molluska

1. Snail Radula w.m. (Figure 6.10)
 - Locate rows of chitinous teeth.

6.18 Cephalopoda

Cephalopods¹⁹ are members of the molluscan class Cephalopoda (Greek plural kephalópoda; “head-feet”) such as a squid, octopus or nautilus. These exclusively marine animals are characterized by bilateral body symmetry, a prominent head, and a set of arms or tentacles modified from the primitive molluscan foot. About 800 living species of cephalopods have been identified. Two important extinct taxa are the Ammonoidea (ammonites) and Belemnidea (belemnites).

Cephalopods are found in all the oceans of Earth. Cephalopods occupy most of the depth of the ocean, from the abyssal plain to the sea surface.

Cephalopods are widely regarded as the most intelligent of the invertebrates, and have well developed senses and large brains (larger than those of gastropods). The nervous system of cephalopods is the most complex of the invertebrates.

The brain is protected in a cartilaginous cranium. The giant nerve fibers of the cephalopod mantle have been widely used for many years as experimental material in neurophysiology; their large diameter (due to lack of myelination) makes them relatively easy to study compared with other animals. Many cephalopods are social creatures.

¹⁹<https://en.wikipedia.org/wiki/Cephalopod>

Cephalopods have advanced vision, can detect gravity with statocysts, and have a variety of chemical sense organs. Octopuses use their arms to explore their environment and can use them for depth perception.

Most cephalopods rely on vision to detect predators and prey, and to communicate with one another. Consequently, cephalopod vision is acute: training experiments have shown that the common octopus can distinguish the brightness, size, shape, and horizontal or vertical orientation of objects. The morphological construction gives cephalopod eyes the same performance as sharks'; however, their construction differs, as cephalopods lack a cornea, and have an everted retina. Cephalopods' eyes are also sensitive to the plane of polarization of light. Surprisingly, given their ability to change color, all octopodes and most cephalopods are considered to be color blind. Coleoid cephalopods (octopus, squid, cuttlefish) have a single photoreceptor type and lack the ability to determine color by comparing detected photon intensity across multiple spectral channels. When camouflaging themselves, they use their chromatophores to change brightness and pattern according to the background they see, but their ability to match the specific color of a background may come from cells such as iridophores and leucophores that reflect light from the environment. They also produce visual pigments throughout their body, and may sense light levels directly from their body.

Cephalopods are the only mollusks with a closed circulatory system. Like most mollusks, cephalopods use hemocyanin, a copper-containing protein, rather than hemoglobin, to transport oxygen. As a result, their blood is colorless when deoxygenated and turns blue when exposed to air.

Cephalopods exchange gases with the seawater by forcing water through their gills, which are attached to the roof of the organism. Water enters the mantle cavity on the outside of the gills, and the entrance of the mantle cavity closes. When the mantle contracts, water is forced through the gills, which lie between the mantle cavity and the funnel. The water's expulsion through the funnel can be used to power jet propulsion. The gills, which are much more efficient than those of other mollusks, are attached to the ventral surface of the mantle cavity.

All living cephalopods have a two-part beak; most have a radula, although it is reduced in most octopus. They feed by capturing prey with their tentacles, drawing it into their mouth and taking bites from it. They have a mixture of toxic digestive juices, some

of which are manufactured by symbiotic algae, which they eject from their salivary glands onto their captured prey held in their mouth. These juices separate the flesh of their prey from the bone or shell. The salivary gland has a small tooth at its end which can be poked into an organism to digest it from within.

The digestive gland itself is rather short. It has four elements, with food passing through the crop, stomach and caecum before entering the intestine. Most digestion, as well as the absorption of nutrients, occurs in the digestive gland, sometimes called the liver. Nutrients and waste materials are exchanged between the gut and the digestive gland through a pair of connections linking the gland to the junction of the stomach and caecum. Cells in the digestive gland directly release pigmented excretory chemicals into the lumen of the gut, which are then bound with mucus passed through the anus as long dark strings, ejected with the aid of exhaled water from the funnel.

Most cephalopods possess a single pair of large nephridia. Filtered nitrogenous waste is produced in the pericardial cavity of the branchial hearts, each of which is connected to a nephridium by a narrow canal. The canal delivers the excreta to a bladder-like renal sac, and also resorbs excess water from the filtrate. Several outgrowths of the lateral vena cava project into the renal sac, continuously inflating and deflating as the branchial hearts beat. This action helps to pump the secreted waste into the sacs, to be released into the mantle cavity through a pore.

6.19 Annelida

The annelids²⁰ (Annelida, from Latin *anellus*, "little ring"), also known as the ringed worms or segmented worms, are a large phylum, with over 17,000 extant species including ragworms, earthworms, and leeches. The species exist in and have adapted to various ecologies – some in marine environments as distinct as tidal zones and hydrothermal vents, others in fresh water, and yet others in moist terrestrial environments.

The annelids are bilaterally symmetrical, triploblastic, coelomate, invertebrate organisms. They also have parapodia for locomotion. Most textbooks still use the traditional division into polychaetes (almost all marine), oligochaetes (which include earthworms) and leech-like species. Cladistic research since 1997 has radically changed this scheme,

²⁰<https://en.wikipedia.org/wiki/Annelid>

viewing leeches as a sub-group of oligochaetes and oligochaetes as a sub-group of polychaetes. In addition, the Pogonophora, Echiura and Sipuncula, previously regarded as separate phyla, are now regarded as sub-groups of polychaetes. Annelids are considered members of the Lophotrochozoa, a “super-phylum” of protostomes that also includes mollusks²¹, brachiopods²¹, flatworms and nemertea²².

The basic annelid form consists of multiple segments. Each segment has the same sets of organs and, in most polychaetes, has a pair of parapodia that many species use for locomotion. Septa separate the segments of many species, but are poorly defined or absent in others, and Echiura and Sipuncula show no obvious signs of segmentation. In species with well-developed septa, the blood circulates entirely within blood vessels, and the vessels in segments near the front ends of these species are often built up with muscles that act as hearts. The septa of such species also enable them to change the shapes of individual segments, which facilitates movement by peristalsis (“ripples” that pass along the body) or by undulations that improve the effectiveness of the parapodia. In species with incomplete septa or none, the blood circulates through the main body cavity without any kind of pump, and there is a wide range of locomotory techniques – some burrowing species turn their pharynges inside out to drag themselves through the sediment.

Although many species can reproduce asexually and use similar mechanisms to regenerate after severe injuries, sexual reproduction is the normal method in species whose reproduction has been studied. The minority of living polychaetes whose reproduction and lifecycles are known produce trochophore larvae, that live as plankton and then sink and metamorphose into miniature adults. Oligochaetes are full hermaphrodites and produce a ring-like cocoon around their bodies, in which the eggs and hatchlings are nourished until they are ready to emerge.

Earthworms are Oligochaetes that support terrestrial food chains both as prey and in some regions, are important in aeration and enriching of soil. The burrowing of marine polychaetes, which may constitute up to a third of all species in near-shore environments, encourages the development of ecosystems by enabling water and oxygen to penetrate the sea floor. In addition to improving soil fertility, annelids serve humans as food and as bait. Scientists observe annelids to monitor the quality of marine and fresh wa-

ter. Although blood-letting is no longer in favor with doctors, some leech species are regarded as endangered species because they have been over-harvested for this purpose in the last few centuries.

Since annelids are soft-bodied, their fossils are rare – mostly jaws and the mineralized tubes that some of the species secreted. Although some late Ediacaran fossils may represent annelids, the oldest known fossil that is identified with confidence comes from about 518 million years ago in the early Cambrian period. Fossils of most modern mobile polychaete groups appeared by the end of the Carboniferous, about 299 million years ago. Palaeontologists disagree about whether some body fossils from the mid Ordovician, about 472 to 461 million years ago, are the remains of oligochaetes, and the earliest indisputable fossils of the group appear in the Tertiary period, which began 65 million years ago.

6.20 Earthworm

An earthworm²³ is a tube-shaped, segmented worm found in the phylum Annelida. Earthworms are commonly found living in soil, feeding on live and dead organic matter. An earthworm’s digestive system runs through the length of its body. It conducts respiration through its skin. It has a double transport system composed of coelomic fluid that moves within the fluid-filled coelom and a simple, closed blood circulatory system. It has a central and a peripheral nervous system. The central nervous system consists of two ganglia above the mouth, one on either side, connected to a nerve cord running back along its length to motor neurons and sensory cells in each segment. Large numbers of chemoreceptors are concentrated near its mouth. Circumferential and longitudinal muscles on the periphery of each segment enable the worm to move. Similar sets of muscles line the gut, and their actions move the digesting food toward the worm’s anus.

Earthworms are hermaphrodites—each individual carries both male and female sex organs. They lack either an internal skeleton or exoskeleton, but maintain their structure with fluid-filled coelom chambers that function as a hydrostatic skeleton.

Depending on the species, an adult earthworm can be from 10 mm long and 1 mm wide to 3 m long and over 25 mm wide, but the typical *Lumbricus*

²¹<https://en.wikipedia.org/wiki/Brachiopod>

²²<https://en.wikipedia.org/wiki/Nemertea>

²³<https://en.wikipedia.org/wiki/Earthworm>

*terrestris*²⁴ grows to about 360 mm long. Probably the longest worm on confirmed records is *Amyntas mekongianus*²⁵ that extends up to 3 m in the mud along the banks of the Mekong River in Southeast Asia.

From front to back, the basic shape of the earthworm is a cylindrical tube, divided into a series of segments (called metamerisms) that compartmentalize the body. Furrows are generally externally visible on the body demarking the segments; dorsal pores and nephridiopores exude a fluid that moistens and protects the worm's surface, allowing it to breathe. Except for the mouth and anal segments, each segment carries bristle-like hairs called lateral setae used to anchor parts of the body during movement; species may have four pairs of setae on each segment or more than eight sometimes forming a complete circle of setae per segment.

Generally, within a species, the number of segments found is consistent across specimens, and individuals are born with the number of segments they will have throughout their lives. The first body segment (segment number 1) features both the earthworm's mouth and, overhanging the mouth, a fleshy lobe called the prostomium, which seals the entrance when the worm is at rest, but is also used to feel and chemically sense the worm's surroundings. Some species of earthworm can even use the prehensile prostomium to grab and drag items such as grasses and leaves into their burrow.

An adult earthworm develops a belt-like glandular swelling, called the clitellum, which covers several segments toward the front part of the animal. This is part of the reproductive system and produces egg capsules. The posterior is most commonly cylindrical like the rest of the body, but depending on the species, may also be quadrangular, octagonal, trapezoidal, or flattened. The last segment is called the periproct; the earthworm's anus, a short vertical slit, is found on this segment.

The exterior of an individual segment is a thin cuticle over skin, commonly pigmented red to brown, which has specialized cells that secrete mucus over the cuticle to keep the body moist and ease movement through soil. Under the skin is a layer of nerve tissue, and two layers of muscles—a thin outer layer of circular muscle, and a much thicker inner layer of longitudinal muscle. Interior to the muscle layer is a fluid-filled chamber called a coelom that by its pressurization provides structure to the worm's boneless

body. The segments are separated from each other by septa (the plural of “septum”) which are perforated transverse walls, allowing the coelomic fluid to pass between segments. A pair of structures called nephrostomes are located at the back of each segment; a nephric tubule leads from each nephrostome through the septum and into the following segment. This tubule then leads to the main body fluid filtering organ, the nephridium or metanephridium, which removes metabolic waste from the coelomic fluid and expels it through pores called nephridiopores on the worm's sides; usually two nephridia (sometimes more) are found in most segments. At the center of a worm is the digestive tract, which runs straight through from mouth to anus without coiling, and is flanked above and below by blood vessels (the dorsal blood vessel and the ventral blood vessel as well as a subneural blood vessel) and the ventral nerve cord, and is surrounded in each segment by a pair of pallial blood vessels that connect the dorsal to the subneural blood vessels.

The earthworm's nervous system has three parts: the central nervous system (CNS), peripheral nervous system and the sympathetic nervous system.

The gut of the earthworm is a straight tube which extends from the worm's mouth to its anus. It is differentiated into a buccal cavity (generally running through the first one or two segments of the earthworm), pharynx (running generally about four segments in length), esophagus, crop, gizzard (usually) and intestine. Food enters in the mouth. The pharynx acts as a suction pump; its muscular walls draw in food. In the pharynx, the pharyngeal glands secrete mucus. Food moves into the esophagus. From there the food passes into the crop and gizzard. In the gizzard, strong muscular contractions grind the food with the help of mineral particles ingested along with the food. Once through the gizzard, food continues through the intestine for digestion. Instead of being coiled like a mammalian intestine, an earthworm's intestine increases surface area to increase nutrient absorption by having many folds running along its length. The intestine has its own pair of muscle layers like the body, but in reverse order—an inner circular layer within an outer longitudinal layer.

The earthworm has a dual circulatory system in which both the coelomic fluid and a closed circulatory system carry the food, waste, and respiratory gases. The closed circulatory system has five main blood vessels: the dorsal (top) vessel, which runs above the digestive tract; the ventral (bottom) vessel, which runs below the digestive tract; the subneural vessel, which

²⁴https://en.wikipedia.org/wiki/Lumbricus_terrestris

²⁵https://en.wikipedia.org/wiki/Amyntas_mekongianus

runs below the ventral nerve cord; and two lateroneural vessels on either side of the nerve cord. The dorsal vessel moves the blood forward, while the other four longitudinal vessels carry the blood rearward. In segments seven through eleven, a pair of aortic arches rings the coelom and acts as hearts, pumping the blood to the ventral vessel that acts as the aorta. The blood consists of ameboid cells and hemoglobin dissolved in the plasma. The second circulatory system derives from the cells of the digestive system that line the coelom.

The excretory system contains a pair of nephridia in every segment, except for the first three and the last ones. The waste in the coelom fluid from a forward segment is drawn in by the beating of cilia of the nephrostome. From there it is carried through the septum (wall) via a tube which forms a series of loops entwined by blood capillaries that also transfer waste into the tubule of the nephrostome. The excretory wastes are then finally discharged through a pore on the worm's side.

Earthworms have no special respiratory organs. Gases are exchanged through the moist skin and capillaries, where the oxygen is picked up by the hemoglobin dissolved in the blood plasma and carbon dioxide is released. Water, as well as salts, can also be moved through the skin by active transport.

Mating occurs on the surface, most often at night. Earthworms are hermaphrodites; that is, they have both male and female sexual organs. The sexual organs are located in segments 9 to 15. Earthworms have one or two pairs of testes contained within sacs. The two or four pairs of seminal vesicles produce, store and release the sperm via the male pores. Ovaries and oviducts in segment 13 release eggs via female pores on segment 14, while sperm is expelled from segment 15. One or more pairs of spermathecae are present in segments 9 and 10 (depending on the species) which are internal sacs that receive and store sperm from the other worm during copulation. As a result, segment 15 of one worm exudes sperm into segments 9 and 10 with its storage vesicles of its mate. Some species use external spermatophores for sperm transfer. Copulation and reproduction are separate processes in earthworms. The mating pair overlap front ends ventrally and each exchanges sperm with the other. The clitellum becomes very reddish to pinkish in color. Sometime after copulation, long after the worms have separated, the clitellum (behind the spermathecae) secretes material which forms a ring around the worm. The worm then backs out of the ring, and as it does so,



Figure 6.11: Clam

it injects its own eggs and the other worm's sperm into it. As the worm slips out of the ring, the ends of the cocoon seal to form a vaguely lemon-shaped incubator (cocoon) in which the embryonic worms develop. They emerge as small, but fully formed earthworms, but lack their sex structures, which develop in about 60 to 90 days. They attain full size in about one year. Scientists predict that the average lifespan under field conditions is four to eight years, while most garden varieties live only one to two years.

6.21 View Living Organisms

1. Turbellaria
2. Rotifers

6.22 Clam Dissection

1. Get a dissecting pan, a scalpel, forceps, and a pointer.
2. Get a clam.
3. Identify the anterior and posterior ends of the clam as well as the dorsal, ventral, and left and right lateral surfaces (Figure 6.11).
4. Place a clam in a dissecting tray with the left side up facing you.
5. Locate the umbo, the bump at the anterior end of the valve. This is the oldest part of the clam shell. Find the hinge ligament which hinges the valves together and observe the growth rings.
6. Locate the adductor muscles. With your blade pointing toward the dorsal edge, slide your scalpel between the two valves & cut through the anterior adductor muscle, cutting as close to the shell as possible.

7. Repeat step 6 in cutting the posterior adductor muscle.
8. Bend the left valve back so it lies flat on the tray. Once both adductor muscles have been cut away from the left shell, the shell can be opened easily.
9. Locate the muscle “scars” on the inner surface of the left valve. The adductor muscles were attached here to hold the clam closed.
10. Identify the mantle, the tissue that lines both valves & covers the soft body of the clam. Find the mantle cavity, the space inside the mantle.
11. Locate two openings on the posterior end of the clam. The more ventral opening is the incurrent siphon that carries water into the clam and the more dorsal opening is the excurrent siphon where wastes and water leave. The large muscle attached to the siphons is called the siphon retractor muscle. There would be another one on the right side. These muscles pull the siphon in. Most clams can retract the siphons completely into the shell.
12. Lift the mantle up. Notice how thin it is. Remove the mantle to expose the gills and the foot. Notice the structure of the gills. Bivalves are filter feeders. The gills are used to strain plankton out of the water, as well as to remove oxygen from the water. There are another pair of gills on the right side of the clam.
13. Observe the muscular foot of the clam, which is ventral to the gills. Note the hatchet shape of the foot used to burrow into mud or sand.
14. Locate the labial palps, flaplike structures that surround and guide food into the clam’s mouth. The palps are anterior to the gills & ventral to the anterior adductor muscle. Between the palps, find the mouth.
15. Carefully make an incision in the middle of the visceral mass to view the internal organs.
16. Locate the spongy, yellowish reproductive organs.
17. Ventral to the umbo, find the digestive gland, a greenish structure that surrounds the stomach.
18. Locate the long, coiled intestine extending from the stomach.
19. Follow the intestine through the clam. Find the area near the dorsal surface that the intestine passes through called the pericardial area. Find the clam’s heart in this area.
20. Continue following the intestine toward the posterior end of the clam. The heart is contained in a thin-walled sac called the pericardium near the top of the clam above the visceral mass. The tube-like structure that runs through the

pericardium is the intestine.

6.23 Cleaning up

1. Dispose of the remains of the clam in the red biohazard bins.
2. Clean the dissection tray and instruments.

6.24 Earthworm Dissection

1. Get a dissecting pan, a scalpel, forceps, and a pointer.
2. Get an earthworm.
3. Position your preserved earthworm dorsal side up and pin it down through the first segment and then again further back behind the clitellum (Figure 6.12).
4. Cut a slit in the dorsal surface near the posterior pin and extend the cut forward to the first segment. Be careful not to cut too deep.
5. Starting at the first segment, cut the septa (thin membranes) that internally divide the segments, so the skin can be laid flat. Use additional pins to hold the integument open and expose the organs.
6. Continue to lay the skin back until you have uncovered a centimeter or so of the intestine.
7. Locate and identify the five pairs of aortic arches, or hearts. Then find the dorsal blood vessel. Look for smaller blood vessels that branch from the dorsal blood vessel.
8. Starting at the anterior end, locate the muscular pharynx (food ingestion). This is followed by a tube-like esophagus which terminates in a crop (the wider organ) which serves as a storage stomach. Posterior to the crop is the gizzard. Gently press on the crop and gizzard to test their firmness. While the crop is soft and thin, the gizzard is muscular (soil is ground up and churned within the gizzard). The gizzard is followed by a long intestine in which both digestion and absorption occur. Undigested material is voided through the anus.
9. To find organs of the nervous system, push aside the digestive and circulatory system organs. Locate the ventral nerve cord. Trace the nerve cord forward to the nerve collar, which circles the pharynx. Find one pair of ganglia under the pharynx and another pair of ganglia above the pharynx. The ganglia above the pharynx serve as the brain of the earthworm.



Figure 6.12: Earthworm dissection

10. Locate the nephridia. There are two in every segment.
11. Locate and identify a pair of ovaries in segment 13.
12. Look for two pairs of tiny testes in segments 10 and 11. To find these organs, you will again have to push aside some parts already dissected.

6.25 Cleaning up

1. Dispose of the remains of the earthworm in the red biohazard bins.
2. Clean the dissection tray and instruments and return them to the place where you picked them up.
3. Clean table tops with red bottled sanitizer
4. Wash hands before leaving class.

6.26 Review Questions

1. What are rotifers?
2. What are flat worms?
3. What are mollusks?
4. What are annelids?
5. What is segmentation?
6. What is a radula?
7. What is a closed circulatory system?
8. What is an open circulatory system?

Chapter 7

Nematoda and Arthropoda

7.1 Nematodes

The nematodes¹ or roundworms constitute the phylum Nematoda (also called Nematelminthes). They are a diverse animal phylum inhabiting a broad range of environments. Nematode species can be difficult to distinguish, and although over 25,000 have been described, of which more than half are parasitic, it is estimated that over 40,000 species exist. Nematodes are classified along with insects and other moulting animals in the clade Ecdysozoa, and, unlike flatworms, have tubular digestive systems with openings at both ends. A characteristic of Nematoda is the one-way digestive tract, with a pseudocoelom (body cavity made up of only an ectoderm and endoderm).

Nematodes have successfully adapted to nearly every ecosystem from marine (salt water) to fresh water, to soils, and from the polar regions to the tropics, as well as the highest to the lowest of elevations. They are ubiquitous in freshwater, marine, and terrestrial environments, where they often outnumber other animals in both individual and species counts, and are found in locations as diverse as mountains, deserts and oceanic trenches. They are found in every part of the earth's lithosphere, even at great depths, 0.9–3.6 km, below the surface of the Earth in gold mines in South Africa. They represent 90% of all animals on the ocean floor. Their numerical dominance, often exceeding a million individuals per square meter and accounting for about 80% of all individual animals on earth, their diversity of life cycles, and their presence at various trophic levels point at an important role in many ecosystems. The many parasitic forms include pathogens in most plants and animals. A third of the genera occur as parasites of vertebrates; about 35 nematode species occur in humans.

Nematodes are small, slender worms: typically approximately 5 to 100 μm thick, and 0.1 to 2.5 mm long. The smallest nematodes are microscopic, while free-living species can reach as much as 5 cm, and some parasitic species are larger still, reaching over 1 m in length. The body is often ornamented with ridges, rings, bristles, or other distinctive structures.

The head of a nematode is relatively distinct. Whereas the rest of the body is bilaterally symmetrical, the head is radially symmetrical, with sensory bristles and, in many cases, solid 'head-shields' radiating outwards around the mouth. The mouth has either three or six lips, which often bear a series of teeth on their inner edges. An adhesive 'caudal gland' is often found at the tip of the tail.

The epidermis is either a syncytium or a single layer of cells, and is covered by a thick collagenous cuticle. The cuticle is often of complex structure, and may have two or three distinct layers. Underneath the epidermis lies a layer of longitudinal muscle cells. The relatively rigid cuticle works with the muscles to create a hydroskeleton as nematodes lack circumferential muscles. Projections run from the inner surface of muscle cells towards the nerve cords; this is a unique arrangement in the animal kingdom, in which nerve cells normally extend fibres into the muscles rather than vice versa.

The oral cavity is lined with cuticle, which is often strengthened with ridges or other structures, and, especially in carnivorous species, may bear a number of teeth. The mouth often includes a sharp stylet, which the animal can thrust into its prey. In some species, the stylet is hollow, and can be used to suck liquids from plants or animals. The oral cavity opens into a muscular, sucking pharynx, also lined with cuticle. Digestive glands are found in this region of the gut, producing enzymes that start to break down the food. In stylet-bearing species, these may even be

¹<https://en.wikipedia.org/wiki/Nematode>

injected into the prey. There is no stomach, with the pharynx connecting directly to a intestine (not surrounded by smooth muscle) that forms the main length of the gut. This produces further enzymes, and also absorbs nutrients through its single cell thick lining. The last portion of the intestine is lined by cuticle, forming a rectum, which expels waste through the anus just below and in front of the tip of the tail. Movement of food through the digestive system is the result of body movements of the worm. The intestine has valves or sphincters at either end to help control the movement of food through the body.

Nitrogenous waste is excreted in the form of ammonia through the body wall, and is not associated with any specific organs. However, the structures for excreting salt to maintain osmoregulation are typically more complex. In many marine nematodes, one or two unicellular ‘renette glands’ excrete salt through a pore on the underside of the animal, close to the pharynx. In most other nematodes, these specialized cells have been replaced by an organ consisting of two parallel ducts connected by a single transverse duct. This transverse duct opens into a common canal that runs to the excretory pore.

Four peripheral nerves run the length of the body on the dorsal, ventral, and lateral surfaces. Each nerve lies within a cord of connective tissue lying beneath the cuticle and between the muscle cells. The ventral nerve is the largest, and has a double structure forward of the excretory pore. The dorsal nerve is responsible for motor control, while the lateral nerves are sensory, and the ventral combines both functions. The nervous system is also the only place in the nematode body that contains cilia, which are all non-motile and with a sensory function. At the anterior end of the animal, the nerves branch from a dense, circular nerve (nerve ring) round surrounding the pharynx, and serving as the brain. Smaller nerves run forward from the ring to supply the sensory organs of the head. The bodies of nematodes are covered in numerous sensory bristles and papillae that together provide a sense of touch. Behind the sensory bristles on the head lie two small pits, or ‘amphids’. These are well supplied with nerve cells, and are probably chemoreception organs. A few aquatic nematodes possess what appear to be pigmented eye-spots, but is unclear whether or not these are actually sensory in nature.

Most nematode species are dioecious, with separate male and female individuals, though some, such as *Caenorhabditis elegans*², are androdioecious, con-

sisting of hermaphrodites and rare males. Both sexes possess one or two tubular gonads. In males, the sperm are produced at the end of the gonad and migrate along its length as they mature. The testis opens into a relatively wide seminal vesicle and then during intercourse into a glandular and muscular ejaculatory duct associated with the vas deferens and cloaca. In females, the ovaries each open into an oviduct (in hermaphrodites, the eggs enter a spermatheca first) and then a glandular uterus. The uteri both open into a common vulva/ vagina, usually located in the middle of the morphologically ventral surface.

Reproduction is usually sexual, though hermaphrodites are capable of self-fertilization. Males are usually smaller than females/ hermaphrodites (often much smaller) and often have a characteristically bent or fan-shaped tail. During copulation, one or more chitinized spicules move out of the cloaca and are inserted into the genital pore of the female. Amoeboid sperm crawl along the spicule into the female worm.

Eggs may be embryonated (containing an embryo) or unembryonated when passed by the female, meaning their fertilized eggs may not yet be developed. A few species are known to be ovoviviparous. The eggs are protected by an outer shell, secreted by the uterus. In free-living roundworms, the eggs hatch into larvae, which appear essentially identical to the adults, except for an underdeveloped reproductive system; in parasitic roundworms, the life cycle is often much more complicated.

Nematodes as a whole possess a wide range of modes of reproduction. Some nematodes, such as *Heterorhabditis* spp., undergo a process called endotokia matricida: intrauterine birth causing maternal death. Some nematodes are hermaphroditic, and keep their self-fertilized eggs inside the uterus until they hatch. The juvenile nematodes will then ingest the parent nematode. This process is significantly promoted in environments with a low food supply.

Nematodes commonly parasitic on humans include ascarids (*Ascaris*), filarias, hookworms, pinworms (*Enterobius*), and whipworms (*Trichuris trichiura*). The species *Trichinella spiralis*³, commonly known as the ‘trichina worm’, occurs in rats, pigs, and humans, and is responsible for the disease trichinosis.

²https://en.wikipedia.org/wiki/Caenorhabditis_elegans

³https://en.wikipedia.org/wiki/Trichinella_spiralis

7.2 *Ascaris*

*Ascaris*⁴ is a genus of parasitic nematode worms known as the “small intestinal roundworms”. One species, *Ascaris lumbricoides*, affects humans and causes the disease ascariasis. Their eggs are deposited in feces and soil. Plants with the eggs on them infect any organism that consumes them. *A. lumbricoides* is the largest intestinal roundworm and is the most common helminth infection of humans worldwide. Infestation can cause morbidity by compromising nutritional status, affecting cognitive processes, inducing tissue reactions such as granuloma to larval stages, and by causing intestinal obstruction, which can be fatal. The body is long, cylindrical, fusiform (pointed at both the ends), body wall is composed of cuticle, epidermis and musculature. Presence of a false body pseudocoelom not lined by epithelium. Digestive system is complete. Respiration by simple diffusion. Nervous system consists of a nerve ring and many longitudinal nerve cords. Only sexual reproduction. Sexes are separate with sexual dimorphism. Males are usually shorter than females.



Figure 7.1: Female *Ascaris*.

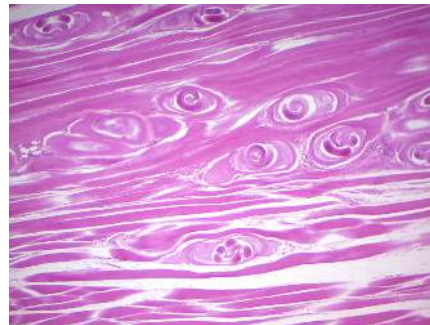


Figure 7.2: *Trichinella* encysted in muscle.

7.3 View Prepared Slides of *Ascaris*

1. Female *Ascaris* x.s. (Figure 7.1)
 - Identify: oviduct, pseudocoelom, intestine with lumen (GVC), uterus, eggs, ventral nerve cord, longitudinal muscles, cuticle, excretory canal

7.4 *Trichinella*

*Trichinella*⁵ is the genus of parasitic roundworms of the phylum Nematoda that cause trichinosis (also known as trichinellosis). Members of this genus are often called trichinella or trichina worms. The genus was first recognised in a larval form in 1835. The L1 larvae live in a modified skeletal muscle cell. The adult worms occupy a membrane-bound portion of columnar epithelium, living as intracellular parasites. Infections with this genus have been reported from more than 150 different naturally or experimentally

infected hosts. It has been shown to have a world-wide distribution in domestic and/or sylvatic animals. *Trichinella* is known as the smallest human nematode parasite, yet it is also the largest of all intracellular parasites. Oral ingestion of larvae-contaminated tissue is the usual route of infection. Cooking pork meat properly or by freezing pork, *Trichinella* infection can be prevented. However, freezing pork is not an effective method for killing larvae.

7.5 View Prepared Slides of *Trichinella*

1. *Trichinella* encysted in muscle (Figure 7.2)
 - Identify: muscle, cyst, larva

⁴<https://en.wikipedia.org/wiki/Ascaris>

⁵<https://en.wikipedia.org/wiki/Trichinella>

7.6 Vinegar eels

*Turbatrix aceti*⁶ (vinegar eels, vinegar nematode) are free-living nematodes that feed on the microbial culture, called mother of vinegar used to create vinegar, and may be found in unfiltered vinegar. Vinegar eels are often given to fry (baby fish) as a live food, like microworms. Although they are harmless and non-parasitic, leaving eels in vinegar is considered objectionable in the United States and is not permitted in vinegar destined for American consumers. Manufacturers normally filter and pasteurize their product prior to bottling, destroying the live bacterial and yeast culture that these nematodes require for sustenance.

7.7 Arthropods

Arthropods⁷ (from arthron, “joint” and pous, “foot”) are invertebrate animals having an exoskeleton (external skeleton), a segmented body, and paired jointed appendages. Arthropods form the phylum Euarthropoda, which includes insects, arachnids, myriapods, and crustaceans. Arthropods are characterized by their jointed limbs and cuticle made of chitin, often mineralized with calcium carbonate. The arthropod body plan consists of segments, each with a pair of appendages. The rigid cuticle inhibits growth, so arthropods replace it periodically by molting.

Their versatility has enabled them to become the most species-rich members of all ecological guilds in most environments. They have over a million described species, making up more than 80% of all described living animal species, some of which, unlike most animals, are very successful in dry environments.

Arthropods range in size from the microscopic crustacean *Stygotantulus* up to the Japanese spider crab. Arthropods’ primary internal cavity is a hemocoel, which accommodates their internal organs, and through which their hemolymph – analogue of blood – circulates; they have open circulatory systems. Like their exteriors, the internal organs of arthropods are generally built of repeated segments. Their nervous system is “ladder-like”, with paired ventral nerve cords running through all segments and forming paired ganglia in each segment.

Their heads are formed by fusion of varying numbers of segments, and their brains are formed by fusion of the ganglia of these segments and encircle the esophagus. The respiratory and excretory systems of arthropods vary, depending as much on their environment as on the subphylum to which they belong.

Their vision relies on various combinations of compound eyes and pigment-pit ocelli: in most species, the ocelli can only detect the direction from which light is coming, and the compound eyes are the main source of information, but the main eyes of spiders are ocelli that can form images and, in a few cases, can swivel to track prey. Arthropods also have a wide range of chemical and mechanical sensors, mostly based on modifications of the many setae (bristles) that project through their cuticles. Arthropods’ methods of reproduction and development are diverse; all terrestrial species use internal fertilization, but this is often by indirect transfer of the sperm via an appendage or the ground, rather than by direct injection.

Aquatic species use either internal or external fertilization. Almost all arthropods lay eggs, but scorpions give birth to live young after the eggs have hatched inside the mother. Arthropod hatchlings vary from miniature adults to grubs and caterpillars that lack jointed limbs and eventually undergo a total metamorphosis to produce the adult form. The level of maternal care for hatchlings varies from nonexistent to the prolonged care provided by scorpions.

The evolutionary ancestry of arthropods dates back to the Cambrian period. The group is generally regarded as monophyletic, and many analyses support the placement of arthropods with cycloneurals (or their constituent clades) in a superphylum Ecdysozoa.

Arthropods contribute to the human food supply both directly as food, and more importantly indirectly as pollinators of crops. Some species are known to spread severe disease to humans, livestock, and crops.

Arthropods belong to the phylum Euarthropoda. The phylum is sometimes called Arthropoda, but strictly this term denotes a (putative - see Tactopoda) clade that also encompasses Phylum Onychophora.

The appendages of arthropods may be either biramous or uniramous. A uniramous limb comprises a single series of segments attached end-to-end. A biramous limb, however, branches into two, and each branch consists of a series of segments attached end-

⁶https://en.wikipedia.org/wiki/Turbatrix_aceti

⁷<https://en.wikipedia.org/wiki/Arthropod>

to-end.

The phylum Euarthropoda is typically subdivided into five subphyla, of which one is extinct:

- Trilobites are a group of formerly numerous marine animals that disappeared in the Permian–Triassic extinction event, though they were in decline prior to this killing blow, having been reduced to one order in the Late Devonian extinction.
- Chelicerates include horseshoe crabs, spiders, mites, scorpions and related organisms. They are characterized by the presence of chelicerae, appendages just above / in front of the mouth. Chelicerae appear in scorpions and horseshoe crabs as tiny claws that they use in feeding, but those of spiders have developed as fangs that inject venom.
- Myriapods comprise millipedes, centipedes, and their relatives and have many body segments, each segment bearing one or two pairs of legs (or in a few cases being legless). They are sometimes grouped with the hexapods.
- Crustaceans are primarily aquatic (a notable exception being woodlice) and are characterized by having biramous appendages. They include lobsters, crabs, barnacles, crayfish, shrimp and many others.
- Hexapods comprise insects and three small orders of insect-like animals with six thoracic legs. They are sometimes grouped with the myriapods, in a group called Uniramia, though genetic evidence tends to support a closer relationship between hexapods and crustaceans.

7.8 Crayfish

Crayfish⁸, also known as crawfish, crawdads, freshwater lobsters, mountain lobsters, mudbugs or yabbies, are freshwater crustaceans resembling small lobsters, to which they are related. They breathe through feather-like gills. Some species are found in brooks and streams where there is running fresh water, while others thrive in swamps, ditches, and paddy fields. Crayfish feed on animals and plants, either living or decomposing, and detritus. Its body is made up of twenty body segments grouped into two main body parts, the cephalothorax and the abdomen. Each segment may possess one pair of appendages, although in various groups these may be reduced or missing. On

average, crayfish grow to 17.5 centimeters in length, but some grow larger. Walking legs have a small claw at the end.

7.9 Crayfish Dissection

1. Get a dissection pan, scissors, forceps, and a pointer.
2. Get a cray fish.
3. Place a crayfish on its side in a dissection tray (Fig. 7.3).
4. Locate the cephalothorax and the abdomen. The carapace, a shield of chitin, covers the dorsal surface of the cephalothorax. On the carapace, observe an indentation, the cervical groove, that extends across the midregion and separates the head and thoracic regions. On the thoracic region, locate the prominent suture or indentation on the cephalothorax that defines a central area separate from the sides. Note the individual segments of the abdomen.
5. Turn the crayfish with its dorsal side upward.
6. Locate the rostrum, which is the pointed extension of the carapace at the head of the animal. Locate the two eyes at the end of stalks.
7. Locate the five pairs of appendages on the head region. First locate the antennules in the most anterior segment. Behind them observe the much longer pair of antennae.
8. Turn the crayfish around with its ventral side upward.
9. Locate the mouth. Observe the mandibles (jaws), behind the antennae.
10. Locate the two pairs of maxillae, which are the last appendages in the cephalic region.
11. On the thoracic portion of the cephalothorax, observe the three pointed maxillipeds.
12. On the abdomen, observe six distinct segments. Observe a pair of swimmerets on each of the first five segments.
13. On the last abdominal segment, observe a pair of pointed appendages modified into a pair of uropods. In the middle of the uropods, locate the triangular-shaped telson.
14. Determine the sex of your specimen. Males (Fig. 7.4 have hardened gonapophyses and hooks on the third pair of legs. Females (7.5 have an opening to a seminal receptacle between the fifth pair of legs.
15. Turn the crayfish around so that its dorsal side is upward.

⁸<https://en.wikipedia.org/wiki/Crayfish>

16. Use one hand to hold the crayfish dorsal side up in the dissecting tray, use scissors to carefully cut through the back of the carapace along the dotted line shown in figure 7.6 below. Cut along the indentations that separate the thoracic portion of the carapace into three regions. Start the cut at the posterior edges of the carapace, and extend it along both sides in the cephalic region.
17. Using the forceps, carefully and slowly lift away the carapace.
18. Place the crayfish on its side (head facing left). Using scissors, cut starting at the base along the dotted line shown in Figure 7.7. Extend the cut line forward toward the rostrum (at the top of the head).
19. Use forceps to carefully lift away the remaining parts of the carapace, exposing the underlying gills and other organs.
20. Locate and identify the organs of the digestive system. Locate the maxillae that pass the pieces of food into the mouth. The food travels down the short esophagus into the stomach. Locate the digestive gland, which produces digestive substances and from which the absorption of nutrients occurs. Undigested material passes into the intestine. Observe that the intestine is attached to the lobed stomach. The undigested material is eliminated from the anus.
21. Locate the gills, which are feather-like structures found underneath the carapace and attached to the chelipeds and walking legs.
22. Locate the dorsal tubular heart and several arteries. Notice the holes in the heart indicating that the crayfish has an open circulatory system in which the blood flows from arteries into sinuses, or spaces, in tissues. The blood flows over the gills before returning to the heart through the holes.
23. Find the ventral nerve cord. Locate a ganglion, one of the enlargements of the ventral nerve cord. Locate the dorsal brain, which is located just behind the compound eyes. Note the two large nerves that lead from the brain, around the esophagus, and join the ventral nerve cord.
24. Locate and identify the organs of the reproductive system. If the specimen is male, locate the testis. The testis is the long, white organ under the heart. If the specimen is a female, locate the bi-lobed ovary. It is in the same relative position as the testis, but the ovary appears as a large, reddish mass under the heart.

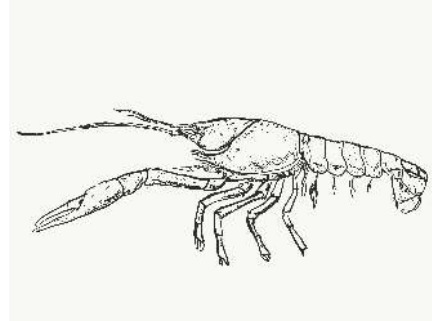


Figure 7.3: Crayfish

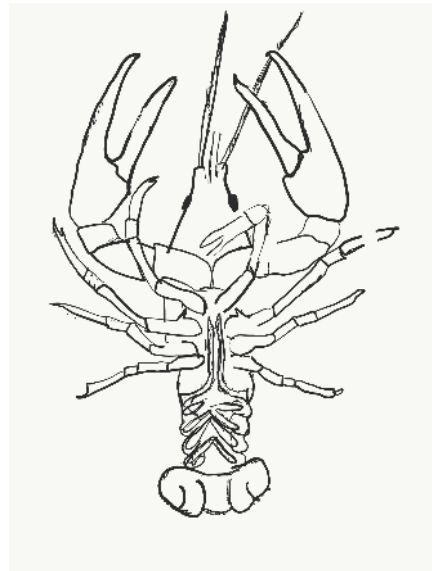


Figure 7.4: Ventral view of a male crayfish. Notice the gonapods and hooks.

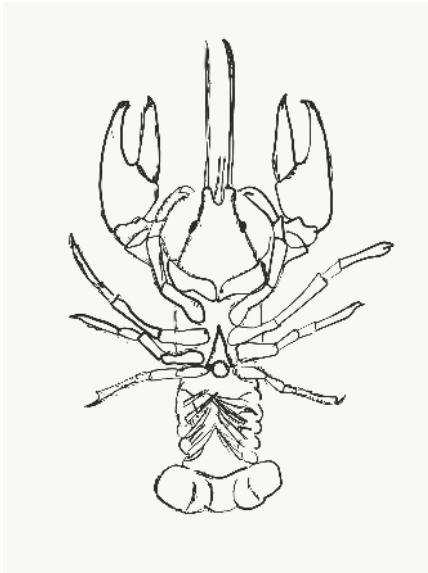


Figure 7.5: Ventral view of a female crayfish. Notice the central opening to the seminal receptacle.

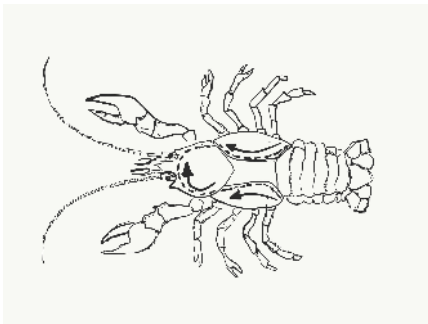


Figure 7.6: Dorsal view of the crayfish. Cut along the dotted lines.

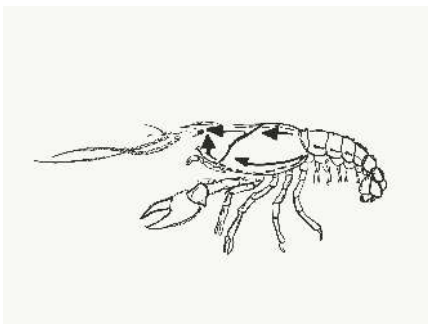


Figure 7.7: Lateral view of the crayfish. Cut along the dotted lines.

7.10 Cleaning up

1. Dispose of the remains of the crayfish in the red biohazard bins.
2. Clean the dissection tray and instruments and return them to the place where you picked them up.
3. Clean table tops with red bottled sanitizer.
4. Wash your hands.

7.11 View Living Organisms

1. Vinegar eels
2. Mixed crustaceans
 - Look for isopods (sow bugs), amphipods, copepods, water fleas (*Daphnia*), ostracods, and fairy shrimp.

7.12 Review Questions

1. What are nematodes?
2. What are arthropods?
3. What is an exoskeleton?
4. What are crustaceans?

Chapter 8

Echinodermata, Hemichordata and Chordata

Echinodermata¹ (the phylum which includes starfish, sea urchins, sea cucumbers, and crinoids) and Hemichordata², form the ambulacraria³, the sister taxon of the chordates. The Chordata⁴ and ambulacraria form the superphylum deuterostomia, composed of the deuterostomes. Deuterostomes are one of the two major divisions of the bilaterians, the other being the protostomes. During the early development of the embryo, in deuterostomes, the blastopore (the first opening to form) becomes the anus whereas in the protostomes, it becomes the mouth. In deuterostomes, the mouth develops at a later stage, at the opposite end of the blastula from the blastopore, and a gut forms connecting the two.

8.1 Echinodermata

Echinoderm is the common name given to any member of the phylum Echinodermata (from Ancient Greek, echinos – “hedgehog” and derma – “skin”) of marine animals. The adults are recognizable by their (usually five-point) radial symmetry, and include such well-known animals as sea stars, sea urchins, sand dollars, and sea cucumbers, as well as the sea lilies or “stone lilies”. Echinoderms are found at every ocean depth, from the intertidal zone to the abyssal zone. The phylum contains about 7000 living species, making it the second-largest grouping of deuterostomes (a superphylum), after the chordates (which include the vertebrates, such as birds, fishes, mammals, and reptiles). Echinoderms are also the largest phylum that has no freshwater or terrestrial

(land-based) representatives.

The first definitive members of the phylum appeared near the start of the Cambrian. One group of Cambrian echinoderms, the cinctans (Homalozoa), which are close to the base of the echinoderm origin, have been found to possess external gills used for filter feeding, like Chordata and Hemichordata.

The echinoderms are important both ecologically and geologically. Ecologically, there are few other groupings so abundant in the biotic desert of the deep sea, as well as shallower oceans. Most echinoderms are able to regenerate tissue, organs, limbs, and reproduce asexually; in some cases, they can undergo complete regeneration from a single limb. Geologically, the value of echinoderms is in their ossified skeletons, which are major contributors to many limestone formations, and can provide valuable clues as to the geological environment. They were the most used species in regenerative research in the 19th and 20th centuries.

Along with the chordates and hemichordates, echinoderms are deuterostomes, one of the two major divisions of the bilaterians, the other being the protostomes. During the early development of the embryo, in deuterostomes, the blastopore (the first opening to form) becomes the anus whereas in the protostomes, it becomes the mouth. In deuterostomes, the mouth develops at a later stage, at the opposite end of the blastula from the blastopore, and a gut forms connecting the two. The larvae of echinoderms have bilateral symmetry but this is lost during metamorphosis when their bodies are reorganized and develop the characteristic radial symmetry of the echinoderm, typically pentamerism (literally: consisting of 5 parts). The characteristics of adult echinoderms are the possession of a water vascular

¹<https://en.wikipedia.org/wiki/Echinoderm>

²<https://en.wikipedia.org/wiki/Hemichordata>

³<https://en.wikipedia.org/wiki/Ambulacraria>

⁴<https://en.wikipedia.org/wiki/Chordata>

system with external tube feet and a calcareous endoskeleton consisting of ossicles connected by a mesh of collagen fibres.

Two main subdivisions are traditionally recognized:

1. Eleutherozoa, which encompasses the Asterozoa (starfish), Ophiurozoa (brittle stars), Echinozoa (sea urchins and sand dollars) and Holothurozoa (sea cucumbers).
2. Platyhelminthes, some of which are sessile while others move around. These consist of the Ctenophora (comb jellies) and Scyphozoa (jellyfish).

Echinoderms have a mesodermal skeleton composed of calcareous plates or ossicles. Each one of these, even the articulating spine of a sea urchin, is composed mineralogically of a crystal of calcite. If solid, these would form a heavy skeleton, so they have a sponge-like porous structure known as stereom. Ossicles may be fused together, as in the test of sea urchins, or may articulate with each other as in the arms of sea stars, brittle stars and crinoids. The ossicles may be flat plates or bear external projections in the form of spines, granules or warts and they are supported by a tough epidermis (skin). Skeletal elements are also deployed in some specialized ways, such as the “Aristotle’s lantern” mouthparts of sea urchins used for grinding, the supportive stalks of crinoids and the structural “lime ring” of sea cucumbers.

The epidermis consists of cells responsible for the support and maintenance of the skeleton, as well as pigment cells, mechanoreceptor cells (which detect motion on the animal’s surface), and sometimes gland cells which secrete sticky fluids or even toxins. The varied and often vivid colors of echinoderms are produced by the action of skin pigment cells.

Echinoderms possess a unique water vascular system. This is a network of fluid-filled canals derived from the coelom (body cavity) that function in gas exchange, feeding, sensory reception and locomotion. This system varies between different classes of echinoderm but typically opens to the exterior through a sieve-like madreporite on the aboral (upper) surface of the animal. The madreporite is linked to a slender duct, the stone canal, which extends to a ring canal that encircles the mouth or esophagus. From this, radial canals extend along the arms of asteroids and adjoin the test in the ambulacral areas of echinoids. Short lateral canals branch off the radial canals, each one ending in an ampulla. Part of the ampulla can protrude through a pore (or a pair of pores in sea

urchins) to the exterior and is known as a podium or tube feet. The water vascular system assists with the distribution of nutrients throughout the animal’s body and is most obviously expressed in the tube feet which can be extended or contracted by the redistribution of fluid between the foot and the internal sac.

Echinoderms possess a simple digestive system which varies according to the animal’s diet. Starfish are mostly carnivorous and have a mouth, esophagus, two-part stomach, intestine and rectum, with the anus located in the center of the aboral body surface. In many species, the large cardiac stomach can be everted and digest food outside the body. In other species, whole food items such as mollusks may be ingested. Brittle stars have a blind gut with no intestine or anus. They have varying diets and expel food waste through their mouth. Sea urchins are herbivores and use their specialized mouthparts to graze, tear and chew algae and sometimes other animal or vegetable material. They have an esophagus, a large stomach and a rectum with the anus at the apex of the test. Sea cucumbers are mostly detritivores, sorting through the sediment with their buccal tentacles which are modified tube feet. Sand and mud accompanies their food through their simple gut which has a long, coiled intestine and a capacious cloaca. Crinoids are passive suspension feeders, catching plankton with their outstretched arms. Boluses of mucus-trapped food are passed to the mouth which is linked to the anus by a loop consisting of a short esophagus and longer intestine.

The coelomic cavities of echinoderms are complex. Aside from the water vascular system, echinoderms have a haemal coelom (or haemal system, the “haemal” being a misnomer), a perivisceral coelom, a gonadal coelom and often also a perihemal coelom (or perihemal system). The water vascular system, haemal system and perihemal system form the tubular coelomic system. Echinoderms are an exception having both a coelomic circulatory system (i.e., the water vascular system) and a haemal circulatory system (i.e., the haemal and perihemal systems). Haemal and perihemal systems are derived from the coelom and form an open and reduced circulatory system. This usually consists of a central ring and five radial vessels. There is no true heart and the blood often lacks any respiratory pigment. Gaseous exchange occurs via dermal branchiae or papulae in starfish, genital bursae in brittle stars, peristomal gills in sea urchins and cloacal trees in sea cucumbers. Exchange of gases also takes place through the tube feet. Echinoderms lack specialized excretory (waste disposal) organs and so nitrogenous waste, chiefly

in the form of ammonia, diffuses out through the respiratory surfaces.

Echinoderms have a simple radial nervous system that consists of a modified nerve net consisting of interconnecting neurons with no central brain, although some do possess ganglia. Nerves radiate from central rings around the mouth into each arm or along the body wall; the branches of these nerves coordinate the movements of the organism and the synchronization of the tube feet. Starfish have sensory cells in the epithelium and have simple eyespots and touch-sensitive tentacle-like tube feet at the tips of their arms. Sea urchins have no particular sense organs but do have statocysts that assist in gravitational orientation, and they have sensory cells in their epidermis, particularly in the tube feet, spines and pedicellariae. Brittle stars, crinoids and sea cucumbers in general do not have sensory organs but some burrowing sea cucumbers of the order Apodida have a single statocyst adjoining each radial nerve and some have an eyespot at the base of each tentacle.

The gonads occupy much of the body cavities of sea urchins and sea cucumbers, while the less voluminous crinoids, brittle stars and starfish have two gonads in each arm. While the ancestral condition is considered to be the possession of one genital aperture, many organisms have multiple gonopores through which eggs or sperm may be released.

8.2 Starfish

Starfish⁵ or sea stars are star-shaped echinoderms belonging to the class Asteroidea. Common usage frequently finds these names being also applied to ophiuroids, which are correctly referred to as brittle stars or “basket stars”. About 1,500 species of starfish occur on the seabed in all the world’s oceans, from the tropics to frigid polar waters. They are found from the intertidal zone down to abyssal depths, 6,000 m below the surface.

Starfish are marine invertebrates. They typically have a central disc and five arms, though some species have a larger number of arms. The aboral or upper surface may be smooth, granular or spiny, and is covered with overlapping plates. Many species are brightly colored in various shades of red or orange, while others are blue, grey or brown. Starfish have tube feet operated by a hydraulic system and a

mouth at the center of the oral or lower surface. They are opportunistic feeders and are mostly predators on benthic invertebrates. Several species have specialized feeding behaviors including eversion of their stomachs and suspension feeding. They have complex life cycles and can reproduce both sexually and asexually. Most can regenerate damaged parts or lost arms and they can shed arms as a means of defense. The Asteroidea occupy several significant ecological roles. Starfish, such as the ochre sea star (*Pisaster ochraceus*) and the reef sea star (*Stichaster australis*), have become widely known as examples of the key-stone species concept in ecology. The tropical crown-of-thorns starfish (*Acanthaster planci*) is a voracious predator of coral throughout the Indo-Pacific region, and the northern Pacific sea star is considered to be one of the world’s 100 worst invasive species.

The fossil record for starfish is ancient, dating back to the Ordovician around 450 million years ago, but it is rather poor, as starfish tend to disintegrate after death. Only the ossicles and spines of the animal are likely to be preserved, making remains hard to locate. With their appealing symmetrical shape, starfish have played a part in literature, legend, design and popular culture.

8.2.1 Sexual reproduction

Most species of starfish are gonochorous⁶, i.e. there are separate male and female individuals. These are usually not distinguishable externally as the gonads cannot be seen, but their sex is apparent when they spawn. Some species are simultaneous hermaphrodites, producing eggs and sperm at the same time and in a few of these, the same gonad, called an ovotestis, produces both eggs and sperm. Other starfish are sequential hermaphrodites. Protandrous individuals of species like *Asterina gibbosa* start life as males before changing sex into females as they grow older. In some species such as *Nepanthia belcheri*, a large female can split in half and the resulting offspring are males. When these grow large enough they change back into females. The lifespan of a starfish varies considerably between species, generally being longer in larger forms and in those with planktonic larvae (up to lifespan more than 30 years).

Some species of starfish have the ability to regenerate lost arms and can regrow an entire new limb given time. A few can regrow a complete new disc

⁵<https://en.wikipedia.org/wiki/Starfish>

⁶<https://en.wikipedia.org/wiki/Gonochorism>

from a single arm, while others need at least part of the central disc to be attached to the detached part. Regrowth can take several months or years, and starfish are vulnerable to infections during the early stages after the loss of an arm.

Each starfish arm contains two gonads that release gametes through openings called gonoducts, located on the central disc between the arms. Fertilization is generally external but in a few species, internal fertilization takes place. In most species, the buoyant eggs and sperm are simply released into the water (free spawning) and the resulting embryos and larvae live as part of the plankton. In others, the eggs may be stuck to the undersides of rocks. In certain species of starfish, the females brood their eggs – either by simply enveloping them or by holding them in specialized structures. In brooding species, the eggs are relatively large, and supplied with yolk, and they generally develop directly into miniature starfish without an intervening larval stage. The developing young are called lecithotrophic because they obtain their nutrition from the yolk as opposed to “planktotrophic” larvae that feed in the water column.

In the tropics, a plentiful supply of phytoplankton is continuously available for starfish larvae to feed on. Spawning takes place at any time of year, each species having its own characteristic breeding season. In temperate regions, the spring and summer brings an increase in food supplies. The first individual of a species to spawn may release a pheromone that serves to attract other starfish to aggregate and to release their gametes synchronously. In other species, a male and female may come together and form a pair. This behavior is called pseudocopulation and the male climbs on top, placing his arms between those of the female. When she releases eggs into the water, he is induced to spawn. Starfish may use environmental signals to coordinate the time of spawning (day length to indicate the correct time of the year, dawn or dusk to indicate the correct time of day), and chemical signals to indicate their readiness to breed. In some species, mature females produce chemicals to attract sperm in the sea water.

Some species of starfish are able to reproduce asexually as adults either by fission of their central discs or by autotomy of one or more of their arms.

Most starfish embryos hatch at the blastula stage. The original ball of cells develops a lateral pouch, the archenteron. The entrance to this is known as the blastopore and it will later develop into the anus. Another invagination of the surface will fuse with the tip of the archenteron as the mouth while the interior sec-

tion will become the gut. At the same time, a band of cilia develops on the exterior. This enlarges and extends around the surface and eventually onto two developing arm-like outgrowths. At this stage, the larva is known as a bipinnaria. The cilia are used for locomotion and feeding, their rhythmic beat wafting phytoplankton towards the mouth.

The next stage in development is a brachiolaria larva and involves the growth of three short, additional arms. These are at the anterior end, surround a sucker and have adhesive cells at their tips. Both bipinnaria and brachiolaria larvae are bilaterally symmetrical. When fully developed, the brachiolaria settles on the seabed and attaches itself with a short stalk formed from the ventral arms and sucker. Metamorphosis now takes place with a radical rearrangement of tissues. The left side of the larval body becomes the oral surface of the juvenile and the right side the aboral surface. Part of the gut is retained but the mouth and anus move to new positions. Some of the body cavities degenerate but others become the water vascular system and the visceral coelom. The starfish is now pentaradially symmetrical. It casts off its stalk and becomes a free-living juvenile starfish about 1 mm in diameter.

8.3 Starfish Dissection

1. Get a dissecting pan, scissors and a pointer.
2. Obtain a preserved starfish.
3. Place the starfish in the dissecting pan with its dorsal or aboral (top) surface upward.
4. Locate the central disc in the center of the starfish. Count and record the number of arms or rays the starfish has.
5. Locate the small, round hard plate called the madreporite on top of the central disc. Water enters through this into the water vascular system.
6. Feel the upper surface of the starfish for spines. These spines protect the starfish and are part of their endoskeleton.
7. Turn the starfish over to its ventral or oral surface.
8. Locate the mouth in the center of the central disc. Find the ring of oral spines surrounding the mouth.
9. Find the groove that extends down the underside of each arm. This is called the ambulacral groove.
10. Feel the numerous, soft tube feet inside each groove. The tube feet are part of the water vas-



Figure 8.1: Starfish with exposed pyloric caeca, gonads and ambulacral ridge.



Figure 8.2: Starfish tube feet.



Figure 8.3: Starfish pedicellariae.

- cular system and aid in movement and feeding.
11. With the starfish's aboral surface facing you, choose three rays and cut off the tip of each ray.
 12. Cut along each side of each ray towards the central disc and then carefully remove the flap of skin (Fig. 8.1).
 13. Inside each arm, locate two long digestive glands called the pyloric caeca. These make enzymes to digest food in the stomach.
 14. Cut a circular flap of skin from the central disc. (You will have to also cut around the madreporite in order to remove this flap.) Observe the stomach under the central disc.
 15. Remove the pyloric caeca from one of the dissected rays.
 16. Find the gonads (testes or ovaries) underneath. These may be small if the starfish is NOT in breeding season.
 17. In the third ray, remove both the pyloric caeca and the gonads to see the water vascular system. Embedded in the soft body wall are skeletal plates called ossicles.
 18. Cut off the tip of a ray to observe the parts of the tube feet. Find the zipper-like ridge that extends the length of the ray. The tube feet are attached to these.
 19. Locate the bulb-like top of a tube foot called the ampulla. This sac works like the top of an eyedropper to create suction. The bottom of the tube foot is a sucker.

8.4 View Prepared Slides of Asterias

1. Starfish tube feet (Figure 8.2)
 - Locate: tube feet, suckers
2. Asterias pedicellariae w.m. (Figure 8.3)
 - Locate: pincer-like structures used to cleanse the skin.

8.5 Hemichordata

Hemichordata⁷ is a phylum of marine deuterostome animals, generally considered the sister group of the echinoderms. They appear in the Lower or Middle Cambrian and include two main classes: Enteropneusta (acorn worms), and Pterobranchia. A third class, Planctosphaeroidea, is known only from the larva of a single species, Planctosphaera pelagica.

Acorn worms are solitary worm-shaped organisms. They generally live in burrows (the earliest secreted tubes) and are deposit feeders, but some species are pharyngeal filter feeders, while the family Torquaratoridae are free living detritivores. Many are well known for their production and accumulation of various halogenated phenols and pyrroles. Pterobranchs are filter-feeders, mostly colonial, living in a collagenous tubular structure called a coenecium.

⁷<https://en.wikipedia.org/wiki/Hemichordata>

The body of acorn worms is worm-shaped and divided into an anterior proboscis, an intermediate collar, and a posterior trunk. The proboscis is a muscular and ciliated organ used in locomotion and in the collection and transport of food particles. The mouth is located between the proboscis and the collar. The trunk is the longest part of the animal. It contains the pharynx, which is perforated with gill slits (or pharyngeal slits), the esophagus, a long intestine, and a terminal anus. It also contains the gonads.

8.6 Chordata

A chordate is an animal belonging to the phylum Chordata⁸; chordates possess a notochord, a hollow dorsal nerve cord, pharyngeal slits, an endostyle, and a muscular post-anal tail, for at least some period of their life cycle. Chordates are deuterostomes, as during the embryo development stage the anus forms before the mouth. They are also bilaterally symmetric coelomates with metameric segmentation and a circulatory system. In the case of vertebrate chordates, the notochord is usually replaced by a vertebral column during development.

Taxonomically, the phylum includes the following subphyla:

- Vertebrata, which includes fish, amphibians, reptiles, birds, and mammals
- Tunicata, which includes salps and sea squirts
- Cephalochordata, which include the lancelets

Of the more than 65,000 living species of chordates, about half are bony fish of the superclass Osteichthyes (boney fish). The world's largest and fastest animals, the blue whale and peregrine falcon respectively, are chordates, as are humans. Fossil chordates are known from at least as early as the Cambrian explosion.

Chordates form a phylum of animals that are defined by having at some stage in their lives all of the following:

- A notochord, a fairly stiff rod of cartilage that extends along the inside of the body. Among the vertebrate sub-group of chordates, the notochord develops into the spine, and in wholly aquatic species this helps the animal to swim by flexing its tail.

- A dorsal neural tube. In fish and other vertebrates, this develops into the spinal cord, the main communications trunk of the nervous system
- Pharyngeal slits. The pharynx is the part of the throat immediately behind the mouth. In fish, the slits are modified to form gills, but in some other chordates they are part of a filter-feeding system that extracts particles of food from the water in which the animals live.
- Post-anal tail. A muscular tail that extends backwards behind the anus.
- An endostyle. This is a groove in the ventral wall of the pharynx. In filter-feeding species it produces mucus to gather food particles, which helps in transporting food to the esophagus. It also stores iodine, and may be a precursor of the vertebrate thyroid gland.

There are soft constraints that separate chordates from certain other biological lineages, but have not yet been made part of the formal definition:

- All chordates are deuterostomes. This means that, during the embryo development stage, the anus forms before the mouth.
- All chordates are based on a bilateral body plan.
- All chordates are coelomates, and have a fluid filled body cavity called a coelom with a complete lining called peritoneum derived from mesoderm (see Brusca and Brusca).

8.7 Craniata (Vertebrata)

Craniates, one of the three subdivisions of chordates, all have distinct skulls. They include the hagfish which have no vertebrae. Michael J. Benton commented that “craniates are characterized by their heads, just as chordates, or possibly all deuterostomes, are by their tails”.

Most are vertebrates, in which the notochord is replaced by the vertebral column. These consist of a series of bony or cartilaginous cylindrical vertebrae, generally with neural arches that protect the spinal cord, and with projections that link the vertebrae. However, hagfish have incomplete braincases and no vertebrae, and are therefore not regarded as vertebrates, but as members of the craniates, the group from which vertebrates are thought to have evolved. However, the cladistic exclusion of hagfish from the vertebrates is controversial, as they may be degenerate vertebrates who have lost their vertebral columns.

⁸<https://en.wikipedia.org/wiki/Chordate>

The position of lampreys is ambiguous. They have complete braincases and rudimentary vertebrae, and therefore may be regarded as vertebrates and true fish. However, molecular phylogenetics, which uses biochemical features to classify organisms, has produced both results that group them with vertebrates and others that group them with hagfish. If lampreys are more closely related to the hagfish than the other vertebrates, this would suggest that they form a clade, which has been named the Cyclostomata (literally: round mouths).

8.8 Tunicates

Most tunicates⁹ appear as adults in two major forms, both of which are soft-bodied filter-feeders that lack the standard features of chordates: “sea squirts” are sessile and consist mainly of water pumps and filter-feeding apparatus; salps float in mid-water, feeding on plankton, and have a two-generation cycle in which one generation is solitary and the next forms chain-like colonies. However, all tunicate larvae have the standard chordate features, including long, tadpole-like tails; they also have rudimentary brains, light sensors and tilt sensors. The third main group of tunicates, Appendicularia (also known as Larvacea) retain tadpole-like shapes and active swimming all their lives, and were for a long time regarded as larvae of sea squirts or salps. The etymology of the term Urochorda(ta) (Balfour 1881) is from the ancient Greek *oura*, “tail”) + Latin *chorda* (“cord”), because the notochord is only found in the tail. The term Tunicata (Lamarck 1816) is recognized as having precedence and is now more commonly used.



Figure 8.4: Ascidians¹⁰

8.9 View Prepared Slides of Tunicates

1. *Ascidian*¹¹ larva w.m. (Figure 8.5)
 - Identify: notochord



Figure 8.5: Ascidian larva.

8.10 Cephalochordates

Cephalochordates are small, “vaguely fish-shaped” animals that lack brains, clearly defined heads and

⁹<https://en.wikipedia.org/wiki/Tunicate>

¹¹<https://en.wikipedia.org/wiki/Ascidacea>

specialized sense organs. These burrowing filter-feeders compose the earliest-branching chordate sub-phylum.

Lancelet¹² (Amphioxus) *Branchiostoma lanceolatum* (European lancelet) is a lancelet in the subphylum Cephalochordata. It is a marine invertebrate with a notochord but no backbone and is used as a model organism to study the evolutionary development of vertebrates.

Is found in shallow seas in the north-east Atlantic Ocean, from Norway, Scotland as well as further south to the Mediterranean Sea and the Black Sea. Its range has expanded through the Suez Canal to the northerly parts of the Indian Ocean and the coasts of East Africa. It burrows in soft substrates such as sand, gravel and shell fragments and is quite particular as to the size of the particles. It occurs from the low tide mark down to about 40 meters.

It has an elongated body, flattened laterally and pointed at both ends. A stiffening rod of tightly packed cells, the notochord, extends the whole length of the body. Above it is a nerve cord with a single frontal eye. The mouth is on the underside of the body and is surrounded by a tuft of 20 or 30 cirri or slender sensory appendages. The gut runs just below the notochord from the mouth to the anus, in front of the tail. There is a flap-like, vertical fin surrounding the pointed tail. Gas exchange takes place as water passes through gill slits in the mid region, and segmented gonads lie just behind these. The animal is pearly white and semi-transparent which enables the internal organs to be seen from outside. Its appearance is similar to a “primitive fish”. It can grow up to 6 cm.

8.11 View Prepared Slides of Lancelet

1. Lancelet w.m. (Figure 8.6)
 - Locate: dorsal hollow nerve cord, notochord, pharyngeal gill slit, buccal cavity, buccal cirri (oral tentacles), atriopore, anus, liver, myotomes, post-anal tail.
2. Lancelet x.s. through pharynx (Figure 8.7)
 - Locate: dorsal hollow nerve cord, notochord, pharyngeal gill slits, myotomes, liver.

¹²<https://en.wikipedia.org/wiki/Lancelet>



Figure 8.6: A young adult lancelet.



Figure 8.7: Lancelet cross section.

8.12 Review Questions

1. What are echinoderms?
2. What are tube feet?
3. What the five characteristics of chordates?
4. What is the notochord?
5. What are tunicates?

Chapter 9

Mammalian Tissues

In biology, tissue¹ is a cellular organizational level between cells and a complete organ. A tissue is an ensemble of similar cells and their extracellular matrix from the same origin that together carry out a specific function. Organs are then formed by the functional grouping together of multiple tissues. The English word is derived from the French *tissu*, meaning something that is woven, from the verb *tisser*, “to weave”.

The study of human and animal tissues is known as histology or, in connection with disease, histopathology. For plants, the discipline is called plant anatomy. The classical tools for studying tissues are the paraffin block in which tissue is embedded and then sectioned, the histological stain, and the optical microscope. In the last couple of decades, developments in electron microscopy, immunofluorescence, and the use of frozen tissue sections have enhanced the detail that can be observed in tissues. With these tools, the classical appearances of tissues can be examined in health and disease, enabling considerable refinement of medical diagnosis and prognosis.

9.1 Animal tissues

Animal tissues are grouped into four basic types: connective, muscle, nervous, and epithelial. Collections of tissues joined in structural units to serve a common function compose organs. While all animals can generally be considered to contain the four tissue types, the manifestation of these tissues can differ depending on the type of organism. For example, the origin of the cells comprising a particular tissue type may differ developmentally for different classifications of animals.

¹[https://en.wikipedia.org/wiki/Tissue_\(biology\)](https://en.wikipedia.org/wiki/Tissue_(biology))

9.2 Connective tissue²

Connective tissues are made up of cells separated by non-living material, which is called an extracellular matrix. This matrix can be liquid or rigid. For example, blood contains plasma as its matrix and bone's matrix is rigid. Connective tissue gives shape to organs and holds them in place. Blood, bone, tendon, ligament, adipose and areolar tissues are examples of connective tissues.

9.3 Muscle tissue³

Muscle cells form the active contractile tissue of the body known as muscle tissue or muscular tissue. Muscle tissue functions to produce force and cause motion, either locomotion or movement within internal organs. Muscle tissue is separated into three distinct categories: visceral or smooth muscle, found in the inner linings of organs; skeletal muscle, typically attached to bones and which generates gross movement; and cardiac muscle, found in the heart where it contracts to pump blood throughout an organism.

9.4 Nervous tissue⁴

Cells comprising the central nervous system and peripheral nervous system are classified as nervous (or neural) tissue. In the central nervous system, neural tissues form the brain and spinal cord. In the peripheral nervous system, neural tissues form the cranial nerves and spinal nerves, inclusive of the motor neurons.

²https://en.wikipedia.org/wiki/Connective_tissue

³https://en.wikipedia.org/wiki/Muscle_tissue

⁴https://en.wikipedia.org/wiki/Nervous_tissue

9.5 Epithelial tissue⁵

The epithelial tissues are formed by cells that cover the organ surfaces such as the surface of skin, the airways, the reproductive tract, and the inner lining of the digestive tract. The cells comprising an epithelial layer are linked via semi-permeable, tight junctions; hence, this tissue provides a barrier between the external environment and the organ it covers. In addition to this protective function, epithelial tissue may also be specialized to function in secretion, excretion and absorption. Epithelial tissue helps to protect organs from microorganisms, injury, and fluid loss.

Functions of epithelial tissue:

- The cells of the body's surface form the outer layer of skin.
- Inside the body, epithelial cells form the lining of the mouth and alimentary canal and protect these organs.
- Epithelial tissues help in absorption of water and nutrients.
- Epithelial tissues help in elimination of waste.
- Epithelial tissues secrete enzymes and/or hormones in the form of glands.

There are many kinds of epithelium, and nomenclature is somewhat variable. Most classification schemes combine a description of the cell-shape in the upper layer of the epithelium with a word denoting the number of layers: either simple (one layer of cells) or stratified (multiple layers of cells). However, other cellular features, such as cilia may also be described in the classification system.

Some common kinds of epithelium are listed below:

- Simple squamous epithelium
- Stratified squamous epithelium
- Simple cuboidal epithelium
- Transitional epithelium
- Pseudostratified columnar epithelium (also known as Ciliated columnar epithelium)
- Columnar epithelium
- Glandular epithelium
- Ciliated columnar epithelium

9.6 Animal Organs

In biology, an organ or viscus is a collection of tissues joined in a structural unit to serve a common function.

⁵<https://en.wikipedia.org/wiki/Epithelium>

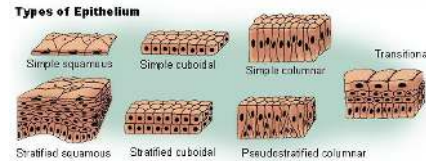


Figure 9.1: Types of epithelia⁶

In anatomy, a viscus is an internal organ, and viscera is the plural form.

Organs are composed of main tissue, parenchyma, and “sporadic” tissues, stroma. The main tissue is that which is unique for the specific organ, such as the myocardium, the main tissue of the heart, while sporadic tissues include the nerves, blood vessels, and connective tissues. The main tissues that make up an organ tend to have common embryologic origins, such as arising from the same germ layer. Functionally related organs often cooperate to form whole organ systems. Organs exist in all higher biological organisms, in particular they are not restricted to animals, but can also be identified in plants. In single-cell organisms like bacteria, the functional analogue of an organ is called organelle.

A hollow organ is a visceral organ that forms a hollow tube or pouch, such as the stomach, intestine, or bladder.

9.7 Animal Organ Systems

Two or more organs working together in the execution of a specific body function form an organ system. The functions of organ systems often share significant overlap. For instance, the nervous and endocrine system both operate via a shared organ, the hypothalamus. For this reason, the two systems are combined and studied as the neuroendocrine system. The same is true for the musculoskeletal system because of the relationship between the muscular and skeletal systems.

Mammals such as humans have a variety of organ systems. These specific systems are also widely studied in human anatomy.

- Cardiovascular system⁷: pumping and channeling blood to and from the body and lungs with heart, blood and blood vessels.
- Digestive system⁸: digestion and processing

⁷https://en.wikipedia.org/wiki/Circulatory_system

⁸https://en.wikipedia.org/wiki/Human_digestive_system

food with salivary glands, esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum and anus.

- Endocrine system⁹: communication within the body using hormones made by endocrine glands such as the hypothalamus, pituitary gland, pineal body or pineal gland, thyroid, parathyroids and adrenals, i.e., adrenal glands.
- Excretory system¹⁰: kidneys, ureters, bladder and urethra involved in fluid balance, electrolyte balance and excretion of urine.
- Lymphatic system¹¹: structures involved in the transfer of lymph between tissues and the blood stream, the lymph and the nodes and vessels that transport it including the Immune system: defending against disease-causing agents with leukocytes, tonsils, adenoids, thymus and spleen.
- Integumentary system¹²: skin, hair and nails.
- Muscular system¹³: movement with muscles.
- Nervous system¹⁴: collecting, transferring and processing information with brain, spinal cord and nerves.
- Reproductive system¹⁵: the sex organs, such as ovaries, fallopian tubes, uterus, vulva, vagina, testes, vas deferens, seminal vesicles, prostate and penis.
- Respiratory system¹⁶: the organs used for breathing, the pharynx, larynx, trachea, bronchi, lungs and diaphragm.
- Skeletal system¹⁷: structural support and protection with bones, cartilage, ligaments and tendons.

9.8 View Prepared Slides of Tissues

1. Lung simple squamous epithelium (Figure 9.2)
 - Identify flattened cells with noticeable nucleus.
2. Stratified squamous epithelium (Figure 9.3)
 - Identify: layers of squamous epithelium cells, with a greater concentration of liv-

ing, nucleate cells towards the side of contact with the rest of the tissues.

3. Simple cuboidal epithelium (Figure 9.4)
 - Identify: cuboidal shaped cells with large, central nucleus. Notice the attachment end versus the end facing the open space.
4. Simple columnar epithelium (Figure 9.5)
 - Identify: rectangular shaped cells with nucleus at the base, goblet cells with mucus, cilia, and basement membrane.
5. Amphibian stratified ciliated columnar epithelium
 - Identify: ciliated columnar epithelial cells
6. Pseudostratified ciliated columnar epithelium (Figure 9.6)
 - Identify: pseudostratification, position of nuclei, goblet cells filled with mucus, and cilia
7. Areolar tissue spread (Figure 9.7)
 - Identify: fibroblasts, collagen fibers, elastic fibers.
8. Dense fibrous irregular
 - Identify: fibroblasts' nuclei, collagen bundles
9. White fibrous tissue human (Figure 9.8)
 - Identify: fibroblasts' nuclei, collagen bundles
10. Adipose tissue (Figure 9.9)
 - Identify: fibroblasts filled with fat, nucleus, cell membrane, intercellular matrix
11. Mammal hyaline cartilage (Figure 9.10)
 - Identify: chondrocytes, lacunae, homogeneous matrix, perichondrium.
12. Trachea monkey (Figure 9.11)
 - Identify: chondrocytes, lacunae, homogeneous matrix, perichondrium.
13. Elastic cartilage human (Figure 9.12)
 - Identify: chondrocytes, lacunae, elastic fibers, perichondrium.
14. Fibrocartilage (Figure 9.13)
 - Identify: chondrocytes, lacunae, collagen fibers
15. Bone ground human (Figure 9.14)
 - Identify: Haversian Systems, osteocytes in lacunae, Haversian Canal, canaliculi, calcified matrix
16. Human blood Wright's smear (Figure 9.15)
 - Identify: erythrocytes (red blood cells) - notice the approximately round shape, and the lack of nucleus -; leukocytes (white blood cells) - up to 5 different types, with nuclei in various shapes-; and the platelets (very small pieces of cells in

⁹https://en.wikipedia.org/wiki/Endocrine_system

¹⁰https://en.wikipedia.org/wiki/Excretory_system

¹¹https://en.wikipedia.org/wiki/Lymphatic_system

¹²https://en.wikipedia.org/wiki/Integumentary_system

¹³https://en.wikipedia.org/wiki/Muscular_system

¹⁴https://en.wikipedia.org/wiki/Nervous_system

¹⁵https://en.wikipedia.org/wiki/Reproductive_system

¹⁶https://en.wikipedia.org/wiki/Respiratory_system

¹⁷<https://en.wikipedia.org/wiki/Skeleton>

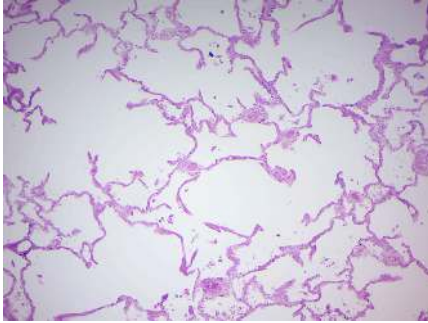


Figure 9.2: Simple squamous epithelium (human lung).

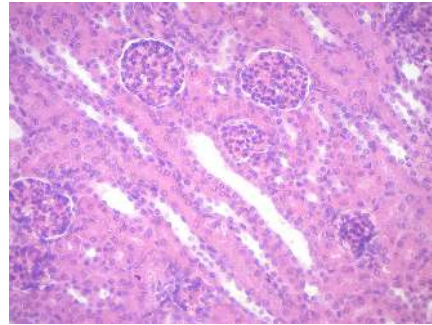


Figure 9.4: Simple cuboidal epithelium (kidney).

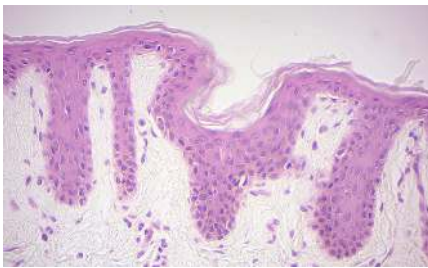


Figure 9.3: Stratified squamous epithelium (human epidermis).

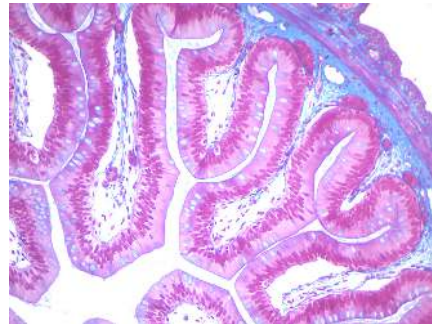


Figure 9.5: Simple columnar epithelium (amphibian).

- the matrix).
17. Sickle cell anemia (Figure 9.16)
 18. Human skeletal muscle (Figure 9.17)
 - Identify: each individual muscle fiber, nuclei, and striations.
 19. Cardiac muscle human
 - Identify: individual cells with one nucleus per cell, striations, intercalated disks, and branched muscle fibers.
 20. Cardiac muscle mammal (Figure 9.18)
 - Identify: individual cells with one nucleus per cell, striations, intercalated disks, and branched muscle fibers.
 21. Smooth muscle mammal (Figure 9.19)
 - Identify: individual cells with one nucleus per cell, homogeneous cytoplasm, shape, and arrangement of the muscle fibers.
 22. Amphibian smooth muscle teased (Figure 9.20)
 - Identify: individual smooth muscle cells with nucleus.
 23. Motor neuron smear (Figure 9.21)
 - Identify: cell body of neuron with nucleus, dendrites, axons (if possible), and neuroglial cells

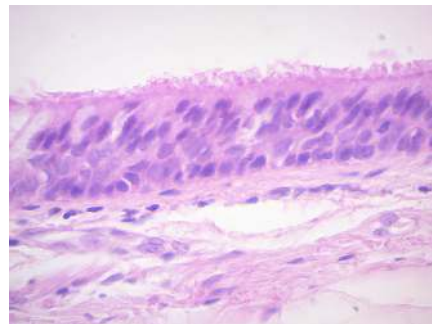


Figure 9.6: Pseudostratified ciliated columnar epithelium.

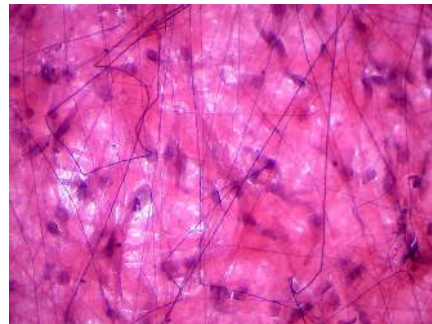


Figure 9.7: Areolar connective tissue.

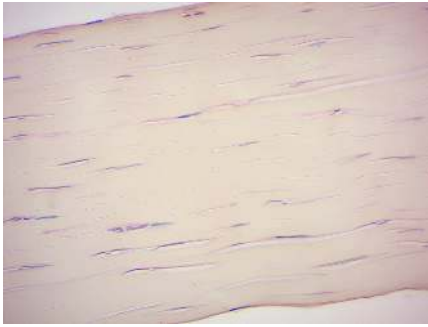


Figure 9.8: White fibrous tissue.

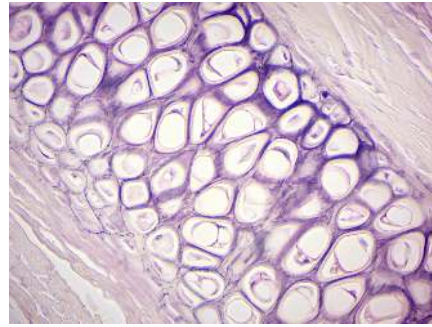


Figure 9.12: Elastic cartilage.

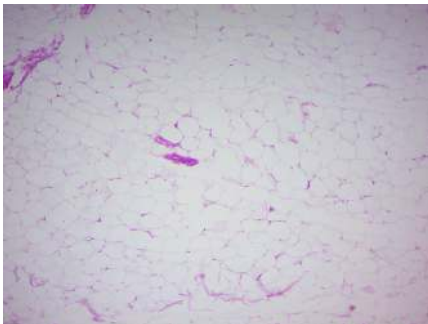


Figure 9.9: Adipose tissue.

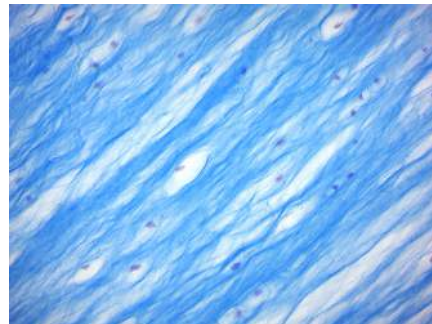


Figure 9.13: Fibrocartilage.

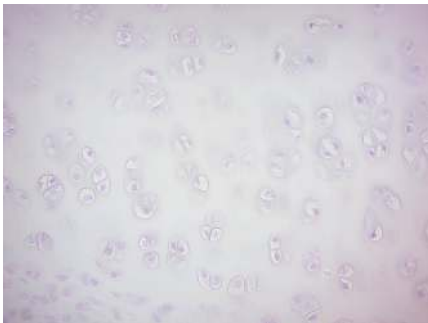


Figure 9.10: Hyaline cartilage.

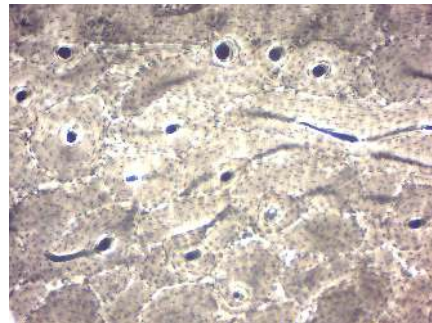


Figure 9.14: Ground bone.

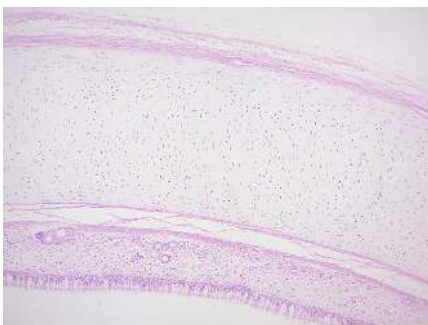


Figure 9.11: Monkey trachea.

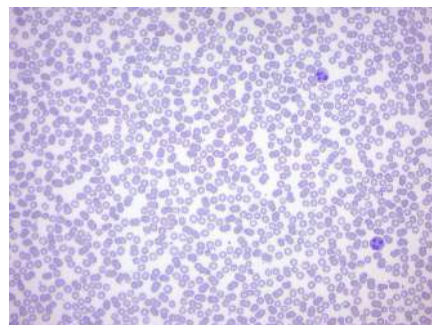


Figure 9.15: Human blood smear.

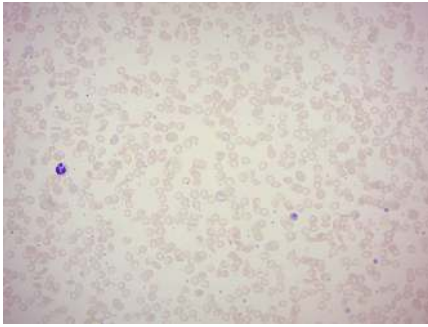


Figure 9.16: Sickle cell blood smear.

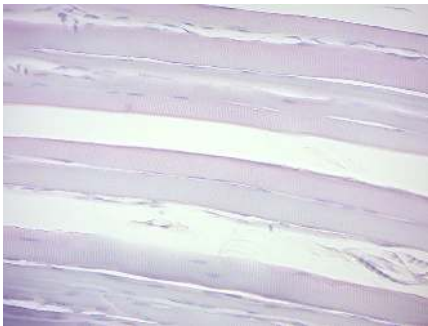


Figure 9.17: Skeletal muscle.

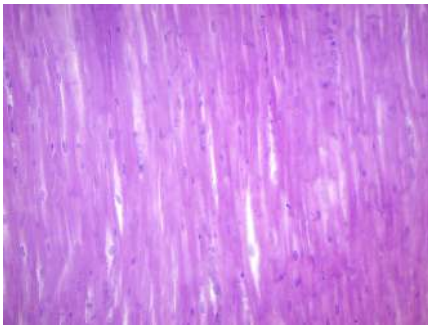


Figure 9.18: Mammalian cardiac muscle.

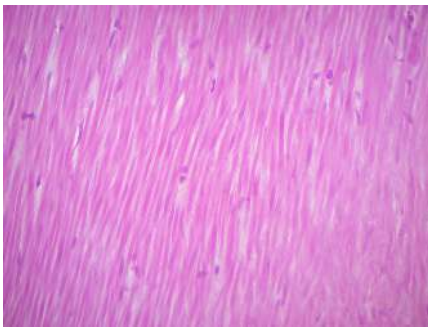


Figure 9.19: Smooth muscle.



Figure 9.20: A teased smooth muscle fiber.

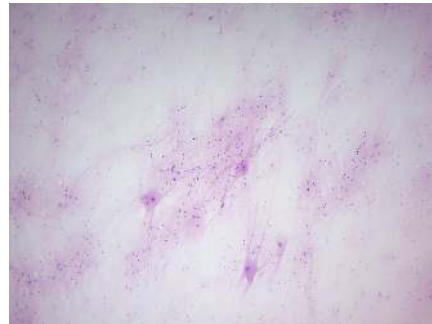


Figure 9.21: Motor neuron smear.

9.9 Review Questions

1. What are tissues?
2. What are the four main types of animal tissues?
3. What is the difference between skeletal and smooth muscle?
4. What is epithelial tissue?
5. What is connective tissue?
6. What are the characteristics that distinguish cardiac muscle from skeletal muscle?

Chapter 10

Mammalian Anatomy I

The domestic pig (*Sus scrofa domesticus* or only *Sus domesticus*), often called swine, hog, or simply pig when there is no need to distinguish it from other pigs, is a large, even-toed ungulate. It is variously considered a subspecies of the wild boar or a distinct species. The domestic pig's head-plus-body-length ranges from 0.9 to 1.8 m, and the adult can weigh between 50 and 350 kg. Compared to other even-toed ungulates¹ (artiodactyls), its head is relatively long, pointed, and free of warts. Artiodactyls are generally herbivorous, but the domestic pig is an omnivore, like its wild relative. When used as livestock, domestic pigs are farmed primarily for the consumption of their meat, called pork. The animal's bones, hide, and bristles are also used in commercial products. Domestic pigs, especially miniature breeds, are often kept as pets. Female pigs reach sexual maturity at 3–12 months of age, and can mate every 18–24 days if they are not pregnant. The pregnancy period averages 112–120 days.



The domestic pig, both as a live animal and source of post-mortem tissues, is one of the most valuable animal models used in biomedical research today, because of its biological, physiological and anatomical similarities to human beings. This is also the reason why we have chosen fetal pigs² for dissection in this lab.

10.1 Fetal Pig Dissection

1. Obtain a dissecting pan, a pair of scissors, a scalpel, forceps, and a pointer.
2. Obtain a fetal pig.

3. Place your fetal pig in the dissecting pan ventral side up.
4. Examine the fetal pig and locate two rows of nipples of mammary glands on the ventral abdominal surface of both males and females.
5. Locate the umbilical cord and make a transverse cut through the umbilical cord.
6. Examine the cut end and locate the two umbilical arteries that carry blood from the fetal pig to the placenta, and the single umbilical vein that delivers nutrient-rich blood back to the fetal pig.
7. Determine the sex of your specimen:
 - Female: The urogenital opening in the female is immediately ventral to the anus and has a small genital papilla marking its location.
 - Male: The scrotal sac is ventral to the anus and a urogenital opening is just posterior to the umbilical cord.
8. Use a piece of string and tie one end of it around the ankle of the left foreleg.
9. Pass the other end of the string under the dissecting pan and tie it to the other foreleg. Stretch the string tightly so that it will hold the pig's legs apart.
10. Tie a second piece of string in the same manner around the hindlegs.
11. Probe the chest area of the pig with your fingers. You should be able to feel the hard sternum (breastbone) and the ridges of the ribcage. Move your fingers down until you feel the bottom edge of the rib cage. This is where the diaphragm separates the thoracic and abdominal cavities.
12. Grab the tip of the umbilical cord with the thumb and index finger of your left hand (if you are right handed), holding the scissors in your left hand, make a careful incision just above of the umbilical cord (towards the head). When

¹https://en.wikipedia.org/wiki/Even-toed_ungulate

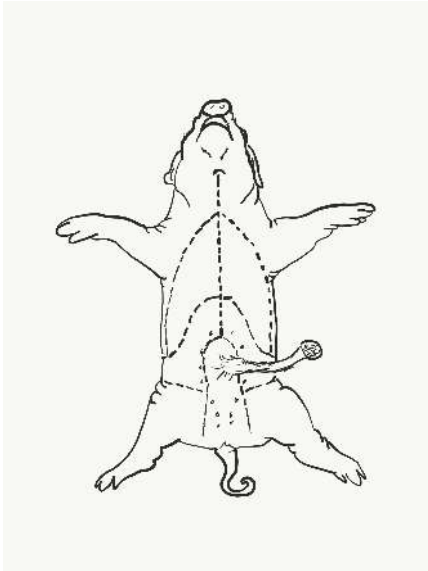


Figure 10.1: Cut along the dotted lines to expose the abdominal and thoracic cavities and the neck area.



Figure 10.2: Overview of the abdominal and thoracic cavities and the neck area.

you see the opening of the abdominal cavity, continue cutting along the lines shown in Figure 10.1.

13. Cut through the skin and the muscle, but be careful not to cut too deep to avoid damaging the internal organs. Use a forceps to hold the tissue away from the organs as you cut. Carry the incision all the way to the pan.
14. Lift up the flaps of skin, peel them back so they lay flat on the pan.
15. Do not remove the umbilical cord, cut around it as indicated.
16. Inspect the abdominal cavity and identify the organs located in this cavity (Figure 10.2).
17. If your pig is a female, identify the uterus and ovaries (Figure 10.3).
18. If your pig is male, dissect the groin area of the pig and identify the testes (Figure 10.4).
19. Use the scissors to cut through the rib cage and the sternum. When you reach the midpoint between the forelegs, make another incision towards the right and left side of the pig all the way down to the pan.
20. Pull back the rib cage and pin the two flaps to the pan to expose the thoracic cavity.
21. Carefully cut along the midline towards the mouth to expose the neck area (Figure 10.5).



Figure 10.3: Close-up of the uterus and ovaries.



Figure 10.4: Close-up of the testes.



Figure 10.5: Close-up of the neck area.

10.2 Cleaning up

1. Dispose of the remains of the pig in the red biohazard bins.
2. Clean the dissection tray and instruments and return them to the place where you picked them up.
3. Clean table tops with red bottled sanitizer
4. Wash hands before leaving class

10.3 Review Questions

1. What are the names of the cavities that are separated by the diaphragm?
2. Where is the pancreas located?
3. What is the shape of the uterus in the pig?
4. What shape the shapes of two major parts of the colon in the pig?
5. What is the name of the part of the colon that where the small intestine fuses with the large intestine?
6. Where is the thyroid gland located?
7. Where is the thymus located?

Chapter 11

Mammalian Anatomy II

11.1 Skin

Skin¹ is the soft outer tissue covering vertebrates. Other animal coverings, such as the arthropod exoskeleton, have different developmental origin, structure and chemical composition. The adjective cutaneous means “of the skin” (from Latin *cutis*, skin). In mammals, the skin is an organ of the integumentary system made up of multiple layers of ectodermal tissue, and guards the underlying muscles, bones, ligaments and internal organs. Skin of a different nature exists in amphibians, reptiles, and birds. All mammals have some hair on their skin, even marine mammals like whales, dolphins, and porpoises which appear to be hairless. The skin interfaces with the environment and is the first line of defense from external factors. For example, the skin plays a key role in protecting the body against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, and the production of vitamin D folates. Severely damaged skin may heal by forming scar tissue. This is sometimes discolored and depigmented. The thickness of skin also varies from location to location on an organism. In humans for example, the skin located under the eyes and around the eyelids is the thinnest skin in the body at 0.5 mm thick, and is one of the first areas to show signs of aging such as “crows feet” and wrinkles. The skin on the palms and the soles of the feet is 4 mm thick and is the thickest skin on the body.

Fur² is the hair covering of non-human mammals, particularly those mammals with extensive body hair that is soft and thick. Primarily, fur augments the insulation the skin provides but can also serve as a secondary sexual characteristic or as camouflage. On some animals, the skin is very hard and thick, and can be processed to create leather. Reptiles and fish

¹<https://en.wikipedia.org/wiki/Skin>

²<https://en.wikipedia.org/wiki/Fur>

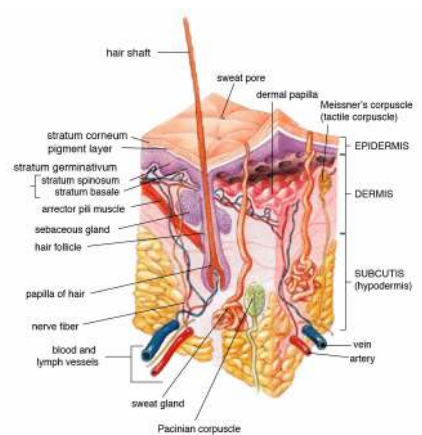


Figure 11.1: Anatomy of human skin.³

have hard protective scales on their skin for protection, and birds have hard feathers, all made of tough -keratins. Amphibian skin is not a strong barrier, especially regarding the passage of chemicals via skin and is often subject to osmosis and diffusive forces. For example, a frog sitting in an anesthetic solution would be sedated quickly, as the chemical diffuses through its skin. Amphibian skin plays key roles in everyday survival and their ability to exploit a wide range of habitats and ecological conditions.

Mammalian skin is composed of two primary layers:

- the epidermis, which provides waterproofing and serves as a barrier to infection; and
- the dermis, which serves as a location for the appendages of skin;

11.2 Epidermis

The epidermis⁴ is composed of the outermost layers of the skin. It forms a protective barrier over the body's surface, responsible for keeping water in the body and preventing pathogens from entering, and is a stratified squamous epithelium, composed of proliferating basal and differentiated suprabasal keratinocytes.

Keratinocytes are the major cells, constituting 95% of the epidermis, while Merkel cells, melanocytes and Langerhans cells are also present. The epidermis can be further subdivided into the following strata or layers (beginning with the outermost layer):

- Stratum corneum
- Stratum lucidum (only in palms and soles)
- Stratum granulosum
- Stratum spinosum
- Stratum germinativum (also called the stratum basale)

Keratinocytes in the stratum basale proliferate through mitosis and the daughter cells move up the strata changing shape and composition as they undergo multiple stages of cell differentiation to eventually lose their nuclei. During that process, keratinocytes will become highly organized, forming cellular junctions (desmosomes) between each other and secreting keratin proteins and lipids which contribute to the formation of an extracellular matrix and provide mechanical strength to the skin. Keratinocytes from the stratum corneum are eventually shed from the surface (desquamation).

The epidermis contains no blood vessels, and cells in the deepest layers are nourished by diffusion from blood capillaries extending to the upper layers of the dermis.

11.3 Basement membrane

The epidermis and dermis are separated by a thin sheet of fibers called the basement membrane, and is made through the action of both tissues. The basement membrane controls the traffic of the cells and molecules between the dermis and epidermis but also serves, through the binding of a variety of cytokines and growth factors, as a reservoir for their controlled release during physiological remodeling or repair processes.

⁴<https://en.wikipedia.org/wiki/Epidermis>

11.4 Dermis

The dermis⁵ is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis provides tensile strength and elasticity to the skin through an extracellular matrix composed of collagen fibrils, microfibrils, and elastic fibers, embedded in hyaluronan and proteoglycans.

It harbors many mechanoreceptors (nerve endings) that provide the sense of touch and heat through nociceptors and thermoreceptors. It also contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal from its own cells as well as for the epidermis.

The dermis is tightly connected to the epidermis through a basement membrane and is structurally divided into two areas: a superficial area adjacent to the epidermis, called the papillary region, and a deep thicker area known as the reticular region.

11.5 Papillary region

The papillary region is composed of loose areolar connective tissue. This is named for its fingerlike projections called papillae that extend toward the epidermis. The papillae provide the dermis with a “bumpy” surface that interdigitates with the epidermis, strengthening the connection between the two layers of skin.

11.6 Reticular region

The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenous, elastic, and reticular fibers that weave throughout it. These protein fibers give the dermis its properties of strength, extensibility, and elasticity. Also located within the reticular region are the roots of the hair, sweat glands, sebaceous glands receptors, nails, and blood vessels.

⁵<https://en.wikipedia.org/wiki/Dermis>

11.7 Subcutaneous tissue

The subcutaneous tissue (also hypodermis) is not part of the skin, and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell types are fibroblasts, macrophages and adipocytes (the subcutaneous tissue contains 50% of body fat). Fat serves as padding and insulation for the body.

Microorganisms like *Staphylococcus epidermidis* colonize the skin surface. The density of skin flora depends on region of the skin. The disinfected skin surface gets recolonized from bacteria residing in the deeper areas of the hair follicle, gut and urogenital openings.

11.8 Muscle

Muscle⁶ is a soft tissue found in most animals. Muscle cells contain protein filaments of actin and myosin that slide past one another, producing a contraction that changes both the length and the shape of the cell. Muscles function to produce force and motion. They are primarily responsible for maintaining and changing posture, locomotion, as well as movement of internal organs, such as the contraction of the heart and the movement of food through the digestive system via peristalsis.

Muscle tissues are derived from the mesodermal layer of embryonic germ cells in a process known as myogenesis. There are three types of muscle, skeletal or striated, cardiac, and smooth. Muscle action can be classified as being either voluntary or involuntary. Cardiac and smooth muscles contract without conscious thought and are termed involuntary, whereas the skeletal muscles contract upon command.

The term muscle is derived from the Latin *musculus* meaning “little mouse” perhaps because of the shape of certain muscles or because contracting muscles look like mice moving under the skin.

The muscular system consists of all the muscles present in a single body. There are approximately 650 skeletal muscles in the human body, but an exact number is difficult to define. The difficulty lies partly in the fact that different sources group the muscles

differently and partly in that some muscles, such as palmaris longus, are not always present.

The muscular system is one component of the musculoskeletal system, which includes not only the muscles but also the bones, joints, tendons, and other structures that permit movement.

11.9 Cartilage

Cartilage⁷ is a resilient and smooth elastic tissue, rubber-like padding that covers and protects the ends of long bones at the joints, and is a structural component of the rib cage, the ear, the nose, the bronchial tubes, the intervertebral discs, and many other body components. It is not as hard and rigid as bone, but it is much stiffer and much less flexible than muscle.

Because of its rigidity, cartilage often serves the purpose of holding tubes open in the body. Examples include the rings of the trachea, such as the cricoid cartilage and carina.

Cartilage is composed of specialized cells called chondrocytes that produce a large amount of collagenous extracellular matrix, abundant ground substance that is rich in proteoglycan and elastin fibers. Cartilage is classified in three types, elastic cartilage (Figure 9.12), hyaline cartilage (Figure 9.10) and fibrocartilage (Figure 9.13), which differ in relative amounts of collagen and proteoglycan.

Cartilage does not contain blood vessels (it is avascular) or nerves (it is aneural). Nutrition is supplied to the chondrocytes by diffusion. The compression of the articular cartilage or flexion of the elastic cartilage generates fluid flow, which assists diffusion of nutrients to the chondrocytes. Compared to other connective tissues, cartilage has a very slow turnover of its extracellular matrix and does not repair.

11.10 Bone

Bones⁸ support and protect the various organs of the body, produce red and white blood cells, store minerals, provide structure and support for the body, and enable mobility. Bones come in a variety of shapes and sizes and have a complex internal and external

⁶<https://en.wikipedia.org/wiki/Muscle>

⁷<https://en.wikipedia.org/wiki/Cartilage>

⁸<https://en.wikipedia.org/wiki/Bone>

structure. They are lightweight yet strong and hard, and serve multiple functions.

Bone tissue (osseous tissue) is a hard tissue, a type of dense connective tissue. It has a honeycomb-like matrix internally, which helps to give the bone rigidity. Bone tissue is made up of different types of bone cells. Osteoblasts and osteocytes are involved in the formation and mineralization of bone; osteoclasts are involved in the resorption of bone tissue. Modified (flattened) osteoblasts become the lining cells that form a protective layer on the bone surface. The mineralized matrix of bone tissue has an organic component of mainly collagen called ossein and an inorganic component of bone mineral made up of various salts. Bone tissue is a mineralized tissue of two types, cortical bone and cancellous bone. Other types of tissue found in bones include bone marrow, endosteum, periosteum, nerves, blood vessels and cartilage.

In the human body at birth, there are over 270 bones but many of these fuse together during development, leaving a total of 206 separate bones in the adult, not counting numerous small sesamoid bones. The largest bone in the body is the femur or thigh-bone, and the smallest is the stapes in the middle ear.

The Latin word for bone is *os*, hence the many terms that use it as a prefix – such as osseous and osteopathy.

Bone is not uniformly solid, but includes a tough matrix. This matrix makes up about 30% of the bone and the other 70% is of salts that give strength to it. The matrix is made up of between 90 and 95% collagen fibers, and the remainder is ground substance. The primary tissue of bone, bone tissue (osseous tissue), is relatively hard and lightweight. Its matrix is mostly made up of a composite material incorporating the inorganic mineral calcium phosphate in the chemical arrangement termed calcium hydroxylapatite (this is the bone mineral that gives bones their rigidity) and collagen, an elastic protein which improves fracture resistance. The collagen of bone is known as ossein. Bone is formed by the hardening of this matrix around entrapped cells. When these cells become entrapped from osteoblasts they become osteocytes.

The hard, outer layer of bones is composed of cortical bone also called compact bone being much denser than cancellous bone. It forms the hard exterior (cortex) of bones. The cortical bone gives bone its smooth, white, and solid appearance. It facilitates bone's main functions: to support the whole

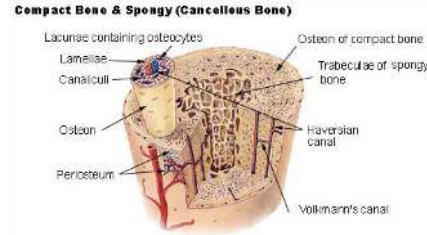


Figure 11.2: Compact and spongy bone.⁹

body, protect organs, provide levers for movement, and store and release chemical elements, mainly calcium. It consists of multiple microscopic columns, each called an osteon. Each column is multiple layers of osteoblasts and osteocytes around a central canal called the Haversian canal. Volkmann's canals at right angles connect the osteons together. The columns are metabolically active, and as bone is reabsorbed and created the nature and location of the cells within the osteon will change. Cortical bone is covered by a periosteum on its outer surface, and an endosteum on its inner surface. The endosteum is the boundary between the cortical bone and the cancellous bone. The primary anatomical and functional unit of cortical bone is the osteon.

Spongy bone tissue is the internal tissue of the skeletal bone and is an open cell porous network. Spongy bone is typically found at the ends of long bones, near to joints and within the interior of vertebrae. Spongy bone is highly vascular and frequently contains red bone marrow where hematopoiesis, the production of blood cells, occurs. The primary anatomical and functional unit of spongy bone is the trabecula. The trabeculae are aligned towards the mechanical load distribution that a bone experiences within long bones such as the femur. Within these spaces are bone marrow and hematopoietic stem cells that give rise to platelets, red blood cells and white blood cells.

11.11 The Kidneys

The kidneys¹⁰ are two bean-shaped organs found on the left and right sides of the body in vertebrates. They are located at the back of the abdominal cavity in the retroperitoneal space. In adult humans, they are about 11 centimeters in length. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached

¹⁰<https://en.wikipedia.org/wiki/Kidney>

to a ureter, a tube that carries excreted urine to the bladder.

The nephron is the structural and functional unit of the kidney. Each adult kidney contains around one million nephrons. The nephron utilizes four processes to alter the blood plasma which flows to it: filtration, reabsorption, secretion, and excretion. Via one or more of these mechanisms, the kidney participates in the control of the volume of various body fluid compartments, fluid osmolality, acid-base balance, various electrolyte concentrations, and removal of toxins. Filtration occurs in the glomerulus: one-fifth of the blood volume that enters the kidneys is filtered. Examples of substances reabsorbed are solute-free water, sodium, bicarbonate, glucose, and amino acids. Examples of substances secreted are hydrogen, ammonium, potassium and uric acid. The kidneys also carry out functions independent of the nephron. For example, they convert a precursor of vitamin D to its active form – calcitriol – and synthesize the hormones erythropoietin and renin.

11.12 Sheep Kidney Dissection

1. Obtain a dissecting pan and a set of dissecting instruments.
2. Place the preserved sheep kidney on your dissecting tray.
3. Cut the kidney in half making a frontal section. Start your incision on the side of the kidney opposite of the hilus and carefully make your way through the cortex, medulla, pelvis, ureter and renal blood vessels.
4. Identify the following parts of the kidney
 - cortex
 - renal column
 - medullary pyramid
 - minor calyx
 - major calyx
 - renal pelvis
 - ureter
 - renal artery
 - renal vein

11.13 Cleaning up

1. Dispose of the dissected kidney in the red bio-hazard bins.
2. Clean the dissection tray and get a sheep heart for dissection.

11.14 The Heart

The heart¹¹ is a muscular organ in most animals, which pumps blood through the blood vessels of the circulatory system. Blood provides the body with oxygen and nutrients, as well as assists in the removal of metabolic wastes. In humans, the heart is located between the lungs, in the middle compartment of the chest.

In humans, other mammals, and birds, the heart is divided into four chambers: upper left and right atria; and lower left and right ventricles. Commonly the right atrium and ventricle are referred together as the right heart and their left counterparts as the left heart. Fish, in contrast, have two chambers, an atrium and a ventricle, while reptiles have three chambers. In a healthy heart blood flows one way through the heart due to heart valves, which prevent back-flow. The heart is enclosed in a protective sac, the pericardium, which also contains a small amount of fluid. The wall of the heart is made up of three layers: epicardium, myocardium, and endocardium.

The heart pumps blood with a rhythm determined by a group of pacemaking cells in the sinoatrial node. These generate a current that causes contraction of the heart, traveling through the atrioventricular node and along the conduction system of the heart. The heart receives blood low in oxygen from the systemic circulation, which enters the right atrium from the superior and inferior venae cavae and passes to the right ventricle. From here it is pumped into the pulmonary circulation, through the lungs where it receives oxygen and gives off carbon dioxide. Oxygenated blood then returns to the left atrium, passes through the left ventricle and is pumped out through the aorta to the systemic circulation—where the oxygen is used and metabolized to carbon dioxide. The human heart beats at a resting rate close to 72 beats per minute. Exercise temporarily increases the rate, but lowers resting heart rate in the long term, and is good for heart health. An adult human heart has a mass of 250–350 grams. The heart is typically the size of a fist. Well-trained athletes can have much larger hearts due to the effects of exercise on the heart muscle, similar to the response of skeletal muscle.

The heart has four chambers, two upper atria, the receiving chambers, and two lower ventricles, the discharging chambers. The atria open into the ventricles via the atrioventricular valves, present in the atrioventricular septum. This distinction is visible

¹¹<https://en.wikipedia.org/wiki/Heart>

also on the surface of the heart as the coronary sulcus. There is an ear-shaped structure in the upper right atrium called the right atrial appendage, or auricle, and another in the upper left atrium, the left atrial appendage. The right atrium and the right ventricle together are sometimes referred to as the right heart. Similarly, the left atrium and the left ventricle together are sometimes referred to as the left heart. The ventricles are separated from each other by the interventricular septum, visible on the surface of the heart as the anterior longitudinal sulcus and the posterior interventricular sulcus.

The heart has four valves, which separate its chambers. One valve lies between each atrium and ventricle, and one valve rests at the exit of each ventricle.

The valves between the atria and ventricles are called the atrioventricular valves. Between the right atrium and the right ventricle is the tricuspid valve. The tricuspid valve has three cusps, which connect to chordae tendinae and three papillary muscles named the anterior, posterior, and septal muscles, after their relative positions. The mitral valve lies between the left atrium and left ventricle. It is also known as the bicuspid valve due to its having two cusps, an anterior and a posterior cusp. These cusps are also attached via chordae tendinae to two papillary muscles projecting from the ventricular wall. These muscles prevent the valves from falling too far back when they close. During the relaxation phase of the cardiac cycle, the papillary muscles are also relaxed and the tension on the chordae tendinae is slight. As the heart chambers contract, so do the papillary muscles. This creates tension on the chordae tendinae, helping to hold the cusps of the atrioventricular valves in place and preventing them from being blown back into the atria.

Two additional semilunar valves sit at the exit of each of the ventricles. The pulmonary valve is located at the base of the pulmonary artery. This has three cusps which are not attached to any papillary muscles. When the ventricle relaxes blood flows back into the ventricle from the artery and this flow of blood fills the pocket-like valve, pressing against the cusps which close to seal the valve. The semilunar aortic valve is at the base of the aorta and also is not attached to papillary muscles. This too has three cusps which close with the pressure of the blood flowing back from the aorta.

Heart tissue, like all cells in the body, needs to be supplied with oxygen, nutrients and a way of removing metabolic wastes. This is achieved by the

coronary circulation, which includes arteries, veins, and lymphatic vessels. Blood flow through the coronary vessels occurs in peaks and troughs relating to the heart muscle's relaxation or contraction.

Heart tissue receives blood from two arteries which arise just above the aortic valve. These are the left main coronary artery and the right coronary artery. The left main coronary artery splits shortly after leaving the aorta into two vessels, the left anterior descending and the left circumflex artery. The left anterior descending artery supplies heart tissue and the front, outer side, and the septum of the left ventricle. It does this by branching into smaller arteries – diagonal and septal branches. The left circumflex supplies the back and underneath of the left ventricle. The right coronary artery supplies the right atrium, right ventricle, and lower posterior sections of the left ventricle.

11.15 Sheep Heart Dissection

1. Obtain a dissecting pan and a set of dissecting instruments.
2. Place the preserved sheep heart on your dissecting tray.
3. Identify the right and left sides of the heart. Look closely and on one side you will see a diagonal line of blood vessels that divide the heart, this line is called the interventricular sulcus. The half that includes all of the apex (pointed end) of the heart is the left side.
4. Locate the coronary arteries and veins that are on the surface of the heart.
5. Find the flaps of dark tissue on the top of the heart. These ear-like flaps are called auricles.
6. The front-most vessel is the pulmonary trunk.
7. Just behind the pulmonary trunk is the aorta. Depending on how the heart was removed, you might also see a branch of the aorta called the brachiocephalic artery.
8. Turn the heart so that you are looking at its dorsal side (the back of the heart.) Find the large opening at the top of the heart next to the right auricle. This is the superior vena cava. You may insert your finger into the vena cava superior to feel the inside of the right atrium.
9. Locate another opening on the backside of the heart on the left side. This is the pulmonary vein. You may insert your finger again to feel the inside of the right atrium.
10. Use a scalpel to make an incision in the heart at the superior vena cava. The incision should

follow the line of the right side of the heart so that you can open just the right side and see the right atrium, the right ventricle, and the tricuspid valve between them.

11. The chordae tendineae are attached to the thin flaps of the tricuspid. They are anchored to the wall of the heart at the papillary muscle.
12. Make a similar incision on the left side of the heart to expose the left atrium, left ventricle, and the bicuspid valve. Notice the chordae tendineae and the papillary muscle on this side of the heart.
13. Insert a probe into the aorta and observe where the probe exits the heart. You may even be able to find the small aortic semilunar valve at the place where the aorta connects to the heart.



Figure 11.3: Artery, vein, capillary.

11.16 Cleaning up

1. Dispose of the dissected heart in the red biohazard bins.
2. Clean the dissection tray and instruments and return them to the place where you picked them up.
3. Clean table tops with red bottled sanitizer
4. Wash your hands.

11.17 View Prepared Slides

1. Cardiac Muscle (Figure 9.18)
 - Identify: branched cardiac muscle cells, nuclei; intercalated disks
2. Cardiac Muscle Human
 - Identify: branched cardiac muscle cells, nuclei; intercalated disks
3. Artery, Vein, Capillary x.s. (Figure 11.3)
 - Identify: artery, vein, capillary
4. Atherosclerosis (Figure 11.4)
 - Locate: arteriosclerotic plaque
5. Kidney (Figure 11.5).
 - Locate: renal corpuscles, proximal and distal tubules, collecting ducts.

In the skin slides listed below identify as many of the following as you can find:

- skin layers: epidermis, dermis, sub-cutaneous
- blood vessel
- dense fibrous irregular connective tissue
- keratinized layer in epidermis
- adipose tissue

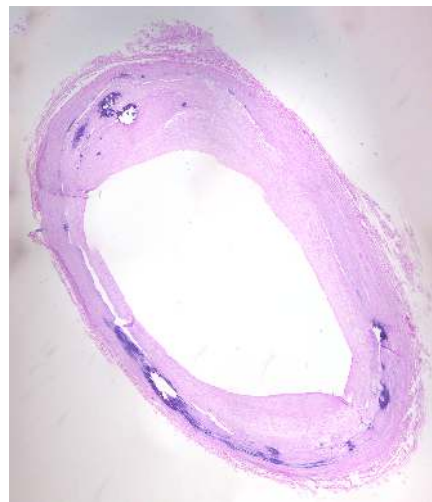


Figure 11.4: Atherosclerosis.

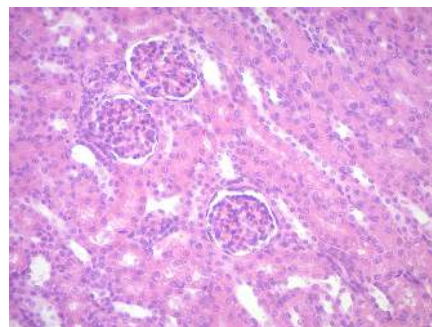


Figure 11.5: Cortex of the kidney.



Figure 11.6: Unpigmented skin.

- smooth muscle of pilli
- stratified squamous epithelium
- hair
- hair follicle
- hair root
- hair papilla
- sweat glands
- sebaceous glands
- Vater-Pacini corpuscle

1. Unpigmented skin (Figure 11.6)
2. Axillary skin (Figure 11.7)
3. Skin, hairy mammal
4. Human scalp hair shafts (Figure 11.8)
5. Cornified skin (Figure 11.9)
6. Compact Bone (Figure 11.10)
 - Identify: Haversian Systems, osteocytes in lacunae, Haversian Canal, canaliculi, calcified matrix
7. Bone Ground Human x.s. (Figure 9.14)
 - Identify: Haversian Systems, osteocytes in lacunae, Haversian Canal, canaliculi, calcified matrix

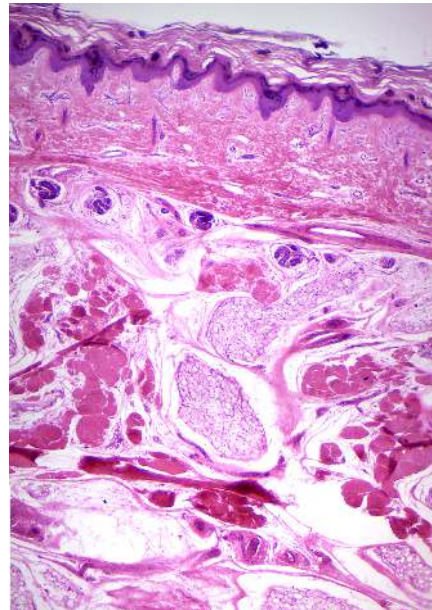


Figure 11.7: Axillary skin.

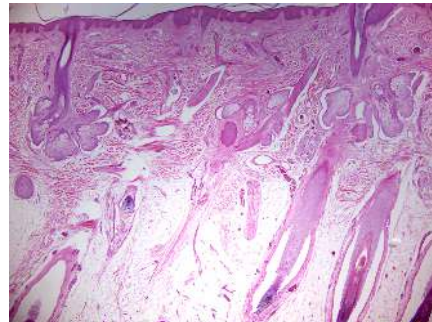


Figure 11.8: Human scalp.

11.18 The human skeleton

The human skeleton¹² (Figure 11.11) is the internal framework of the body. It is composed of around 270 bones at birth and around 206 bones by adulthood after some bones get fused together. The bone mass in the skeleton reaches maximum density around age 21. The human skeleton can be divided into the axial skeleton and the appendicular skeleton. The axial skeleton is formed by the vertebral column, the rib cage, the skull and other associated bones. The appendicular skeleton, which is attached to the axial skeleton, is formed by the shoulder girdle, the pelvic girdle and the bones of the upper and lower limbs.

¹²https://en.wikipedia.org/wiki/Human_skeleton

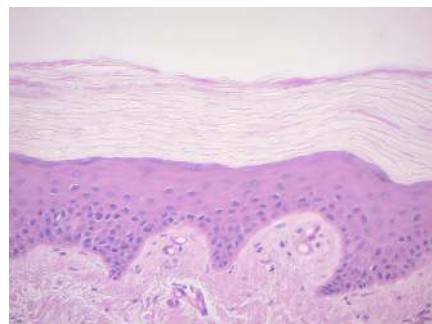


Figure 11.9: Cornified skin.

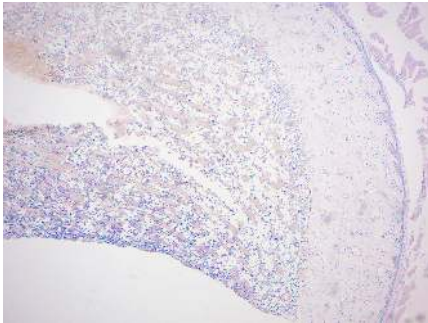


Figure 11.10: Compact Bone.

The human skeleton performs six major functions; support, movement, protection, production of blood cells, storage of minerals, and endocrine regulation.

The human skeleton is not as sexually dimorphic as that of many other primate species, but subtle differences between sexes in the morphology of the skull, dentition, long bones, and pelvis exist. In general, female skeletal elements tend to be smaller and less robust than corresponding male elements within a given population. The human female pelvis is also different from that of males in order to facilitate child-birth. Unlike most primates, human males do not have penile bones.

11.19 Locate the following bones of the human skeleton:

1. Axial skeleton:

Skull Bones:

Frontal	Mandible
Parietal	Maxilla
Temporal	Zygomatic
Occipital	Nasal

Vertebral Column:

7 cervical	1 fused sacrum
12 thoracic	1 fused coccyx
5 lumbar	

Ribs - 12 pairs; notice the two pairs of "floating" ribs

Sternum

Hyoid bone (not shown on small plastic models)

2. Appendicular skeleton:

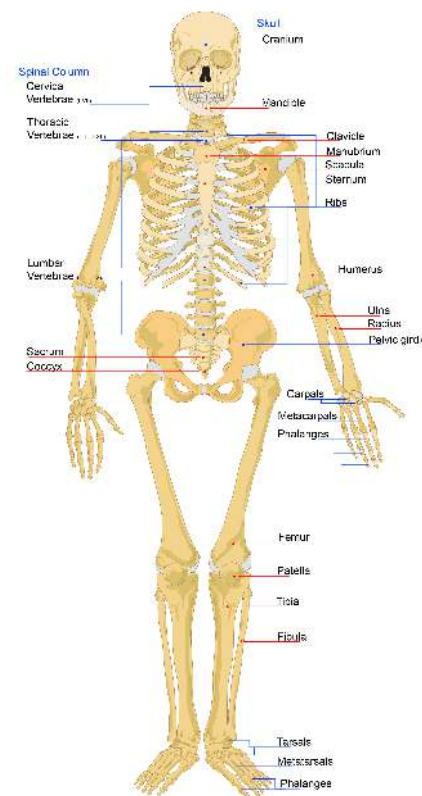


Figure 11.11: The human skeleton.¹³

Pectoral girdle:

Clavicle	Ulna
Scapula	Carpals
Humerus	Metacarpals
Radius	Phalanges

Pelvic girdle:

Coccyx	Fibula
Femur	Tarsals
Patella	Metatarsals
Tibia	Phalanges

3. Skulls:

- Adult: locate the following bones: frontal, occipital, temporal parietal, mandible, maxilla, zygomatic, nasal, sphenoid, ethmoid. Also locate the immovable joints known as sutures.
- Fetal: locate the fontanel ("soft spot")
- Female and Male Pelvic Bones: Notice the pubic arch angle and other characteristics and determine which are female and which are male. Locate the ilium, pubis, and ischium bones. Locate the pubic symphysis (classified as a slightly moveable joint) located between the pubic bones.

3. The basic functional unit of the kidney is the _____.
4. The blood is filtered in the _____ of the kidney.
5. The valve between the right atrium and ventricle is called the _____ valve.
6. The valve between the left atrium and ventricle is called the _____ valve.
7. What is the difference between arteries and veins?
8. The most superficial layer of the skin is called the _____.
9. The hard, outer layer of bones is composed of cortical bone also called _____ bone.
10. _____ bone tissue is the internal tissue of the skeletal bone and is an open cell porous network.

11.20 Comparison of Vertebrate Skeletons

1. Compare the human skeleton to the following:

Bony Fish	Bird
Frog	Bat
Snake	Cat
Turtle	Monkey

2. Locate the following bones in each skeleton (if possible):

Humerus	Metacarpals	Fibula
Radius	Phalanges	Tarsals
Ulna	Femur	Metatarsals

11.21 Review Questions

1. Describe the journey of the blood through the heart.
2. Heart muscle receives blood from two arteries which arise just above the aortic valve. These are the _____ and the _____.

Chapter 12

Reproduction in Animals and Gametogenesis

Nearly all animals undergo some form of sexual reproduction. They produce haploid gametes by meiosis. The smaller, motile gametes are spermatozoa and the larger, non-motile gametes are ova. The gametes fuse to form zygotes, which develop via multiple successive mitoses and differentiation into new individuals.

Some animals are also capable of asexual reproduction. This may take place through parthenogenesis (from the Greek *parthenos*, “virgin”, + *genesis*, “creation”), the development of an embryo from an unfertilized egg cell. During sexual reproduction, mating with a close relative (inbreeding) generally leads to reduced biological fitness, i.e. the organism’s reduced ability to survive and perpetuate its genetic material. Inbreeding results in more recessive traits manifesting themselves, as the genomes of pair-mates are more similar. Animals have evolved numerous diverse mechanisms for avoiding close inbreeding and promoting out-crossing.

12.1 Gametogenesis

Gametogenesis¹ is a biological process by which diploid or haploid precursor cells undergo cell division and differentiation to form mature haploid gametes. Depending on the biological life cycle of the organism, gametogenesis occurs by meiotic division of diploid gametocytes into various gametes, or by mitosis, as we have seen in plants, for example.

Animals produce gametes directly through meiosis in organs called gonads (testis in males and ovaries

in females). Males and females of a species that reproduces sexually have different forms of gametogenesis

- spermatogenesis (male)
- oogenesis (female)

Spermatogenesis² is the process in which an animal produces spermatozoa from spermatogonial stem cells by way of mitosis and meiosis. The initial cells in this pathway are called spermatogonia, which yield primary spermatocytes by mitosis. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two spermatids by Meiosis II. These develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa.

Spermatozoa are the mature male gametes in many sexually reproducing organisms. Thus, spermatogenesis is the male version of gametogenesis, of which the female equivalent is oogenesis. In mammals, it occurs in the seminiferous tubules of the male testes in a stepwise fashion. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. It starts at puberty and usually continues uninterrupted until death, although a slight decrease can be discerned in the quantity of produced sperm with increase in age

Spermatogenesis takes place within several structures of the male reproductive system. The initial stages occur within the testes and progress to the epididymis where the developing gametes mature and are stored until ejaculation. The seminiferous

¹<https://en.wikipedia.org/wiki/Gametogenesis>

²<https://en.wikipedia.org/wiki/Spermatogenesis>

tubules of the testes are the starting point for the process, where spermatogonial stem cells adjacent to the inner tubule wall divide in a centripetal direction—beginning at the walls and proceeding into the innermost part, or lumen—to produce immature sperm. Maturation occurs in the epididymis. The location [Testes/Scrotum] is specifically important as the process of spermatogenesis requires a lower temperature to produce viable sperm, specifically 1-8°C lower than normal body temperature of 37°C. For humans, the entire process of spermatogenesis is variously estimated as taking between 74 days and approximately 120 days. Including the transport on ductal system, it takes 3 months. Testes produce 200 to 300 million spermatozoa daily. However, only about half or 100 million of these become viable sperm.

Oogenesis³ is the differentiation of the ovum (egg cell) into a cell competent to further development when fertilized. It is developed from the primary oocyte by maturation. Oogenesis starts with the process of developing oogonia, which occurs via the transformation of primordial follicles into primary oocytes, a process called oocytogenesis. Oocytogenesis is complete either before or shortly after birth. It is commonly believed that, when oocytogenesis is complete, no additional primary oocytes are created, in contrast to the male process of spermatogenesis, where gametocytes are continuously created. In other words, primary oocytes reach their maximum development at ~20 weeks of gestational age, when approximately seven million primary oocytes have been created; however, at birth, this number has already been reduced to approximately 1-2 million. The succeeding phase of ootidogenesis occurs when the primary oocyte develops into an ootid. This is achieved by the process of meiosis. In fact, a primary oocyte is, by its biological definition, a cell whose primary function is to divide by the process of meiosis. However, although this process begins at prenatal age, it stops at prophase I. In late fetal life, all oocytes, still primary oocytes, have halted at this stage of development, called the dictyate. After menarche, these cells then continue to develop, although only a few do so every menstrual cycle. Meiosis I of ootidogenesis begins during embryonic development, but halts in the diplotene stage of prophase I until puberty. For those primary oocytes that continue to develop in each menstrual cycle, however, synapsis occurs and tetrads form, enabling chromosomal crossover to occur. As a result of meiosis I, the primary oocyte has now

developed into the secondary oocyte and the first polar body. Immediately after meiosis I, the haploid secondary oocyte initiates meiosis II. However, this process is also halted at the metaphase II stage until fertilization, if such should ever occur. When meiosis II has completed, an ootid and another polar body have now been created. Synchronously with ootidogenesis, the ovarian follicle surrounding the ootid has developed from a primordial follicle to a preovulatory one. Both polar bodies disintegrate at the end of Meiosis II, leaving only the ootid, which then eventually undergoes maturation into a mature ovum. The function of forming polar bodies is to discard the extra haploid sets of chromosomes that have resulted as a consequence of meiosis.

12.2 Embryogenesis and Embryonic development

Embryogenesis⁴ is the process by which the embryo forms and develops. In mammals, the term refers chiefly to early stages of prenatal development, whereas the terms fetus and fetal development describe later stages. Embryogenesis starts with the fertilization of the egg cell (ovum) by a sperm cell, (spermatozoon). Once fertilized, the ovum is referred to as a zygote, a single diploid cell. The zygote undergoes mitotic divisions with no significant growth (a process known as cleavage) and cellular differentiation, leading to development of a multicellular embryo. At least four initial cell divisions occur, resulting in a dense ball of at least sixteen cells called the morula. The different cells derived from cleavage, up to the blastula stage, are called blastomeres. After the 7th cleavage has produced 128 cells, the embryo is called a blastula. The blastula is usually a spherical layer of cells (the blastoderm) surrounding a fluid-filled or yolk-filled cavity (the blastocoel). During gastrulation cells migrate to the interior of the blastula, subsequently forming two (in diploblastic animals) or three (triploblastic) germ layers. The embryo during this process is called a gastrula. The germ layers are referred to as the ectoderm, mesoderm and endoderm. In diploblastic animals only the ectoderm and the endoderm are present. In bilateral animals, the blastula develops in one of two ways that divides the whole animal kingdom into two halves. If in the blastula the first pore (blastopore) becomes the mouth of the animal, it is a protostome; if the first pore becomes the anus

³<https://en.wikipedia.org/wiki/Oogenesis>

⁴<https://en.wikipedia.org/wiki/Embryogenesis>

then it is a deuterostome. The protostomes include most invertebrate animals, such as insects, worms and mollusks, while the deuterostomes include the vertebrates. In due course, the blastula changes into a more differentiated structure called the gastrula. The gastrula with its blastopore soon develops three distinct layers of cells (the germ layers) from which all the bodily organs and tissues then develop:

- The endoderm, the innermost layer, gives a rise to the digestive organs, the gills, lungs or swim bladder if present, and kidneys or nephrites.
- The mesoderm, the middle layer, gives rise to the muscles, skeleton if any, and blood system.
- The ectoderm, the outer layer of cells, gives rise to the nervous system, including the brain, and skin or carapace and hair, bristles, or scales.

Somitogenesis⁵ is the process by which somites (primitive segments) are produced. These segmented tissue blocks differentiate into skeletal muscle, vertebrae, and dermis of all vertebrates. At some point after the different germ layers are defined, organogenesis begins. The first stage in vertebrates is called neurulation, where the neural plate folds forming the neural tube. Other common organs or structures that arise at this time include the heart and somites, but from now on embryogenesis follows no common pattern among the different taxa of the animal kingdom. In most animals, organogenesis along with morphogenesis will result in a larva. The hatching of the larva, which must then undergo metamorphosis, marks the end of embryonic development.

12.3 Comparative embryology

Comparative embryology⁶ is the branch of embryology that compares and contrasts embryos of different species. It is used to show how all animals are related. Many things are compared (such as whether or not the organism has a notochord or gill arches). Many components go into comparative embryology, and much information about the developmental similarities between species can be taken from its study, from which many conclusions can be drawn. These similarities among species are called homologous structures, which are structures that have the same or similar function and mechanism, having evolved from a common ancestor. The goal of comparative embryology is to make sense of how an embryo develops, and of how all animals are related.

⁵<https://en.wikipedia.org/wiki/Somitogenesis>

⁶https://en.wikipedia.org/wiki/Comparative_embryology

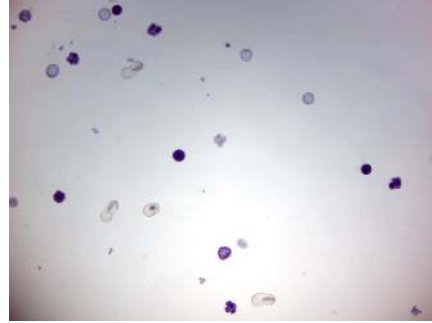


Figure 12.1: Starfish development.

Comparative embryology also supports evolutionary theory, in the sense that all vertebrates develop similarly. The conclusion is that all vertebrates must have a common ancestor.

12.4 View Prepared Slides

12.5 Starfish embryology

1. Starfish development slide (Figure 12.1)
 - Locate: unfertilized egg, fertilized egg, 2-cell, 4-cell, 8-cell, morula, blastula, early gastrula, late gastrula, bipinnaria larva. In a late gastrula, locate the primary germ layers (ectoderm, mesoderm, endoderm), archenteron (primitive gut), blastopore and blastocoel.

12.6 Frog embryology

1. Frog early cleavage (Figure 12.2)
 - Count the cells in this early from embryo.
2. Frog blastula (Figure 12.3)
3. Frog gastrulation: yolk plug stage (Figure 12.4)
 - Locate: yolk plug, ectoderm, endoderm, archenteron (gastrocoel)
4. Frog gastrula (Figure 12.5)
5. Frog early neural groove x.s. (Figure 12.6)
 - Locate neural groove, ectoderm, endoderm, mesoderm, archenteron, location of presumptive notochord
6. Frog late neural tube x.s. (Figure 12.7)
 - Locate: neural tube, ectoderm, endoderm, mesoderm, coelom, archenteron, notochord

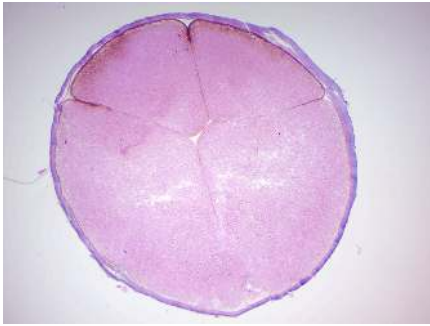


Figure 12.2: Frog early cleavage.

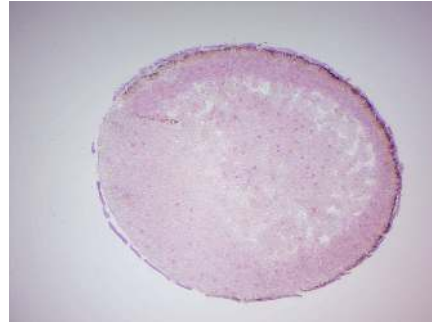


Figure 12.5: Frog gastrula.



Figure 12.3: Frog blastula.



Figure 12.6: Frog neural groove.

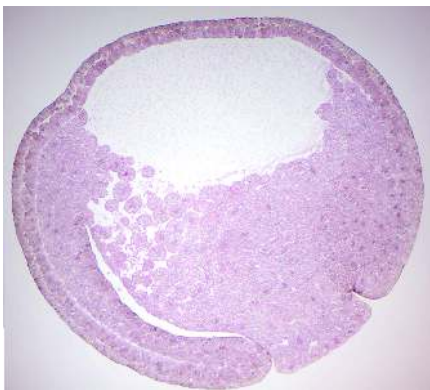


Figure 12.4: Frog gastrulation with yolk plug.

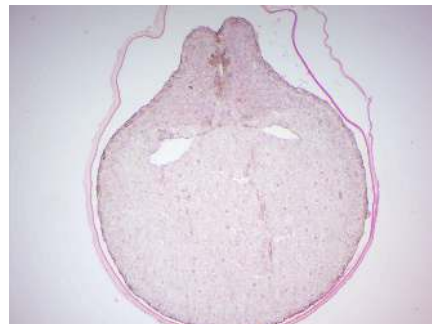


Figure 12.7: Frog neural tube.



Figure 12.8: Chick embryo at 33 hours.

12.7 Chicken embryology



Notice that these slides are very thick, since they contain whole mounts (w.m.) of chicken embryos. Use only the 4× (low power) objective of your microscope to avoid crushing these slides.

1. Chick embryo 33 hrs (Figure 12.8).
 - Locate: head, amniotic fold, forebrain, midbrain, hindbrain, heart, somites, neural tube. What structures will develop from the somites later?
2. Chick embryo 48 hrs (Figure 12.9).
 - Locate: same structures as above plus: optic cup with lens, ventricle and atrium of the heart, auditory vesicle, tail, and tail amniotic fold, notochord.
3. Chick embryo 72 hrs (Figure 12.10).
 - Locate: same structures as above plus; allantois, hind limb bud.
4. Chick Embryo 96 hrs (Figure 12.11).
 - Locate: same structures as above.

12.8 Mammalian reproductive organs and gametes

1. Cat ovary (Figure 12.12)



Figure 12.9: Chick embryo at 48 hours.



Figure 12.10: Chick embryo at 72 hours.

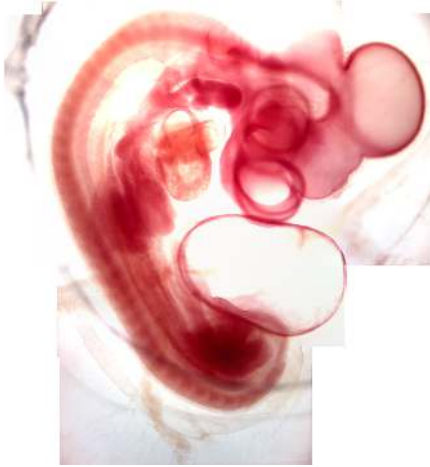


Figure 12.11: Chick embryo at 96 hours.

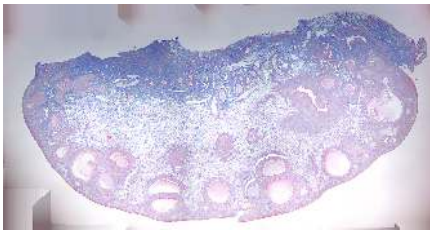


Figure 12.12: Cat ovary.

- Identify primary, secondary and Graffian follicles
- 2. Graafian follicle (Figure 12.14).
- 3. Cat ovary Corpus Luteum
 - Locate corpus luteum
- 4. Uterus (Figure 12.13)
 - Identify: endometrium, myometrium, perimetrium
- 5. Monkey testis (Figure 12.15)
 - Locate: semimiferous tubules, spermatozoa
- 6. Human testis (Figure 12.16)
 - Locate: semimiferous tubules, spermatozoa
- 7. Human sperm smear (Figure 12.17)
 - Identify: sperm, sperm head, sperm neck and sperm tail



Figure 12.13: Uterus.

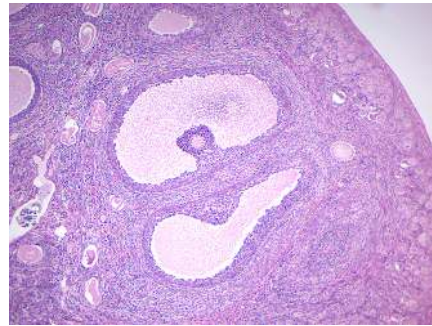


Figure 12.14: Graafian follicle in cat ovary.

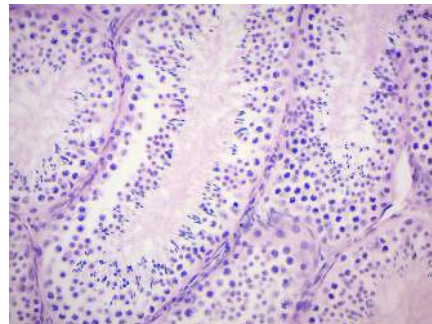


Figure 12.15: Monkey testis.

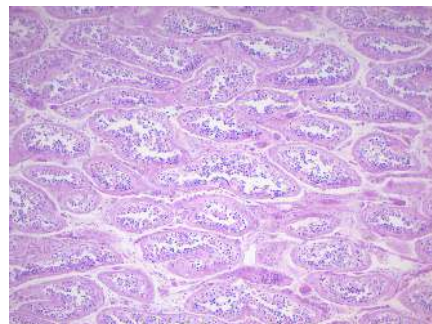


Figure 12.16: Human testis.

12.9 Review Questions

1. What are gametes?
2. Spermatogenesis is the process in which an animal produces _____.



Figure 12.17: Human sperm.

3. Oogenesis is the process in which an animal produces _____.
4. The gametes fuse to form _____, which develop via multiple successive mitoses and differentiation into new individuals.
5. In bilateral animals, the blastula develops in one of two ways that divides the whole animal kingdom into two halves.
6. If in the blastula the first pore (blastopore) becomes the mouth of the animal, it is a _____; if the first pore becomes the anus then it is a _____.
7. In triploblastic animals, the three tissue (germ) layers of the gastrula are the
 - (a) _____
 - (b) _____
 - (c) _____
8. Somitogenesis is the process by which _____ are produced.
9. In frog embryology, what structures are formed during neurulation?

Appendix A

Explanations of Important Terms And Concepts

A.1 Autotrophs and Heterotrophs

An autotroph¹ (“self-feeding”, from the Greek *autos* “self” and *trophe* “nourishing”) or producer, is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings, generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis). They are the producers in a food chain, such as plants on land or algae in water. Autotrophs can reduce carbon dioxide to make organic compounds for biosynthesis and also create a store of chemical energy. Most autotrophs use water as the reducing agent, but some can use other hydrogen compounds such as hydrogen sulfide.

Autotrophs can be photoautotrophs or chemoautotrophs. Phototrophs use light as an energy source, while chemotrophs use electron donors as a source of energy, whether from organic or inorganic sources; however, in the case of autotrophs, these electron donors come from inorganic chemical sources. Such chemotrophs are lithotrophs. Lithotrophs use inorganic compounds, such as hydrogen sulfide, elemental sulfur, ammonium and ferrous iron, as reducing agents for biosynthesis and chemical energy storage. Photoautotrophs and lithoautotrophs use a portion of the ATP produced during photosynthesis or the oxidation of inorganic compounds to reduce NADP^+ to NADPH to form organic compounds.

A heterotroph² Greek *héteros* = “other” plus *trophe* = “nutrition”) is an organism that ingests or absorbs organic carbon (rather than fix carbon from

inorganic sources such as carbon dioxide) in order to be able to produce energy and synthesize compounds to maintain its life. Ninety-five percent or more of all types of living organisms are heterotrophic, including all animals and fungi and some bacteria and protists.

Detritivores³ are heterotrophs which obtain nutrients by consuming detritus (decomposing plant and animal parts as well as feces). Saprotrophs (also called lysotrophs) are chemoheterotrophs that use extracellular digestion in processing decayed organic matter. It is a term most often associated with fungi. The process is most often facilitated through the active transport of such materials through endocytosis within the internal mycelium and its constituent hyphae.

A.2 Biological Life Cycle

A biological life cycle⁴ is a series of changes in form that an organism undergoes, returning to the starting state. Transitions of form may involve growth, asexual reproduction, or sexual reproduction. In some organisms, different “generations” of the species succeed each other during the life cycle. For plants and many algae, there are two multicellular stages, and the life cycle is referred to as alternation of generations. Life cycles that include sexual reproduction involve alternating haploid (n) and diploid ($2n$) stages, i.e., a change of ploidy is involved. To return from a diploid stage to a haploid stage, meiosis must occur. In regard to changes of ploidy, there are 3 types of life cycles that we will encounter in this course:

¹<https://en.wikipedia.org/wiki/Autotroph>

²<https://en.wikipedia.org/wiki/Heterotroph>

³<https://en.wikipedia.org/wiki/Detritivore>

⁴https://en.wikipedia.org/wiki/Biological_life_cycle

1. **haplontic life cycle** (e.g. in fungi): the haploid stage is multicellular and the diploid stage is a single cell, meiosis is “zygotic”.
2. **diplontic life cycle** (e.g. in animals): the diploid stage is multicellular and haploid gametes are formed, meiosis is “gametic”.
3. **haplodiplontic life cycle** (e.g. in plants): multicellular diploid (e.g. the sporophyte in plants) and haploid stages (e.g. the gametophyte in plants) occur, meiosis is “sporic”.

The cycles differ in when mitosis (growth) occurs. Zygotic meiosis and gametic meiosis have one mitotic stage: mitosis occurs during the n (haploid) phase in zygotic meiosis (e.g. in fungi) and during the $2n$ (diploid) phase in gametic meiosis (e.g. in animals). Therefore, zygotic and gametic meiosis are collectively termed haplobiontic (single mitotic phase, not to be confused with haplontic). Sporic meiosis (e.g. in plants), on the other hand, has mitosis in two stages, both the diploid and haploid stages, termed diplobiontic (not to be confused with diplontic).

A.3 Asexual and Sexual Reproduction

Reproduction⁵ (or procreation or breeding) is the biological process by which new individual organisms – “offspring” – are produced from their “parents”. Reproduction is a fundamental feature of all known life; each individual organism exists as the result of reproduction. There are two forms of reproduction: asexual and sexual.

Asexual reproduction is a process by which organisms create genetically similar or identical copies of themselves without the contribution of genetic material from another organism.

Sexual reproduction creates a new organism by combining the genetic material of two organisms. Most animals and plants reproduce sexually. Each of two parent organisms contributes half of the offspring’s genetic makeup by creating haploid gametes. Most organisms form two different types of gametes. In these anisogamous species, the two sexes are referred to as male (producing sperm or microspores) and female (producing ova or megaspores). In isogamous species, the gametes are similar or identical in form (isogametes). Because both gametes look alike, they cannot be classified as “male” or “female.” Instead, organisms undergoing

isogamy are said to have different mating types, most commonly noted as “+” and “–” strains. Sexual reproduction in fungi differs in many aspects from sexual reproduction in animals or plants. Sexually compatible fungi combine by fusing their hyphae together. Many fungi go through a dikaryotic stage, in which the nuclei inherited from the two parents do not combine immediately after cell fusion, but remain separate in the hyphal cells. Sexually reproducing organisms have different sets of genes for every trait (called alleles). Offspring inherit one allele for each trait from each parent. Thus, offspring have a combination of the parents’ genes.

A.4 Multicellularity

Multicellular organisms⁶ are organisms that consist of more than one cell, in contrast to unicellular organisms. Multicellularity allows an organism to exceed the size limits normally imposed by diffusion: single cells with increased size have a decreased surface-to-volume ratio and have difficulty absorbing sufficient nutrients and transporting them throughout the cell. Multicellular organisms thus have the competitive advantages of an increase in size without its limitations. They can have longer lifespans as they can continue living when individual cells die. Multicellularity also permits increasing complexity by allowing differentiation of cell types within one organism. One hypothesis for the origin of multicellularity is that a group of function-specific cells aggregated into a slug-like mass, which moved as a multicellular unit. This is essentially what slime molds do. Another hypothesis is that a primitive cell underwent nucleus division, thereby becoming a coenocyte. A membrane would then form around each nucleus (and the cellular space and organelles occupied in the space), thereby resulting in a group of connected cells in one organism (this mechanism is observable in the fruit fly *Drosophila*). A third hypothesis is that as a unicellular organism divided, the daughter cells failed to separate, resulting in a conglomeration of identical cells in one organism, which could later develop specialized tissues. This is what plant and animal embryos do as well as colonial choanoflagellates.

⁵<https://en.wikipedia.org/wiki/Reproduction>

⁶https://en.wikipedia.org/wiki/Multicellular_organism

A.5 Species

In biology, a species⁷ is the basic unit of biological classification and a taxonomic rank, as well as a unit of biodiversity. Scientists and others need a species definition which allows them to work, regardless of the theoretical difficulties. If as Linnaeus thought, species were fixed, there would be no problem, but evolutionary processes cause species to change continually, and to grade into one another. A species is often defined as the largest group of organisms in which two individuals can produce fertile offspring, typically by sexual reproduction. While this definition is often adequate, when looked at more closely it is problematic. For example, with hybridization, in a species complex of hundreds of similar microspecies, or in a ring species, the boundaries between closely related species become unclear. Among organisms that reproduce only asexually, the concept of a reproductive species breaks down, and each clone is potentially a microspecies. Problems also arise when dealing with fossils, since reproduction cannot be examined. Other ways of defining species include their karyotype, DNA sequence, morphology, behavior or ecological niche.

All species are given a two-part name, a “binomial” (see below). The first part of a binomial is the genus to which the species belongs. The second part is called the specific name or the specific epithet (in botanical nomenclature, also sometimes in zoological nomenclature). For example, *Boa constrictor* is one of four species of the *Boa* genus.

Species were seen from the time of Aristotle until the 18th century as fixed kinds that could be arranged in a hierarchy, the great chain of being. In the 19th century, biologists grasped that species could evolve given sufficient time. Charles Darwin’s 1859 book *On The Origin of Species*⁸ explained how species could arise by natural selection. That understanding was greatly extended in the 20th century through genetics and population ecology. Genetic variability arises from mutations and recombination, while organisms themselves are mobile, leading to geographical isolation and genetic drift⁹ with varying selection pressures. Genes can sometimes be exchanged between species by horizontal gene transfer¹⁰; new species can arise rapidly through

hybridization¹¹ and polyploidy¹²; and species may become extinct for a variety of reasons. Viruses are a special case, driven by a balance of mutation and selection, and can be treated as quasispecies.

As a practical matter, species concepts may be used to define species that are then used to measure biodiversity, though whether this is a good measure is disputed, as other measures are possible.

The commonly used names for kinds of organisms are often ambiguous: “cat” could mean the domestic cat, *Felis catus*, or the cat family, Felidae. Another problem with common names is that they often vary from place to place, so that puma, cougar, catamount, panther, painter and mountain lion all mean *Puma concolor* in various parts of America, while “panther” may also mean the jaguar (*Panthera onca*) of Latin America or the leopard (*Panthera pardus*) of Africa and Asia. In contrast, the scientific names of species are chosen to be unique and universal; they are in two parts used together: the genus as in *Puma*, and the specific epithet as in *concolor*. A species is given a taxonomic name when a type specimen is described formally, in a publication that assigns it a unique scientific name. The description typically provides means for identifying the new species, differentiating it from other previously described and related or confusable species and provides a validly published name (in botany) or an available name (in zoology) when the paper is accepted for publication. The type material is usually held in a permanent repository, often the research collection of a major museum or university, that allows independent verification and the means to compare specimens. Describers of new species are asked to choose names that, in the words of the International Code of Zoological Nomenclature, are “appropriate, compact, euphonious, memorable, and do not cause offence.”

Biologists and taxonomists have made many attempts to define species, beginning from morphology and moving towards genetics. Early taxonomists such as Linnaeus had no option but to describe what they saw: this was later formalized as the typological or morphological species concept. Mayr emphasized reproductive isolation, but this, like other species concepts, is hard or even impossible to test. Many of the concepts are quite similar or overlap, so they are not easy to count.

The evolutionary process by which biological populations evolve to become distinct or reproductively

⁷<https://en.wikipedia.org/wiki/Species>

⁸https://en.wikipedia.org/wiki/On_the_Origin_of_Species

⁹https://en.wikipedia.org/wiki/Genetic_drift

¹⁰https://en.wikipedia.org/wiki/Horizontal_gene_transfer

¹¹[https://en.wikipedia.org/wiki/Hybrid_\(biology\)](https://en.wikipedia.org/wiki/Hybrid_(biology))

¹²<https://en.wikipedia.org/wiki/Polyploid>

isolated as species is called speciation¹³. Charles Darwin described the role of natural selection in speciation in his 1859 book *On The Origin of Species*. Speciation depends on a measure of reproductive isolation¹⁴, a reduced gene flow. This occurs most easily in allopatric speciation¹⁵, where populations are separated geographically and can diverge gradually as mutations accumulate. Reproductive isolation is threatened by hybridization, but this can be selected against once a pair of populations have incompatible alleles of the same gene, as described in the Bateson–Dobzhansky–Muller model. A different mechanism, phyletic speciation, involves one lineage gradually changing over time into a new and distinct form, without increasing the number of resultant species.

A species is extinct when the last individual of that species dies, but it may be functionally extinct well before that moment. It is estimated that over 99 percent of all species that ever lived on Earth, some five billion species, are now extinct. Some of these were in mass extinctions such as those at the ends of the Permian, Triassic and Cretaceous periods. Mass extinctions had a variety of causes including volcanic activity, climate change, and changes in oceanic and atmospheric chemistry, and they in turn had major effects on Earth's ecology, atmosphere, land surface, and waters. Another form of extinction is through the assimilation of one species by another through hybridization.

A.6 Evolution

Evolution¹⁶ is change in the heritable characteristics of biological populations over successive generations. Evolutionary processes give rise to biodiversity at every level of biological organization, including the levels of species, individual organisms, and molecules.

Repeated formation of new species (speciation), change within species (anagenesis), and loss of species (extinction) throughout the evolutionary history of life on Earth are demonstrated by shared sets of morphological and biochemical traits, including shared DNA sequences. These shared traits are more similar among species that share a more recent common ancestor, and can be used to reconstruct a biological “tree of life” based on evolutionary relationships

(phylogenetics), using both existing species and fossils. The fossil record includes a progression from early biogenic graphite, to microbial mat fossils, to fossilized multicellular organisms. Existing patterns of biodiversity have been shaped both by speciation and by extinction.

Charles Darwin¹⁷ developed his theory of “natural selection” from 1838 onwards and was writing up his “big book” on the subject when Alfred Russel Wallace¹⁸ sent him a version of virtually the same theory in 1858. Their separate papers were presented together at an 1858 meeting of the Linnaean Society of London. At the end of 1859, Darwin's book “*On the Origin of Species*” explained natural selection in detail and in a way, that led to an increasingly wide acceptance of Darwin's concepts of evolution at the expense of alternative theories.

According to Ernst Mayr¹⁹, Darwin's theory actually consists of a number of different theories that can be best understood when they are clearly distinguished from each other. Mayr distinguished five independent theories:

1. The non-constancy of species (the basic theory of evolution)
2. The descent of all organisms from common ancestors (branching evolution)
3. The gradualness of evolution (no saltations, no discontinuities)
4. The multiplication of species (the origin of diversity)
5. Natural selection

The first and second theories were widely accepted by biologists rather quickly following Darwin's publication. The other three theories were not widely accepted until the arrival of the so-called modern synthesis in the 20th century (see below).

Evolution by natural selection²⁰ is a process demonstrated by the observation that more offspring are produced than can possibly survive, along with three facts about populations: 1) traits vary among individuals with respect to morphology, physiology, and behavior (phenotypic variation), 2) different traits confer different rates of survival and reproduction (differential fitness), and 3) traits can be passed from generation to generation (heritability of fitness). Thus, in successive generations members of a population are replaced by progeny of parents better adapted to survive and reproduce in the

¹³<https://en.wikipedia.org/wiki/Speciation>

¹⁴https://en.wikipedia.org/wiki/Reproductive_isolation

¹⁵https://en.wikipedia.org/wiki/Allopatric_speciation

¹⁶<https://en.wikipedia.org/wiki/Evolution>

¹⁷https://en.wikipedia.org/wiki/Charles_Darwin

¹⁸https://en.wikipedia.org/wiki/Alfred_Russel_Wallace

¹⁹https://en.wikipedia.org/wiki/Ernst_Mayr

²⁰https://en.wikipedia.org/wiki/Natural_selection

biophysical environment in which natural selection takes place.

The four most widely recognized evolutionary processes are natural selection (including sexual selection), genetic drift²¹, mutation²² and gene migration²³ due to genetic admixture. Natural selection and genetic drift sort variation; mutation and gene migration create variation.

The mechanisms of reproductive heritability and the origin of new traits remained a mystery. Towards this end, Darwin developed his provisional theory of pangenesis. In 1865, Gregor Mendel²⁴ reported that traits were inherited in a predictable manner through the independent assortment and segregation of elements (later known as genes). Mendel's laws of inheritance eventually supplanted most of Darwin's pangenesis theory. August Weismann²⁵ made the important distinction between germ cells that give rise to gametes (such as sperm and egg cells) and the somatic cells of the body, demonstrating that heredity passes through the germ line only. Hugo de Vries²⁶ connected Darwin's pangenesis theory to Weismann's germ/soma cell distinction and proposed that Darwin's pangenes were concentrated in the cell nucleus and when expressed they could move into the cytoplasm to change the cells structure. de Vries was also one of the researchers who made Mendel's work well-known, believing that Mendelian traits corresponded to the transfer of heritable variations along the germline. de Vries developed a mutation theory to explain how new variants originate. This led to a temporary rift between those who accepted Darwinian evolution and biometricians who allied with de Vries. In the 1930s, pioneers in the field of population genetics, such as Ronald Fisher²⁷, Sewall Wright²⁸ and J. B. S. Haldane²⁹ set the foundations of evolution onto a robust statistical philosophy. The false contradiction between Darwin's theory, genetic mutations, and Mendelian inheritance was thus reconciled.

In the 1920s and 1930s the so-called modern synthesis³⁰ connected natural selection and population genetics, based on Mendelian inheritance, into a unified theory that applied generally to any branch of

biology. The modern synthesis explained patterns observed across species in populations, through fossil transitions in paleontology, and complex cellular mechanisms in developmental biology. The publication of the structure of DNA³¹ by James Watson³² and Francis Crick³³ in 1953 demonstrated a physical mechanism for inheritance. Molecular biology improved our understanding of the relationship between genotype and phenotype. Advancements were also made in phylogenetic systematics, mapping the transition of traits into a comparative and testable framework through the publication and use of evolutionary trees. In 1973, evolutionary biologist Theodosius Dobzhansky³⁴ penned that "nothing in biology makes sense except in the light of evolution," because it has brought to light the relations of what first seemed disjointed facts in natural history into a coherent explanatory body of knowledge that describes and predicts many observable facts about life on this planet.

All life on Earth shares a common ancestor known as the last universal common ancestor³⁵ (LUCA), which lived approximately 3.5–3.8 billion years ago.

In terms of practical application, an understanding of evolution has been instrumental to developments in numerous scientific and industrial fields, including agriculture, human and veterinary medicine, and the life sciences in general. Discoveries in evolutionary biology have made a significant impact not just in the traditional branches of biology but also in other academic disciplines, including biological anthropology, and evolutionary psychology.

A.7 Phylum

In biology, a phylum³⁶ (plural: phyla) is a level of classification or taxonomic rank below Kingdom and above Class. Traditionally, in botany the term division has been used instead of phylum. Depending on definitions, the animal kingdom Animalia or Metazoa contains approximately 33 phyla, the plant kingdom Plantae contains about 14, and the fungus kingdom Fungi contains about 8 phyla. Current research in phylogenetics (see below) is uncovering the relationships between phyla, which are contained in larger

²¹https://en.wikipedia.org/wiki/Genetic_drift

²²<https://en.wikipedia.org/wiki/Mutation>

²³https://en.wikipedia.org/wiki/Gene_flow

²⁴https://en.wikipedia.org/wiki/Gregor_Mendel

²⁵https://en.wikipedia.org/wiki/August_Weismann

²⁶https://en.wikipedia.org/wiki/Hugo_de_Vries

²⁷

²⁸https://en.wikipedia.org/wiki/Sewall_Wright

²⁹https://en.wikipedia.org/wiki/J._B._S._Haldane

³⁰[https://en.wikipedia.org/wiki/Modern_synthesis_\(20th_century\)](https://en.wikipedia.org/wiki/Modern_synthesis_(20th_century))

³¹<https://en.wikipedia.org/wiki/DNA>

³²https://en.wikipedia.org/wiki/James_Watson

³³https://en.wikipedia.org/wiki/Francis_Crick

³⁴https://en.wikipedia.org/wiki/Theodosius_Dobzhansky

³⁵https://en.wikipedia.org/wiki/Last_universal_common_ancestor

³⁶<https://en.wikipedia.org/wiki/Phylum>

clades, like Ecdysozoa and Embryophyta.

The term phylum was coined by Ernst Haeckel³⁷ from the Greek phylon, “race, stock,” related to phyle, “tribe, clan.” In plant taxonomy, August W. Eichler³⁸ (1883) classified plants into five groups named divisions, a term that remains in use today for groups of plants, algae and fungi.

Informally, phyla can be thought of as groupings of organisms based on general specialization of body plan. At its most basic, a phylum can be defined in two ways: as a group of organisms with a certain degree of morphological or developmental similarity (the phenetic definition), or a group of organisms with a certain degree of evolutionary relatedness (the phylogenetic definition).

A.8 Phylogenetics

In biology, phylogenetics³⁹ (Greek: phylé, phylon = tribe, clan, race + genetikós = origin, source, birth) is the study of the evolutionary history and relationships among individuals or groups of organisms (e.g. species, or populations). These relationships are discovered through phylogenetic inference methods that evaluate observed heritable traits, such as DNA sequences or morphology under a model of evolution of these traits. The result of these analyses is a phylogeny (also known as a phylogenetic tree) – a diagrammatic hypothesis about the history of the evolutionary relationships of a group of organisms. The tips of a phylogenetic tree can be living organisms or fossils, and represent the “end”, or the present, in an evolutionary lineage. Phylogenetic analyses have become central to understanding biodiversity, evolution, ecology, and genomes.

Taxonomy is the identification, naming and classification of organisms. It is usually richly informed by phylogenetics, but remains a methodologically and logically distinct discipline. The degree to which taxonomies depend on phylogenies (or classification depends on evolutionary development) differs depending on the school of taxonomy: phenetics (Greek: phainein - to appear) ignores phylogeny altogether and attempts to classify organisms based on overall similarity, usually in morphology or other observable traits, regardless of their phylogeny or evolutionary relation; cladistics (phylogenetic systematics) tries to reproduce phylogeny in its classification without loss

of information; evolutionary taxonomy tries to find a compromise between them.

A.9 Taxonomy

Taxonomy⁴⁰ (from Ancient Greek taxis, meaning ‘arrangement’, and nomia, meaning ‘method’) is the science of defining and naming groups of biological organisms on the basis of shared characteristics. Organisms are grouped together into taxa (singular: taxon) and these groups are given a taxonomic rank; groups of a given rank can be aggregated to form a super-group of higher rank, thus creating a taxonomic hierarchy. The principal ranks in modern use are domain, kingdom, phylum (division is sometimes used in botany in place of phylum), class, order, family, genus and species. The Swedish botanist Carl Linnaeus⁴¹ (1707–1778) is regarded as the father of taxonomy, as he developed a system known as Linnaean taxonomy for categorization of organisms and binomial nomenclature for naming organisms.

With the advent of such fields of study as phylogenetics, cladistics, and systematics, the Linnaean system has progressed to a system of modern biological classification based on the evolutionary relationships between organisms, both living and extinct.

Linnaeus ushered in a new era of taxonomy. With his major works *Systema Naturae* 1st Edition in 1735, *Species Plantarum* in 1753, and *Systema Naturae* 10th Edition, he revolutionized modern taxonomy. His works implemented a standardized binomial naming system for animal and plant species, which proved to be an elegant solution to a chaotic and disorganized taxonomic literature. He not only introduced the standard of class, order, genus, and species, but also made it possible to identify plants and animals from his book, by using the smaller parts of the flower. Thus, the Linnaean system was born, and is still used in essentially the same way today as it was in the 18th century. Currently, plant and animal taxonomists regard Linnaeus’ work as the “starting point” for valid names (at 1753 and 1758 respectively). Names published before these dates are referred to as “pre-Linnaean”, and not considered valid (with the exception of spiders published in *Svenska Spindlar*). Even taxonomic names published by Linnaeus himself before these dates are considered pre-Linnaean.

³⁷https://en.wikipedia.org/wiki/Ernst_Haeckel

³⁸https://en.wikipedia.org/wiki/August_W._Eichler

³⁹<https://en.wikipedia.org/wiki/Phylogenetics>

⁴⁰[https://en.wikipedia.org/wiki/Taxonomy_\(biology\)](https://en.wikipedia.org/wiki/Taxonomy_(biology))

⁴¹https://en.wikipedia.org/wiki/Carl_Linnaeus

Whereas Linnaeus classified for ease of identification, the idea of the Linnaean taxonomy as translating into a sort of dendrogram of the Animal and Plant Kingdoms was formulated toward the end of the 18th century, well before *On the Origin of Species* was published. Among early works exploring the idea of a transmutation of species were Erasmus Darwin's⁴² 1796 *Zoönomia* and Jean-Baptiste Lamarck's⁴³ *Philosophie Zoologique* of 1809. The idea was popularized in the Anglophone world by the speculative but widely read *Vestiges of the Natural History of Creation*⁴⁴, published anonymously by Robert Chambers⁴⁵ in 1844.

With Darwin's theory, a general acceptance quickly appeared that a classification should reflect the Darwinian principle of common descent. Tree of life representations became popular in scientific works, with known fossil groups incorporated. One of the first modern groups tied to fossil ancestors was birds. Using the then newly discovered fossils of *Archaeopteryx* and *Hesperornis*, Thomas Henry Huxley pronounced that they had evolved from dinosaurs, a group formally named by Richard Owen⁴⁶ in 1842. The resulting description, that of dinosaurs "giving rise to" or being "the ancestors of" birds, is the essential hallmark of evolutionary taxonomic thinking. As more and more fossil groups were found and recognized in the late 19th and early 20th centuries, paleontologists worked to understand the history of animals through the ages by linking together known groups. With the modern evolutionary synthesis of the early 1940s, an essentially modern understanding of the evolution of the major groups was in place.

The cladistic method has emerged since the 1960s. In 1958, Julian Huxley⁴⁷ used the term clade. Later, in 1960, Cain and Harrison introduced the term cladistic. The salient feature is arranging taxa in a hierarchical evolutionary tree, ignoring ranks. A taxon is called monophyletic, if it includes all the descendants of an ancestral form. Groups that have descendant groups removed from them (e.g. dinosaurs, with birds as offspring group) are termed paraphyletic, while groups representing more than one branch from the tree of life are called polyphyletic. The International Code of Phylogenetic

⁴²https://en.wikipedia.org/wiki/Erasmus_Darwin

⁴³https://en.wikipedia.org/wiki/Jean-Baptiste_Lamarck

⁴⁴https://en.wikipedia.org/wiki/Vestiges_of_the_Natural_History_of_Creation

⁴⁵[https://en.wikipedia.org/wiki/Robert_Chambers_\(publisher,_born_1802\)](https://en.wikipedia.org/wiki/Robert_Chambers_(publisher,_born_1802))

⁴⁶https://en.wikipedia.org/wiki/Richard_Owen

⁴⁷https://en.wikipedia.org/wiki/Julian_Huxley

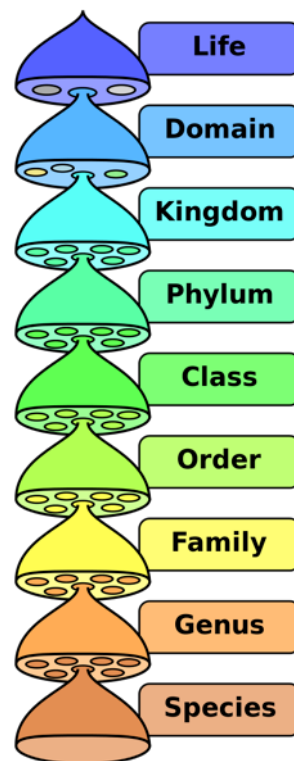


Figure A.1: Biological classification.⁵²

Nomenclature or PhyloCode is intended to regulate the formal naming of clades. Linnaean ranks will be optional under the PhyloCode, which is intended to coexist with the current, rank-based codes.

Well before Linnaeus, plants and animals were considered separate Kingdoms. Linnaeus used this as the top rank, dividing the physical world into the plant, animal and mineral kingdoms. As advances in microscopy made classification of microorganisms possible, the number of kingdoms increased, five and six-kingdom systems being the most common.

Domains are a relatively new grouping. First proposed in 1977, Carl Woese's⁴⁸ three-domain system was not generally accepted until later. One main characteristic of the three-domain method is the separation of Archaea⁴⁹ and Bacteria⁵⁰, previously grouped into the single kingdom Bacteria (a kingdom also sometimes called Monera), with the Eukaryota⁵¹ for all organisms whose cells contain a nucleus.

Biological classification is a critical component of the taxonomic process. As a result, it informs the

⁴⁸https://en.wikipedia.org/wiki/Carl_Woese

⁴⁹<https://en.wikipedia.org/wiki/Archaea>

⁵⁰<https://en.wikipedia.org/wiki/Bacteria>

⁵¹<https://en.wikipedia.org/wiki/Eukaryote>

user as to what the relatives of the taxon are hypothesized to be. Biological classification uses taxonomic ranks, including among others (in order from most inclusive to least inclusive): Domain, Kingdom, Phylum, Class, Order, Family, Genus, and Species

The “definition” of a taxon is encapsulated by its description or its diagnosis or by both combined. There are no set rules governing the definition of taxa, but the naming and publication of new taxa is governed by sets of rules. In zoology, the nomenclature for the more commonly used ranks (superfamily to subspecies), is regulated by the International Code of Zoological Nomenclature (ICZN Code). In the fields of botany, phycology, and mycology, the naming of taxa is governed by the International Code of Nomenclature for algae, fungi, and plants (ICN).

The initial description of a taxon involves five main requirements:

1. The taxon must be given a name based on the 26 letters of the Latin alphabet (a binomial for new species, or uninomial for other ranks).
2. The name must be unique (i.e. not a homonym).
3. The description must be based on at least one name-bearing type specimen.
4. It should include statements about appropriate attributes either to describe (define) the taxon or to differentiate it from other taxa (the diagnosis, ICZN Code, Article 13.1.1, ICN, Article 38). Both codes deliberately separate defining the content of a taxon (its circumscription) from defining its name.
5. These first four requirements must be published in a work that is obtainable in numerous identical copies, as a permanent scientific record.

However, often much more information is included, like the geographic range of the taxon, ecological notes, chemistry, behavior, etc. How researchers arrive at their taxa varies: depending on the available data, and resources, methods vary from simple quantitative or qualitative comparisons of striking features, to elaborate computer analyses of large amounts of DNA sequence data.

An “authority” may be placed after a scientific name. The authority is the name of the scientist or scientists who first validly published the name. For example, in 1758 Linnaeus gave the Asian elephant the scientific name *Elephas maximus*, so the name is sometimes written as “*Elephas maximus* Linnaeus, 1758”. The names of authors are frequently abbreviated: the abbreviation L., for Linnaeus, is commonly used. In botany, there is, in fact, a regulated

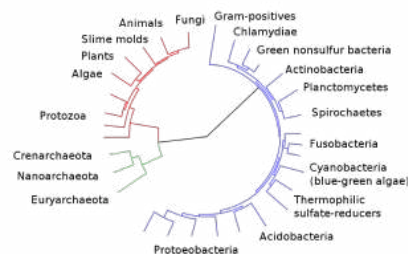


Figure A.2: The tree of life.⁵³

list of standard abbreviations (see list of botanists by author abbreviation). The system for assigning authorities differs slightly between botany and zoology. However, it is standard that if a species’ name or placement has been changed since the original description, the original authority’s name is placed in parentheses.

A.10 Cladistics

Cladistics⁵⁴ (from Greek *klados*, i.e., “branch”) is an approach to biological classification in which organisms are categorized based on shared derived characteristics that can be traced to a group’s most recent common ancestor and are not present in more distant ancestors. Therefore, members of a group are assumed to share a common history and are considered to be closely related.

The original methods used in cladistic analysis and the school of taxonomy derived from the work of the German entomologist Willi Hennig⁵⁵, who referred to it as phylogenetic systematics (also the title of his 1966 book); the terms “cladistics” and “clade” were popularized by other researchers. Cladistics in the original sense refers to a particular set of methods used in phylogenetic analysis, although it is now sometimes used to refer to the whole field.

The following terms, coined by Hennig, are used to identify shared or distinct character states among groups of organisms:

- A **plesiomorphy** (“close form”) or ancestral state is a character state that a taxon has retained from its ancestors. When two or more taxa that are not nested within each other share a plesiomorphy, it is a **symplesiomorphy** (from *syn-*, “together”). Symplesiomorphies do not

⁵⁴<https://en.wikipedia.org/wiki/Cladistics>

⁵⁵https://en.wikipedia.org/wiki/Willi_Hennig

mean that the taxa that exhibit that character state are necessarily closely related. For example, Reptilia is traditionally characterized by (among other things) being cold-blooded (i.e., not maintaining a constant high body temperature), whereas birds are warm-blooded. Since cold-bloodedness is a plesiomorphy, inherited from the common ancestor of traditional reptiles and birds, and thus a symplesiomorphy of turtles, snakes and crocodiles (among others), it does not mean that turtles, snakes and crocodiles form a clade that excludes the birds.

- An **apomorphy** (“separate form”) or derived state is an innovation. It can thus be used to diagnose a clade – or even to help define a clade name in phylogenetic nomenclature. Features that are derived in individual taxa (a single species or a group that is represented by a single terminal in a given phylogenetic analysis) are called autapomorphies (from auto-, “self”). Autapomorphies express nothing about relationships among groups; clades are identified (or defined) by synapomorphies (from syn-, “together”). For example, the possession of digits that are homologous with those of *Homo sapiens* is a synapomorphy within the vertebrates. The tetrapods can be singled out as consisting of the first vertebrate with such digits homologous to those of *Homo sapiens* together with all descendants of this vertebrate (an apomorphy-based phylogenetic definition). Importantly, snakes and other tetrapods that do not have digits are nonetheless tetrapods: other characters, such as amniotic eggs and diapsid skulls, indicate that they descended from ancestors that possessed digits which are homologous with ours.
- A **character state** is homoplastic or “an instance of homoplasy” if it is shared by two or more organisms but is absent from their common ancestor or from a later ancestor in the lineage leading to one of the organisms. It is therefore inferred to have evolved by convergence or reversal. Both mammals and birds are able to maintain a high constant body temperature (i.e., they are warm-blooded). However, the accepted cladogram explaining their significant features indicates that their common ancestor is in a group lacking this character state, so the state must have evolved independently in the two clades. Warm-bloodedness is separately a synapomorphy of mammals (or a larger clade) and of birds (or a larger clade), but it

is not a synapomorphy of any group including both these clades. Hennig’s Auxiliary Principle states that shared character states should be considered evidence of grouping unless they are contradicted by the weight of other evidence; thus, homoplasy of some feature among members of a group may only be inferred after a phylogenetic hypothesis for that group has been established.

The terms plesiomorphy and apomorphy are relative; their application depends on the position of a group within a tree. For example, when trying to decide whether the tetrapods form a clade, an important question is whether having four limbs is a synapomorphy of the earliest taxa to be included within Tetrapoda: did all the earliest members of the Tetrapoda inherit four limbs from a common ancestor, whereas all other vertebrates did not, or at least not homologously? By contrast, for a group within the tetrapods, such as birds, having four limbs is a plesiomorphy. Using these two terms allows a greater precision in the discussion of homology, in particular allowing clear expression of the hierarchical relationships among different homologous features.

It can be difficult to decide whether a character state is in fact the same and thus can be classified as a synapomorphy, which may identify a monophyletic group, or whether it only appears to be the same and is thus a homoplasy, which cannot identify such a group. There is a danger of circular reasoning: assumptions about the shape of a phylogenetic tree are used to justify decisions about character states, which are then used as evidence for the shape of the tree. Phylogenetics uses various forms of parsimony to decide such questions; the conclusions reached often depend on the dataset and the methods. Such is the nature of empirical science, and for this reason, most cladists refer to their cladograms as hypotheses of relationship. Cladograms that are supported by a large number and variety of different kinds of characters are viewed as more robust than those based on more limited evidence.

A monophyletic⁵⁶ group is a group of organisms that forms a clade, which consists of all the descendants of a common ancestor. Monophyletic groups are typically characterized by shared derived characteristics (synapomorphies), which distinguish organisms in the clade from other organisms. The arrangement of the members of a monophyletic group is called a monophyly.

⁵⁶<https://en.wikipedia.org/wiki/Monophyly>

A.13 Bilaterian Animals

The remaining animals form a monophyletic group called the Bilateria⁶³. For the most part, they are bilaterally symmetric, and often have a specialized head with feeding and sensory organs. The body is triploblastic, i.e. all three germ layers are well-developed, and tissues form distinct organs. The digestive chamber has two openings, a mouth and an anus, and there is also an internal body cavity called a coelom or pseudocoelom. There are exceptions to each of these characteristics, however—for instance adult echinoderms are radially symmetric, and certain parasitic worms have extremely simplified body structures.

Genetic studies have considerably changed our understanding of the relationships within the Bilateria. Most appear to belong to two major lineages: the deuterostomes and the protostomes, the latter of which includes the Ecdysozoa, and Lophotrochozoa. The Chaetognatha or arrow worms have been traditionally classified as deuterostomes, though recent molecular studies have identified this group as a basal protostome lineage.

In addition, there are a few small groups of bilaterians with relatively cryptic morphology whose relationships with other animals are not well-established. For example, recent molecular studies have identified Acoelomorpha⁶⁴ and Xenoturbella⁶⁵ as forming a monophyletic group, but studies disagree as to whether this group evolved from within deuterostomes, or whether it represents the sister group to all other bilaterian animals (Nephrozoa⁶⁶). Other groups of uncertain affinity include the Rhombozoa⁶⁷ (also known as Dicyemida) and Orthonectida⁶⁸. One phyla, the Monoblastozoa, was described by a scientist in 1892, but so far there have been no evidence of its existence.

⁶³<https://en.wikipedia.org/wiki/Bilateria>

⁶⁴<https://en.wikipedia.org/wiki/Acoelomorpha>

⁶⁵<https://en.wikipedia.org/wiki/Xenoturbella>

⁶⁶<https://en.wikipedia.org/wiki/Nephrozoa>

⁶⁷<https://en.wikipedia.org/wiki/Dicyemida>

⁶⁸<https://en.wikipedia.org/wiki/Orthonectida>

A.14 Deuterostomes and Protostomes

Deuterostomes⁶⁹ differ from protostomes⁷⁰ in several ways. Animals from both groups possess a complete digestive tract. However, in protostomes, the first opening of the gut to appear in embryological development (the archenteron) develops into the mouth, with the anus forming secondarily. In deuterostomes the anus forms first, with the mouth developing secondarily. In most protostomes, cells simply fill in the interior of the gastrula to form the mesoderm, called schizocoelous development, but in deuterostomes, it forms through invagination of the endoderm, called enterocoelic pouching. Deuterostome embryos undergo radial cleavage⁷¹ during cell division, while protostomes undergo spiral cleavage.

All this suggests the deuterostomes and protostomes are separate, monophyletic lineages. The main phyla of deuterostomes are the Echinodermata and Chordata. The former are radially symmetric and exclusively marine, such as starfish, sea urchins, and sea cucumbers. The latter are dominated by the vertebrates, animals with backbones. These include fish, amphibians, reptiles, birds, and mammals.

In addition to these, the deuterostomes also include the Hemichordata, or acorn worms, which are thought to be closely related to Echinodermata forming a group known as Ambulacraria. Although they are not especially prominent today, the important fossil graptolites may belong to this group.

A.15 Ecdysozoa

The Ecdysozoa⁷² are protostomes, named after the common trait of growth by moulting or ecdysis. The largest animal phylum belongs here, the Arthropoda, including insects, spiders, crabs, and their kin. All these organisms have a body divided into repeating segments, typically with paired appendages. Two smaller phyla, the Onychophora⁷³ and Tardigrada⁷⁴, are close relatives of the arthropods and share these traits. The ecdysozoans also include the Nematoda or roundworms, perhaps the second largest animal

⁶⁹<https://en.wikipedia.org/wiki/Deuterostome>

⁷⁰<https://en.wikipedia.org/wiki/Protostome>

⁷¹[https://en.wikipedia.org/wiki/Cleavage_\(embryo\)](https://en.wikipedia.org/wiki/Cleavage_(embryo))

⁷²<https://en.wikipedia.org/wiki/Ecdysozoa>

⁷³<https://en.wikipedia.org/wiki/Onychophora>

⁷⁴<https://en.wikipedia.org/wiki/Tardigrade>

phylum. Roundworms are typically microscopic, and occur in nearly every environment where there is water. A number are important parasites. Smaller phyla related to them are the Nematomorpha⁷⁵ or horsehair worms, and the Kinorhyncha⁷⁶, Priapulida⁷⁷, and Loricifera⁷⁸. These groups have a reduced coelom, called a pseudocoelom.

A.16 Lophotrochozoa

The Lophotrochozoa⁷⁹, evolved within Protostomia, include two of the most successful animal phyla, the Mollusca and Annelida. The former, which is the second-largest animal phylum by number of described species, includes animals such as snails, clams, and squids, and the latter comprises the segmented worms, such as earthworms and leeches. These two groups have long been considered close relatives because of the common presence of trochophore larvae, but the annelids were considered closer to the arthropods because they are both segmented. Now, this is generally considered convergent evolution, owing to many morphological and genetic differences between the two phyla. Lophotrochozoa also includes the Nemertea⁸⁰ or ribbon worms, the Sipuncula⁸¹, and several phyla that have a ring of ciliated tentacles around the mouth, called a lophophore. These were traditionally grouped together as the lophophorates. but it now appears that the lophophorate group may be paraphyletic, with some closer to the nemerteans and some to the molluscs and annelids. They include the Brachiopoda⁸² or lamp shells, which are prominent in the fossil record, the Entoprocta⁸³, the Phoronida⁸⁴, and possibly the Bryozoa⁸⁵ or moss animals.

A.17 Platyzoa

The Platyzoa⁸⁶ include the phylum Platyhelminthes, the flatworms. These were originally considered some

of the most primitive Bilateria, but it now appears they developed from more complex ancestors. A number of parasites are included in this group, such as the flukes and tapeworms. Flatworms are acoelomates, lacking a body cavity, as are their closest relatives, the microscopic Gastrotricha⁸⁷. The other platyzoan phyla are mostly microscopic and pseudocoelomate. The most prominent are the Rotifera or rotifers, which are common in aqueous environments. They also include the Acanthocephala⁸⁸ or spiny-headed worms, the Gnathostomulida⁸⁹, Micrognathozoa, and possibly the Cyclophora⁹⁰. These groups share the presence of complex jaws, from which they are called the Gnathifera.

A.18 Binomial Nomenclature

Binomial nomenclature⁹¹, also called binominal nomenclature or binary nomenclature, is a formal system of naming species of living things by giving each a name composed of two parts, both of which use Latin grammatical forms, although they can be based on words from other languages. Such a name is called a binomial name (which may be shortened to just “binomial”), a binomen, binominal name or a scientific name; more informally it is also called a Latin name. The first part of the name identifies the genus to which the species belongs; the second part - the specific name or specific epithet - identifies the species within the genus. For example, humans belong to the genus *Homo* and within this genus to the species *Homo sapiens*. The formal introduction of this system of naming species is credited to Carl Linnaeus, effectively beginning with his work *Species Plantarum* in 1753. But Gaspard Bauhin⁹², in as early as 1623, had introduced in his book *Pinax theatri botanici* (English, *Illustrated exposition of plants*) many names of genera that were later adopted by Linnaeus.

The application of binomial nomenclature is now governed by various internationally agreed codes of rules, of which the two most important are the International Code of Zoological Nomenclature (ICZN) for animals and the International Code of Nomenclature for algae, fungi, and plants (ICN). Although the general principles underlying binomial nomenclature

⁷⁵<https://en.wikipedia.org/wiki/Nematomorpha>

⁷⁶<https://en.wikipedia.org/wiki/Kinorhyncha>

⁷⁷<https://en.wikipedia.org/wiki/Priapulida>

⁷⁸<https://en.wikipedia.org/wiki/Loricifera>

⁷⁹<https://en.wikipedia.org/wiki/Lophotrochozoa>

⁸⁰<https://en.wikipedia.org/wiki/Nemertea>

⁸¹<https://en.wikipedia.org/wiki/Sipuncula>

⁸²<https://en.wikipedia.org/wiki/Brachiopod>

⁸³<https://en.wikipedia.org/wiki/Entoprocta>

⁸⁴<https://en.wikipedia.org/wiki/Phoronid>

⁸⁵<https://en.wikipedia.org/wiki/Bryozoa>

⁸⁶<https://en.wikipedia.org/wiki/Platyzoa>

⁸⁷<https://en.wikipedia.org/wiki/Gastrotrich>

⁸⁸<https://en.wikipedia.org/wiki/Acanthocephala>

⁸⁹<https://en.wikipedia.org/wiki/Gnathostomulid>

⁹⁰<https://en.wikipedia.org/wiki/Symbion>

⁹¹https://en.wikipedia.org/wiki/Binomial_nomenclature

⁹²https://en.wikipedia.org/wiki/Gaspard_Bauhin

are common to these two codes, there are some differences, both in the terminology they use and in their precise rules.

In modern usage, the first letter of the first part of the name, the genus, is always capitalized in writing, while that of the second part is not, even when derived from a proper noun such as the name of a person or place. Similarly, both parts are italicized when a binomial name occurs in normal text (or underlined in handwriting). Thus the binomial name of the annual phlox (named after botanist Thomas Drummond) is now written as *Phlox drummondii*. When handwritten, a binomial name should be underlined; for example, *Homo sapiens*.

In scientific works, the “authority” for a binomial name is usually given, at least when it is first mentioned, and the date of publication may be specified.

A.19 The Geologic Time Scale

The geologic time scale⁹³ (GTS) is a system of chronological dating that relates geological strata (stratigraphy) to time. It is used by geologists, paleontologists, and other Earth scientists to describe the timing and relationships of events that have occurred during Earth’s history. The tables of geologic time spans, presented here, agree with the nomenclature, dates and standard color codes set forth by the International Commission on Stratigraphy (ICS).

The primary defined divisions of time are eons, in sequence the Hadean, the Archean, the Proterozoic and the Phanerozoic. The first three of these can be referred to collectively as the Precambrian supereon. Eons are divided into eras, which are in turn divided into periods, epochs and ages.

Studies of strata, the layering of rocks and earth, gave naturalists an appreciation that Earth may have been through many changes during its existence. These layers often contained fossilized remains of unknown creatures, leading some to interpret a progression of organisms from layer to layer.

Nicolas Steno⁹⁴ in the 17th century was one of the first naturalists to appreciate the connection between fossil remains and strata. His observations led him to formulate important stratigraphic concepts (i.e., the “law of superposition” and the “principle of original horizontality”). In the 1790s, William

Smith⁹⁵ hypothesized that if two layers of rock at widely differing locations contained similar fossils, then it was very plausible that the layers were the same age. William Smith’s nephew and student, John Phillips⁹⁶, later calculated by such means that Earth was about 96 million years old.

In the mid-18th century, the naturalist Mikhail Lomonosov suggested that Earth had been created separately from, and several hundred thousand years before, the rest of the universe. Lomonosov’s ideas were mostly speculative. In 1779 the Comte du Buffon⁹⁷ tried to obtain a value for the age of Earth using an experiment: He created a small globe that resembled Earth in composition and then measured its rate of cooling. This led him to estimate that Earth was about 75,000 years old.

Other naturalists used these hypotheses to construct a history of Earth, though their timelines were inexact as they did not know how long it took to lay down stratigraphic layers. In 1830, geologist Charles Lyell⁹⁸, developing ideas found in James Hutton’s⁹⁹ works, popularized the concept that the features of Earth were in perpetual change, eroding and reforming continuously, and the rate of this change was roughly constant. This was a challenge to the traditional view, which saw the history of Earth as static, with changes brought about by intermittent catastrophes. Many naturalists were influenced by Lyell to become “uniformitarians” who believed that changes were constant and uniform.

Early work on developing the geologic time scale was dominated by British geologists, and the names of the geologic periods reflect that dominance. The “Cambrian”, (the classical name for Wales) and the “Ordovician”, and “Silurian”, named after ancient Welsh tribes, were periods defined using stratigraphic sequences from Wales. The “Devonian” was named for the English county of Devon, and the name “Carboniferous” was an adaptation of “the Coal Measures”, the old British geologists’ term for the same set of strata. The “Permian” was named after Perm, Russia, because it was defined using strata in that region by Scottish geologist Roderick Murchison. However, some periods were defined by geologists from other countries. The “Triassic” was named in 1834 by a German geologist Friedrich

⁹⁵[https://en.wikipedia.org/wiki/William_Smith_\(geologist\)](https://en.wikipedia.org/wiki/William_Smith_(geologist))

⁹⁶[https://en.wikipedia.org/wiki/John_Phillips_\(geologist\)](https://en.wikipedia.org/wiki/John_Phillips_(geologist))

⁹⁷[https://en.wikipedia.org/wiki/Georges-Louis_Leclerc, Comte_de_Buffon](https://en.wikipedia.org/wiki/Georges-Louis_Leclerc,_Comte_de_Buffon)

⁹⁸https://en.wikipedia.org/wiki/Charles_Lyell

⁹⁹https://en.wikipedia.org/wiki/James_Hutton

⁹³https://en.wikipedia.org/wiki/Geologic_time_scale

⁹⁴https://en.wikipedia.org/wiki/Nicolas_Steno

Von Alberti¹⁰⁰ from the three distinct layers (Latin trias meaning triad)—red beds, capped by chalk, followed by black shales—that are found throughout Germany and Northwest Europe, called the ‘Trias’. The “Jurassic” was named by a French geologist Alexandre Brongniart for the extensive marine limestone exposures of the Jura Mountains. The “Cretaceous” (from Latin creta meaning ‘chalk’) as a separate period was first defined by Belgian geologist Jean Baptiste d’Omalius d’Halloy in 1822, using strata in the Paris basin and named for the extensive beds of chalk (calcium carbonate deposited by the shells of marine invertebrates) found in Western Europe.

British geologists were also responsible for the grouping of periods into eras and the subdivision of the Tertiary and Quaternary periods into epochs. In 1841 John Phillips published the first global geologic time scale based on the types of fossils found in each era. Phillips’ scale helped standardize the use of terms like Paleozoic (“old life”) which he extended to cover a larger period than it had in previous usage, and Mesozoic (“middle life”) which he invented.

When geologists first recognized that rock strata represented successive time periods, time scales could be estimated only very imprecisely since estimates of rates of change were uncertain. While creationists had been proposing dates of around six or seven thousand years for the age of Earth based on the Bible, early geologists were suggesting millions of years for geologic periods, and some were even suggesting a virtually infinite age for Earth. Geologists and paleontologists constructed the geologic table based on the relative positions of different strata and fossils, and estimated the time scales based on studying rates of various kinds of weathering, erosion, sedimentation, and lithification. Until the discovery of radioactivity in 1896 and the development of its geological applications through radiometric dating during the first half of the 20th century, the ages of various rock strata and the age of Earth were the subject of considerable debate.

The first geologic time scale that included absolute dates was published in 1913 by the British geologist Arthur Holmes¹⁰¹. He greatly furthered the newly created discipline of geochronology and published the world-renowned book *The Age of the Earth* in which he estimated Earth’s age to be at least 1.6 billion years.

¹⁰⁰https://en.wikipedia.org/wiki/Friedrich_August_von_Alberti

¹⁰¹https://en.wikipedia.org/wiki/Arthur_Holmes

Today, we know that the age of the Earth is approximately 4.54 ± 0.05 billion years. This dating is based on evidence from radiometric age-dating of meteorite material and is consistent with the radiometric ages of the oldest-known terrestrial and lunar samples.

By their chemical nature, rock minerals contain certain elements and not others; but in rocks containing radioactive isotopes, the process of radioactive decay generates exotic elements over time. By measuring the concentration of the stable end product of the decay, coupled with knowledge of the half-life and initial concentration of the decaying element, the age of the rock can be calculated. Typical radioactive end products are argon from decay of potassium-40, and lead from decay of uranium and thorium. If the rock becomes molten, as happens in Earth’s mantle, such nonradioactive end products typically escape or are redistributed. Thus the age of the oldest terrestrial rock gives a minimum for the age of Earth, assuming that no rock has been intact for longer than the Earth itself.

Following the development of radiometric age-dating in the early 20th century, measurements of lead in uranium-rich minerals showed that some were in excess of a billion years old. The oldest such minerals analyzed to date—small crystals of zircon from the Jack Hills of Western Australia—are at least 4.404 billion years old. Calcium–aluminum-rich inclusions—the oldest known solid constituents within meteorites that are formed within the Solar System—are 4.567 billion years old, giving a lower limit for the age of the solar system.

It is hypothesized that the accretion of Earth began soon after the formation of the calcium–aluminum-rich inclusions and the meteorites. Because the exact amount of time this accretion process took is not yet known, and the predictions from different accretion models range from a few million up to about 100 million years, the exact age of Earth is difficult to determine. It is also difficult to determine the exact age of the oldest rocks on Earth, exposed at the surface, as they are aggregates of minerals of possibly different ages.

A.20 Plant Anatomy

Plant anatomy¹⁰³ is the study of the structure of plant cells and tissues, whereas plant morphology is

¹⁰³https://en.wikipedia.org/wiki/Plant_anatomy

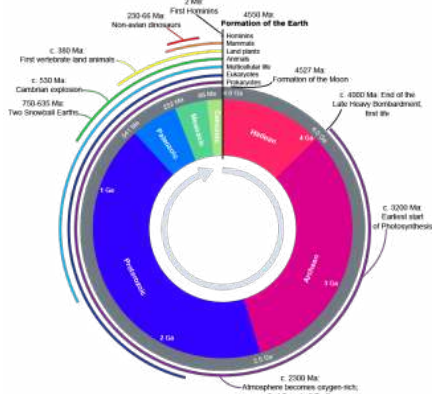


Figure A.4: Geologic clock.¹⁰²

the study of their external form. All plants are multicellular eukaryotes, their DNA stored in nuclei. The characteristic features of plant cells that distinguish them from those of animals and fungi include a primary cell wall composed of the polysaccharides cellulose, hemicellulose and pectin, larger vacuoles than in animal cells and the presence of plastids with unique photosynthetic and biosynthetic functions as in the chloroplasts. Other plastids contain storage products such as starch (amyloplasts) or lipids (elaioplasts).

The bodies of vascular plants including club-mosses, ferns and seed plants (gymnosperms and angiosperms) generally have aerial and subterranean subsystems. The shoots consist of stems bearing green photosynthesizing leaves and reproductive structures. The underground vascularized roots bear root hairs at their tips and generally lack chlorophyll. Non-vascular plants, the liverworts, hornworts and mosses do not produce ground-penetrating vascular roots and most of the plant participates in photosynthesis. The sporophyte generation is nonphotosynthetic in liverworts but may be able to contribute part of its energy needs by photosynthesis in mosses and hornworts.

The root system and the shoot system are interdependent – the usually nonphotosynthetic root system depends on the shoot system for food, and the usually photosynthetic shoot system depends on water and minerals from the root system. Cells in each system are capable of creating cells of the other and producing adventitious shoots or roots. Stolons and tubers are examples of shoots that can grow roots. Roots that spread out close to the surface, such as those of willows, can produce shoots and ultimately new plants. In the event that one of the systems is lost, the other can often regrow it. In fact it is possible to grow an entire plant from a single leaf, as is

the case with *Saintpaulia*, or even a single cell – which can dedifferentiate into a callus (a mass of unspecialized cells) that can grow into a new plant. In vascular plants, the xylem and phloem are the conductive tissues that transport resources between shoots and roots. Roots are often adapted to store food such as sugars or starch, as in sugar beets and carrots.

Stems mainly provide support to the leaves and reproductive structures, but can store water in succulent plants such as cacti, food as in potato tubers, or reproduce vegetatively as in the stolons of strawberry plants or in the process of layering. Leaves gather sunlight and carry out photosynthesis. Large, flat, flexible, green leaves are called foliage leaves. Gymnosperms, such as conifers, cycads, Ginkgo, and gnetophytes are seed-producing plants with open seeds. Angiosperms are seed-producing plants that produce flowers and have enclosed seeds. Woody plants, such as azaleas and oaks, undergo a secondary growth phase resulting in two additional types of tissues: wood (secondary xylem) and bark (secondary phloem and cork). All gymnosperms and many angiosperms are woody plants. Some plants reproduce sexually, some asexually, and some via both means.

A.21 Plant Tissues

In plant anatomy, tissues are categorized broadly into three tissue systems:

1. **Epidermis** - Cells forming the outer surface of the leaves and of the young plant body.
2. **Vascular tissue** - The primary components of vascular tissue are the xylem and phloem. These transport fluid and nutrients internally.
3. **Ground tissue** - Ground tissue is less differentiated than other tissues. Ground tissue manufactures nutrients by photosynthesis and stores reserve nutrients.

Plant tissues can also be divided differently into two types:

1. **Meristematic tissue** consists of actively dividing cells, and leads to increase in length and thickness of the plant. The primary growth of a plant occurs only in certain, specific regions, such as in the tips of stems or roots. It is in these regions that meristematic tissue is present.
2. **Permanent tissue** is formed when cells from meristematic tissues that take up a specific

role lose the ability to divide. This process of taking up a permanent shape, size and a function is called cellular differentiation. Cells of meristematic tissue differentiate to form different types of permanent tissue.

There are 3 types of permanent tissues:

1. **Parenchyma** (para - 'beside'; chyma - 'in filling, loose, unpacked') is the bulk of a substance. In plants, it consists of relatively unspecialised living cells with thin cell walls that are usually loosely packed so that intercellular spaces are found between cells of this tissue. This tissue provides support to plants and also stores food. In some situations, a parenchyma contains chlorophyll and performs photosynthesis, in which case it is called a chlorenchyma. In aquatic plants, large air cavities are present in parenchyma to give support to them to float on water. Such a parenchyma type is called aerenchyma.
2. **Collenchyma** is Greek word where "Collen" means gum and "chyma" means infusion. It is a living tissue of primary body like Parenchyma. Cells are thin-walled but possess thickening of cellulose, water and pectin substances (pectocellulose) at the corners where number of cells join together. This tissue gives a tensile strength to the plant and the cells are compactly arranged and have very little inter-cellular spaces. It occurs chiefly in hypodermis of stems and leaves. It is absent in monocots and in roots. Collenchymatous tissue acts as a supporting tissue in stems of young plants. It provides mechanical support, elasticity, and tensile strength to the plant body. It helps in manufacturing sugar and storing it as starch. It is present in the margin of leaves and resist tearing effect of the wind.
3. **Sclerenchyma** is Greek word where "Sclerenes" means hard and "chyma" means infusion. This tissue consists of thick-walled, dead cells. These cells have hard and extremely thick secondary walls due to uniform distribution of lignin. Lignin deposition is so thick that the cell walls become strong, rigid and impermeable to water.

A.22 Coelom

The coelom¹⁰⁴ is the main body cavity in most animals and is positioned inside the body to surround and contain the digestive tract and other organs. The word coelom comes from Greek: *koilos* hollow, cavity. Coelom formation begins in the gastrula stage. The developing digestive tube of an embryo forms as a blind pouch called the archenteron.

In Protostomes, the coelom forms by a process known as schizocoely. The archenteron initially forms, and the mesoderm splits into two layers: the first attaches to the body wall or ectoderm, forming the parietal layer and the second surrounds the endoderm or alimentary canal forming the visceral layer. The space between the parietal layer and the visceral layer is known as the coelom or body cavity. Examples of protostome coelomates include earthworms, snails, clams, slugs and octopuses.

In Deuterostomes, the coelom forms by enterocoely: mesoderm buds from the walls of the archenteron and hollows to become the coelomic cavities. Some examples of deuterostome coelomates are sea urchins, fish, sea stars and humans.

A coelom can absorb shock or provide a hydrostatic skeleton. It can also support an immune system in the form of coelomocytes that may either be attached to the wall of the coelom or may float about in it freely. The fluid inside the coelom is known as coelomic fluid. The coelomic fluid serves several functions; it acts as a hydroskeleton, it allows free movement and growth of internal organs, it serves for transport of gases, nutrients and waste products between different parts of the body, it allows storage of sperm and eggs during maturation and it acts as a reservoir for waste.

In the past, some zoologists grouped bilaterian animal phyla based on characteristics related to the coelom for practical purposes, knowing, and explicitly stating, that these groups were not phylogenetically related. Animals were classified in three informal groups according to the type of body cavity they possess, in a non-taxonomic, utilitarian way, as the Acoelomata, Pseudocoelomata, and Coelomata. These groups were never intended to represent related animals, or a sequence of evolutionary traits.

However, this scheme was followed by a number of college textbooks and some general classifications,

¹⁰⁴<https://en.wikipedia.org/wiki/Coelom>

but is now almost totally abandoned as a formal classification.

- **Coelomate animals** or Coelomata (also known as eucoelomates — “true coelom”) have a body cavity called a coelom with a complete lining called peritoneum derived from mesoderm (one of the three primary tissue layers). The complete mesoderm lining allows organs to be attached to each other so that they can be suspended in a particular order while still being able to move freely within the cavity. Most bilateral animals, including all the vertebrates, are coelomates.
- **Pseudocoelomate animals** have a pseudocoelom (literally “false cavity”), which is a fluid filled body cavity. Tissue derived from mesoderm partly lines the fluid filled body cavity of these animals. Thus, although organs are held in place loosely, they are not as well organized as in a coelomate. All pseudocoelomates are protostomes; however, not all protostomes are pseudocoelomates. An example of a Pseudocoelomate is the roundworm. Pseudocoelomate animals are also referred to as Hemocoel and Blastocoelomate.
- **Acoelomate animals**, like flatworms, have no body cavity at all. Semi-solid mesodermal tissues between the gut and body wall hold their organs in place.

Appendix B

A Refresher on How to Use The Microscope

A microscope¹ (from the Ancient Greek: mikrós, “small” and skopeîn, “to look” or “see”) is an instrument used to see objects that are too small to be seen by the naked eye. Microscopic means invisible to the eye unless aided by a microscope.

There are many types of microscopes, and they may be grouped in different ways. One way is to describe the way the instruments interact with a sample to create images, either by sending a beam of light or electrons to a sample in its optical path, or by scanning across, and a short distance from, the surface of a sample using a probe. The most common microscope (and the first to be invented) is the optical microscope², which uses light to pass through a sample to produce an image.

The objective lens of a microscope (Figure B.1) is a cylinder containing one or more lenses that are typically made of glass. It is essentially a high-powered magnifying glass which is brought very close to the specimen being examined. The objective collects light from the sample so that it comes to a focus inside the microscope tube. This creates an enlarged image of the specimen.

The eyepieces, or ocular lenses (Figure B.2), are the lenses that are closest to your eyes when you look through the microscope. The objective lens or mirror collects light and brings it to focus creating an image. The eyepiece is placed near the focal point of the objective to magnify this image. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further



Figure B.1: The microscope objectives.

¹<https://en.wikipedia.org/wiki/Microscope>

²https://en.wikipedia.org/wiki/Optical_microscope



Figure B.2: The oculars (eye pieces) of the microscope.



Figure B.4: The stage with the slide holder and central opening showing the condenser lens.



Figure B.3: The coarse (big wheel) and fine (small wheel) focus adjustment knobs.



Figure B.5: The condenser (below the stage), the horizontal stage and slide holder adjustment knobs (hanging down from the stage), the light on switch and light intensity adjustment knob (in the back).

enlargement. The amount of magnification depends on the focal length of the eyepiece. The ocular in our microscopes have a $10\times$ magnification.

Our microscopes have four objective lenses with different magnifications, screwed into the circular “nosepiece” which you rotate to select the required lens. These lenses are color coded for easier use. The least powerful lens is called the scanning objective lens and is a $4\times$ objective. The second lens is referred to as the small objective lens and is $10\times$ lens. The most powerful lens out of the four are referred to as the large objective lenses and are $40\times$ and $100\times$. The $100\times$ objective is an oil-immersion lens. This objective is specially designed for use with refractive index matching oil, which must fill the gap between the objective lens and the specimen.

The stage is a platform below the objective which supports the specimen being viewed. Adjustment knobs (on the left side of the microscope) move the stage up and down with separate adjustment for coarse and fine focusing (Figure B.3). In the center of the stage is a hole through which light passes to illuminate the specimen (Figure B.4). The stage has arms to hold slides (rectangular glass plates on

which the specimen is mounted).

The stage moves up and down for focus. Always start with the lowest magnification in order to center the specimen on the stage. After moving to a higher magnification re-focus using the fine focus knob. You may also have to adjust the horizontal positions using the horizontal stage and slide holder adjustment knobs hanging down on the right side of the stage (Figure B.5). Our microscopes, an adjustable LEDs light source (knob on the right side). The condenser is a lens designed to focus light from the illumination source onto the sample. The light source and condenser also each include a diaphragm to influence the quality and intensity of the illumination. For our purposes, the diaphragms should always be completely open. Adjust the light intensity only using the knob on the right side of the frame of the microscope (the black knob below the green switch in Figure B.4).

We have prepared a number of videos that introduce our microscopes to you and also demonstrate how you can capture images of the slides that you are viewing and transfer them to your own equipment.

Name of Video Youtube address Introduction to microscope³

Description of student and instructor microscope⁴

Turn on tablet and view slide⁵

Student image capture option 1⁶

Student image capture option 2⁷

B.1 Starting up the microscope

The tablets and microscopes must be turned on in a specific order:

1. The microscope must be plugged in. If already plugged in, proceed to step 3.
2. The Motic logo will appear on tablet. After a few seconds, the charging symbol appears.
3. Press and hold the power button on the tablet for 6 seconds.
4. The Motic symbol will appear again and tablet will start up.
5. Turn on the microscope using the switch on the lower right side.

B.2 Turning off the microscope

The tablets and microscopes must be turned off in a specific order:

1. Turn off the microscope using the switch on the lower right side.
2. While the microscope is still plugged in, turn off the tablet by holding the power button down for a few seconds. Select power off.
3. The Motic logo will appear on tablet.
4. Wait until the charging symbol appears.
5. Leave the microscope on the student bench and plugged in after turning it off.
6. Place the cover over the microscope.

³<https://youtu.be/2bRc3u9PMDA>

⁴<https://youtu.be/qQKL4ULRM>

⁵<https://youtu.be/wOB2BSQBZFA>

⁶<https://youtu.be/jdx1SR5dvJc>

⁷<https://youtu.be/v5w-yuL8vg0>