Parasitology Lab Manual

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Dedication

Heartfelt thanks to my former parasitology teachers: the late Ingemar Larson (Augustana College), Brent Nickol and John Janovy Jr. (University of Nebraska), and Janine Caira (University of Connecticut).

Much of the content of this lab manual is derived from the parasitology course taught by Janine Caira.

All images in this manual are from the author.

Contents

Laboratory #1: Microscope use

I. Introduction

II. Laboratory notebook components. Goal: Create your own study tool!

A. Lab handouts and answer sheets. Goal: Answer questions to understand material.

i. Be sure to read your lab handout text and view the lab figures! This is a goldmine of information that should be used…
ii. Answer the questions that are embedded within the lab handouts as you do the lab in the room.
iii. Lab answer sheet policy: Lab handout answer sheets are to be turned in exactly 1 week after the lab exercise. Late labs will not be accepted (unless there is an excused absence)!

B. Entries. Goal: Organize the information.

- Use a loose-leaf notebook (3-ringed binder)
- Text should be on lined paper
- Number each entry
- Each entry should include:
    i. Scientific name
    ii. Life cycle stage
    iii. Type of preparation (e.g., whole mount, tissue section, fecal smear, blood smear, spleen smear, etc.)
    iv. Size: in micrometers (μm) or millimeters (mm); whichever is most appropriate; be certain to indicate units (if possible)
    v. Distinctive features; description; in description refer to plates as appropriate. E.g., "looks like cotton candy" (to help you remember)
- Use separate pages for entries for each different lab. On your sheets the entries should be given the same number as listed in the lab handout.
- Only items indicated with an asterisk in the lab handouts need formal drawings (plates); the structures you should label in each drawing are indicated in the lab handouts.

C. Plates. Goal: Drawing as a means of intense observation.

i. Use pencil only! Credit will not be given to drawings in pen.
ii. Use only 1 side of white blank paper. Drawing paper is recommended.
iii. Label with a ruler.
iv. Include scientific name and scale bar that indicates size of the specimen. Also include the life cycle stage and type of preparation.
v. Specimens should be illustrated with the anterior end up (top of page).
vi. Once each plate is completed, get it initialed by me. *Do not remove your slide from the microscope until after I have initialed the drawing.*

III. Laboratory notebook grading format:

A. Notebook grades will be assigned for each of the 3 lab notebook components
   i. Plates: 15 points (general appearance, accuracy including use of stipples, labels, and legends). 30 total points.
   ii. Entries: 10 points (general completeness; format). 20 total points
   iii. Laboratory handouts: 5 points each lab (accuracy). 60 total points. This will be based on answers to question, and completion of any added activities, such as dissections.

B. Compound microscope use:

A. Use of the ocular micrometer: The first five or six labs involve studying some very tiny parasitic "protistans". Determining the size of these organisms requires us to calibrate our microscopes with a microscopic ruler, known as an ocular micrometer. It is a tiny white ruler on a glass slide in which subdivisions within millimeters are shown as micrometers. There are 1000 micrometers in a millimeter.

We can use an ocular micrometer on the slide to figure out what the total length is of a second ruler that is positioned within the ocular lens of the microscope. This second ruler is called a reticle, or ocular ruler. It is a black ruler. You should be able to see it when you look in the microscope. It has a total of 10 large subdivisions and 100 small subdivisions.

You will be able to compare the size of a specimen you view in the microscope with the ocular ruler. However, the length between subdivisions on the ocular ruler depends on the degree of magnification. This will make more sense once you view the ocular micrometer at different magnifications.

Before calibrating your microscope, think about the diameter of your field of view:

Which of the objectives provides the broadest (largest) field of view?______________

Now, calibrate your ocular ruler at each of the four magnifications on your compound microscope. Do this by placing the glass slide with the ocular micrometer on the stage, centering, and focusing. You should be able to see the ruler and finely focus on the line with its subdivisions. With the ocular micrometer in focus, use it to determine the total length of your ocular ruler, as well as the distance between each large subdivision and the distance between each small subdivision. Begin with the 10x objective (100x total magnification) and then do the other magnifications. Complete the following information in the table, labeling your measurements (e.g., micrometers):

(After your lab has been graded, cut out this portion of the page and tape it somewhere prominent on your lab notebook so that you can refer to it the rest of the semester!

Total magnification	**Ocular ruler/reticle–** total length	**Ocular micrometer–** Distance between Large subdivisions	**Ocular micrometer–** Distance between Small subdivisions
40x			
100x			
400x			
*1000x			

***Note**: This is the objective lens that you will use with oil. It is called **oil immersion**. If you have not done this before, ask for my help.

1. Whole mount of a thorny-headed worm (Phylum Acanthocephala, *Leptorhynchoides theacutus* or similar). *Slide box slide 1.* These specimens were collected from the intestine of a local gamefish species (e.g., Largemouth bass, bluegill) at the SUNY Biological Field Station in Cooperstown. These slides were actually prepared by SUNY Oneonta students conducting research! This is the adult stage of a parasitic worm species that utilizes a small crustacean as its first (intermediate) host and a fish as its final (definitive host) in its life cycle.

Examine the specimen with your microscope and complete the entry for it on a separate piece of paper (for proper entry format, see "II. B." on page one).

Now, using either 40x or 100x magnification to view the worm, measure its total length:___________________________. You can check your answer by taking the slide off the microscope and measuring the worm with a millimeter ruler—the answers should be similar! Using 400x magnification, measure the length of one of its hooks as accurately as possible:________________________

***2. *Entamoeba histolytica*– cyst. Fecal smear. Plate.** *Slide box slide 2* (See figure and textbook pg. 106 Figs. 7.1 & 7.2). This is a species of parasitic ameba (Phylum Archamoebae) that is found in the digestive tract of humans and other animals. Protozoans of the digestive tract usually exhibit two forms, an active feeding dividing form called the **trophozoite**, which perishes rapidly outside the host, and a non-feeding excysted form, better suited for survival in the external environment. This stage is usually called the **cyst**. We will examine more parasitic amebas next week.

The purpose of examining this specimen today is to become familiar with searching for tiny specimens with the 100x (oil immersion objective), and to become familiar with drawing a plate.

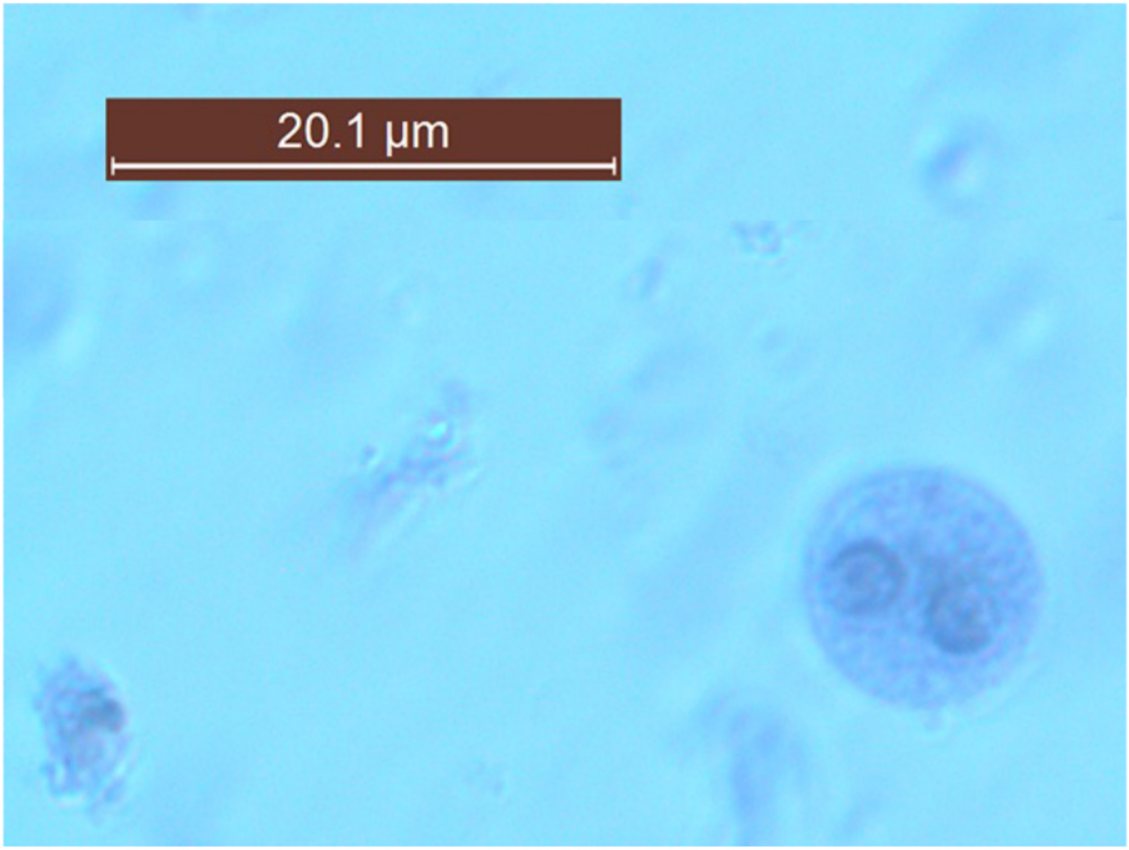
Begin by focusing on your slide at 100x magnification. You must focus clearly on the field of fecal debris before you can increase magnification. Once focused, go up to 400x and begin to search for tiny sphere-shaped entities that might be cysts. It will be hard at first to distinguish the cysts from all the other objects you view! This will improve with practice. Once you have found what you believe is a cyst, center it and continue to 1000x (oil immersion) magnification, carefully adding a drop of oil onto the slide when the microscope nosepiece is rotated so that the slide is halfway between the 40x and 100x objective.

Once you have found a specimen that you would like to draw, have me or the "TA" verify it. Then proceed to make a plate on a separate sheet of paper (see guidelines above under "II. B." on page 1). Label the following structures: cell membrane, cytoplasm, nucleus, and endosome and chromatoid bars that are smooth. **Be sure** your plate drawing is complete. In addition to the drawing itself, it should consist of labels, label lines, a scale bar with label to show specimen size, magnification, and a title. I will initial the plate drawing **before** you remove the slide of *Entamoeba coli* from your microscope.

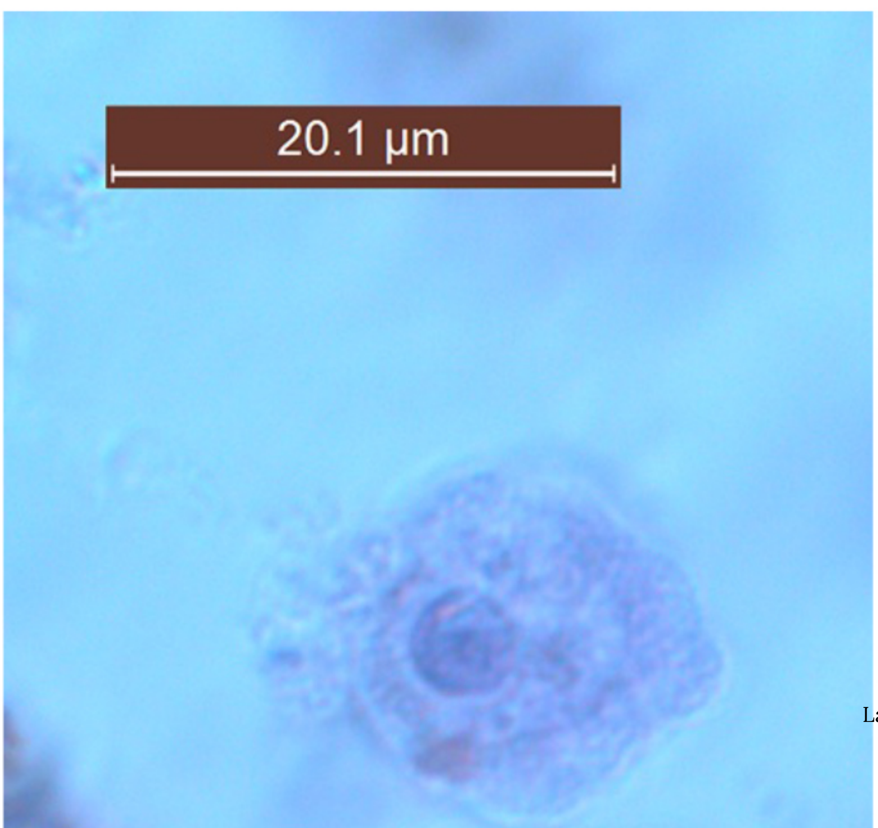
The diagnostic stage of *E. histolytica* is the ______________________________ .

***3. *Entamoeba histolytica*. Trophozoite. Fecal smear. Plate.** *Slide box slide 3.* (See figure and textbook pg. 106 Fig. 7.1) Trophozoites of this species can invade mucosal tissue and cause the characteristic flask-shaped ulcers in the wall of the large intestine (see #3 below). Red blood cells can often be observed inside the food vacuoles. Note the following structures: nucleus, nuclear membrane chromatin, endosome, food vacuole, cell membrane, and pseudopod. Note the central endosome within the nucleus and the relatively even arrangement of the nuclear membrane chromatin. These features help distinguish this potentially pathogenic species from the commensalistic *Entamoeba coli*.

Provide an example of a site other than the large intestine where you might find trophozoites of *E. histolytica*: ____________________________



L1 entry 2 *Entamoeba histolytica* cyst



Laboratory #2: Parasitic protistans: Amebas and others

Most if not all of the animal species harbor one or more species of protozoan in their digestive tract. Although most may be considered commensals, several are parasites, and can cause considerable harm to the host. The human digestive tract may be infected with up to 12 species of protozoans. Today we will mostly focus on protozoans of the digestive tract. However, in the following three labs you will see that humans and other animals may be infected with protozoans in a diversity of other sites, including the reproductive tract, circulatory system, the skin, the brain, and elsewhere.

Protozoans of the digestive tract usually exhibit two forms, an active feeding, dividing form called the **trophozoite**, which does not survive for long outside of the host, and a non-feeding encysted form that can survive in the external environment. This stage is typically called the **cyst**.

The objective today is to study the specimens (with the aid of your textbook and other figures) and to learn the distinguishing features of each species. You will need to measure the size of each specimen you examine. Although you are required to make plates of a subset of the specimens you examine, you are encouraged to make sketches for your entries. Sketches are valuable for future reference. Be sure to make a notebook entry for each of the specimens you see.

I. **Phylum Archamoebae** (Amoebae) [called Phylum Rhizopoda in some editions of your text] Members of the phylum Archameobae (= Rhizopoda=Sarcodina) are characterized by their use of protoplasmic or cytoplasmic streaming to form pseudopodia that are used in locomotion. Some species in this phylum possess flagella at certain temporary life cycle stages. Most species in this phylum are free-living, but some species are parasitic and are fairly important.

Study the specimens that have been provided and note characteristics that can serve to distinguish the species in both the trophozoite and cyst stages. You should be familiar with the following features of amoebae: endoplasm, ectoplasm, nucleus, endosome (=nucleolus, but use the former term), chromatoid bars, cysta wall, food vacuole, glycogen vacuole, and pseudopod. Use the chart provided in attached Figure 1 to assist you in distinguish the various species of amoebae.

1. *Entamoeba histolytica.* Cyst. Fecal smear. *Slide box slide* 2 (See figure and textbook pg. 106 Figs. 7.1 & 7.2). You viewed this specimen in *Lab* 1. *Locate*: up to 4 nuclei, nuclear membrane chromatin, endosome, chromatoid bar, cyst wall. Note that this species possesses four nuclei and chromatoid bars that are smooth.

2. *Entamoeba histolytica.* Trophozoite. Fecal smear. *Slide box slide* 3 (See figure & text pg. 106 Fig. 7.1) You viewed this specimen in *Lab* 1. Trophozoites of this species can invade mucosal tissue and cause the charaxteristic flask-shaped ulcers in the wall of the large intestine (see #3 below). Red blood cells can often be observed inside the food vacuoles. Note the following structures: nucleus, nuclear membrane chromatin, endosome, food vacuole, cell membrane, and pseudopod. Note the central endosome within the nucleus and the relatively even arrangement of the nuclear membrane chromatin. These features help distinguish this potentially pathogenic species from the commensalistic *Entamoeba coli*.

Provide an example of a body organ other than the large intestine where you might find trophozoites of *E. histolytica*: ____________________________

3. *Entamoeba histolytica.* Section of large intestine with trophozoites. Demonstration. (text pg. 109 Fig. 7.5) This is an example of the characteristic flask-shaped ulcer. For your notebook entry, record the diameter of this ulcer, in micrometers.

What is the ontogenetic stage of the *E. histolytica* cells that are inside this ulcer?__________________

***4. *Entamoeba coli.* Cyst. Fecal smear.** *Slide box slide* 4 (see figure & text pg. 112 Fig. 7.8). You studied and drew this specimen last week.

How would you distinguish cysts of this species from those of *E. histolytica*? Provide 2 distinguishing features:_______________________________ & _____________________________ .

***5. *Entamoeba coli.* Trophozoite. Fecal smear.** *Slide box slide* 5 (see figure & text pg. 112 Fig. 7.7) Note how "junky" the cytoplasm is. This species often engulfs intestinal debris. Be sure to locate the nucleus, nuclear membrane chromatin, endosome, pseudopod, food vacuole & cell membrane. Note the eccentric endosome within the nucleus and the uneven nuclear membrane chromatin. Compare these features to the features of trophs of *E. histolytica*.

Provide an example of a potential food item of this ontogenetic stage of this organism:__________________________________

6. *Entamoeba gingivalis.* Trophozoite. Smear from oral swab. Demonstration. (see figure & text pg. 112 Fig. 7.9).

What is the site of infection of this species? _______________________________

How is this species transmitted? _______________________________

Does this species encyst? ______________________________

7. *Iodamoeba buetschlii.* Cyst. Fecal smear. Slide tray. (see figure & text pg. 113 Fig. 7.11). Note the large glycogen vacuole, large endosome and achromatic strands extending from the endosome to the nuclear membrane in this species.

What domestic animal also hosts this species? _______________________________

II. Phylum Heterolobosea

The phylogenetic relationships of many of the major prototist groupings (e.g., phyla) are still being resolved. As a result, the taxonomy (names) is changing as well. Although still called "amebas" and treated with other ameba species in your textbook, the following two species belong to a different grouping known as Heterolobosea.

8. *Naegleria fowleri.* Trophozoites in brain tissue. Histological section. Demonstration.

(text pg. 115 Fig. 7.13 & 7.14). This is an example of a facultative parasite. This species normally lives in soil and water but can facultatively (i.e., if given the chance) become parasitic. As a parasite it produces primary amoebic meningoencephalitis (PAM) in humans and can result in death. This amoeba is unusual in its ability to produce a cyst and a biflagellated form, in addition to the amoeboid trophozoite.

What is the normal habitat of these organisms?__________________________

III. Phylum Retortamonada

The members of this phylum are distinctive in that they lack mitochondria and golgi. Most species of this phylum are inhabitants of the intestinal tracts of vertebrate hosts. All species in this group, whether parasitic or free-living, inhabit anoxic environments. Most species possess a resistant cyst stage and a more fragile feeding stage called the trophozoite.

Unfortunately, we have only a single species of the phylum to show you in lab today. But be sure to keep in mind there are several other species that can be of veterinary or commercial significance.

9. *Giardia lamblia / Giardia duodenalis* Trophozoite. Fecal smear. *Slide box slide 6* (see figure & text pg. 89). This species can be pathogenic in humans. This species and its close relatives can also be found in the intestinal tracts of a diversity of other mammal species. Recognize the following structures: nuclei, adhesive disc, median bodies (if visible), anterior flagella, posterior flagella, ventral flagella, and caudal flagella.

Where , in what body organ, would you expect to find this stage of this species? ________________________

What structure does this organism remain attached to its host? __________________________

10. *Giardia lamblia / Giardia duodenalis* . Cyst. Fecal smear. Slide tray. (see figure & text pg. 89). Locate the following: nuclei, cyst wall, flagella, and median bodies (if visible).

Where would you expect to find this stage of this species? __________________________

V. Phylum Chromista

The opalinids possess numerous rows of flagella over the entire body surface and consequently have traditionally been placed with the Ciliophora. However, recent detailed studies with electron microscopy suggest that they are different from ciliophorans in several ways. For example, they lack infraciliature and possess two or many nuclei of similar structure and size. There is some evidence that suggests reproduction of opalinids is controlled or cued by host hormones.

11. *Opalina.* Trophozoites. Whole mount from frog rectum. Slide tray. (see figure & text pg. 102 Fig. 6.18). Note the numerous nuclei of similar size and shape as well as the many flagella.

How does this species get transmitted from host to host? That is, how does it leave the body of one host, and how does it enter the body of the next host? ________________

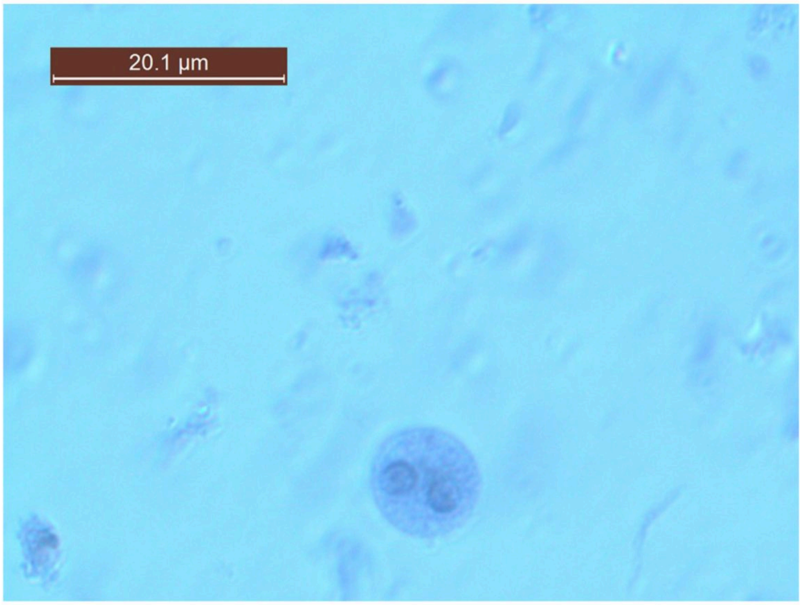
VI. Phylum Ciliata – (ciliates)

Ciliates possess multiple cilia that are united via a network of organelles known as infraciliature, which lies below the pellicle. They also possess two types of nuclei (usually a macronucleus and a micronucleus). A contractile vacuole is usually also present.

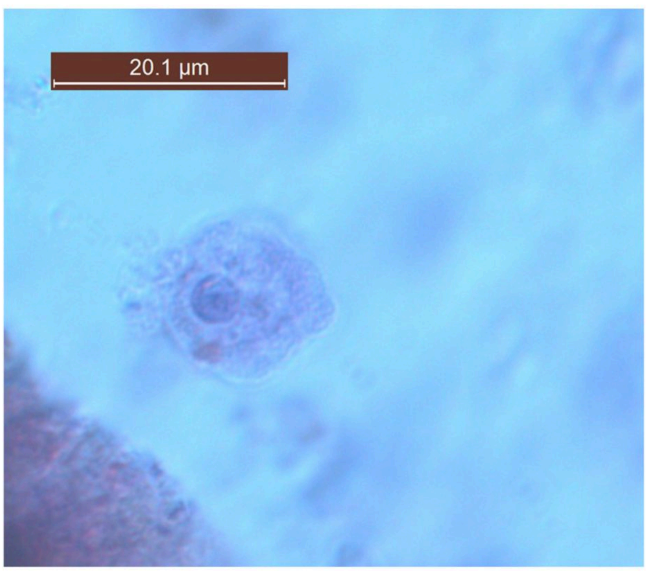
12. *Balantidium coli.* Trophozoites. Fecal smear. Demonstration. (see figure & text pg. 168 Figs. 10.2 & 10.3). This is the largest protozoan parasite of humans. Note the sausage-shaped macronucleus, round micronucleus, and numerous cilia.

What structures would this organism use for locomotion? ________________________

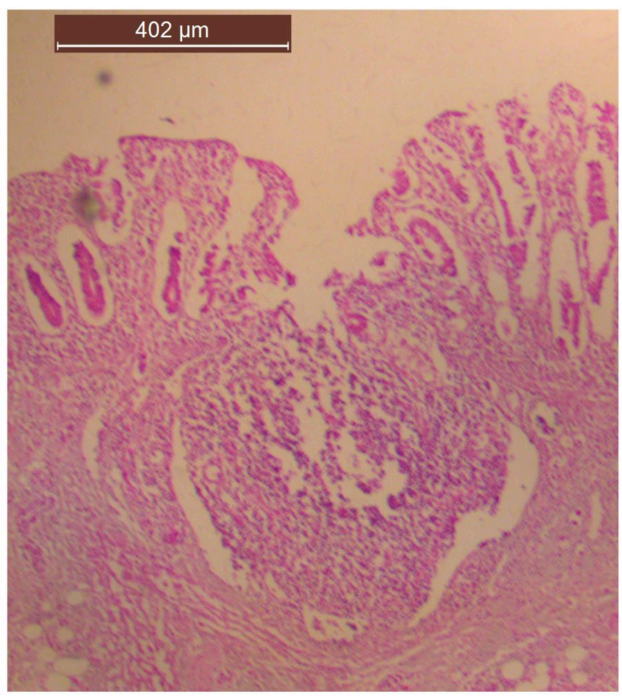
Lab 2



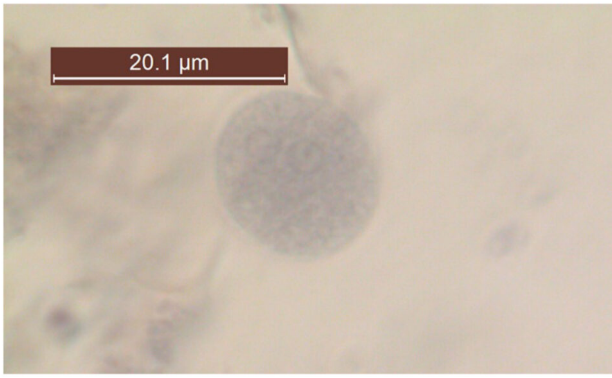
L2 entry 1 *Entamoeba histolytica* cyst



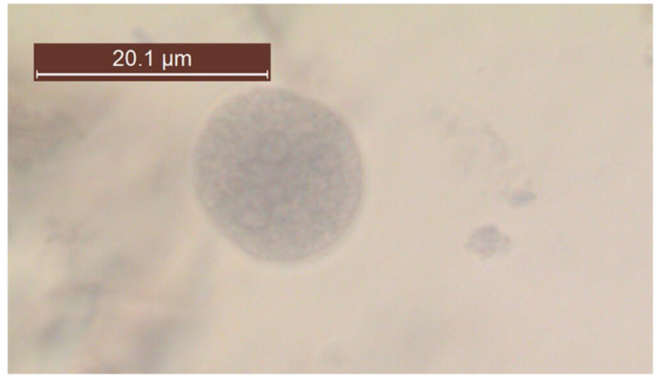
L2 entry 2 *Entamoeba histolytica* troph



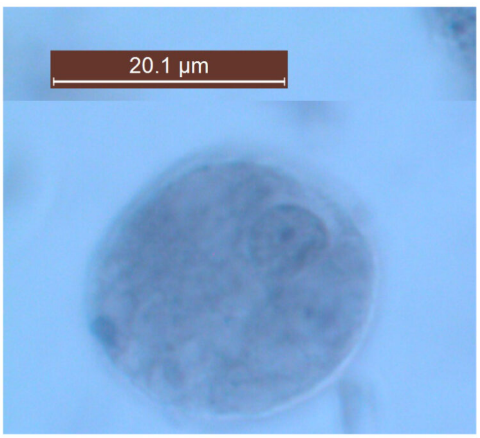
L2 Entry 3 *Entamoeba histolytica* intestinal ulcer



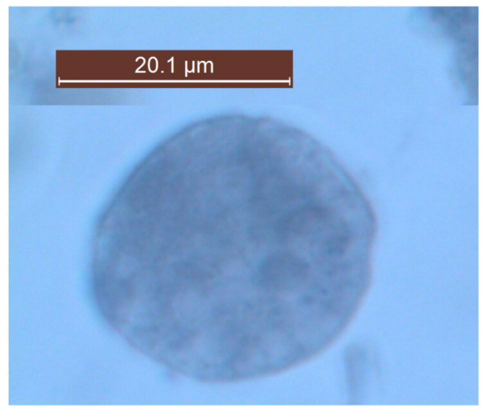
L2 entry 4 *Entamoeba coli* cyst 1st plane



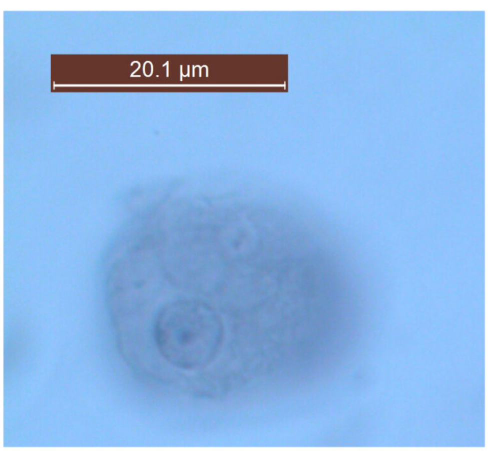
L2 entry 4 *Entamoeba coli* cyst 2nd plane



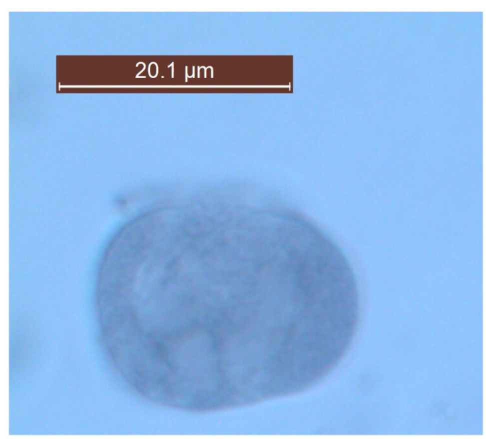
L2 Entry 5 *Entamoeba coli* 2nd troph 1st plane



L2 Entry 5 *Entamoeba coli* 2nd troph 2nd plane

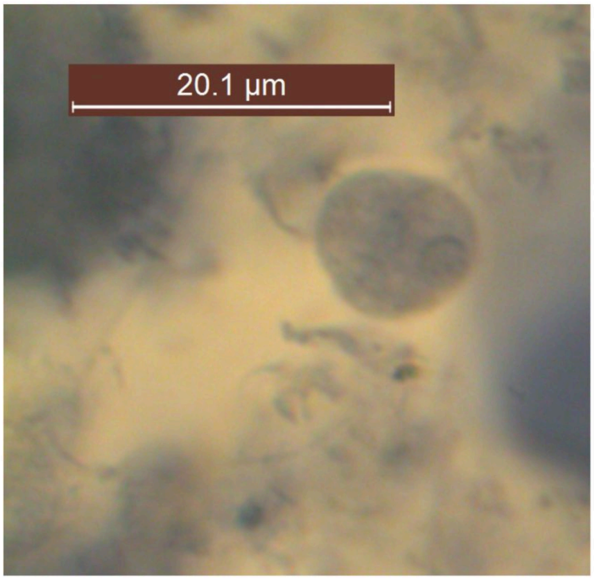


L2 Entry 5 *Entamoeba coli* troph 1st plane

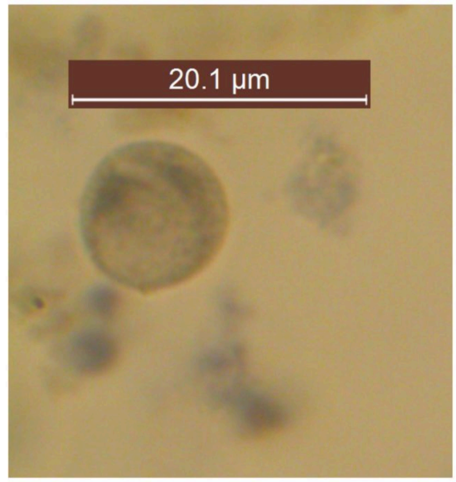


L2 Entry 5 *Entamoeba coli* troph 2nd plane

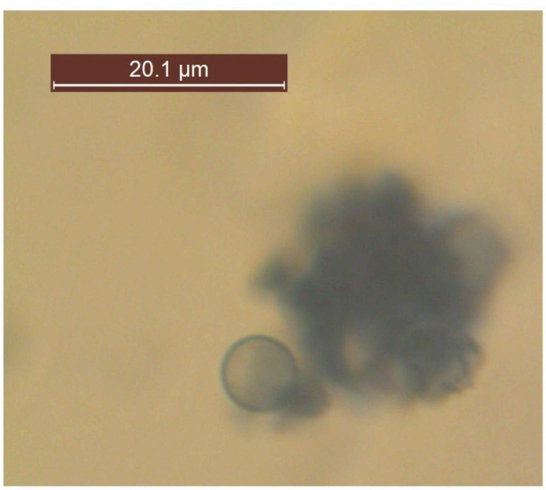
Lab 2



L2 Entry 6 *Entamoeba gingivalis* troph

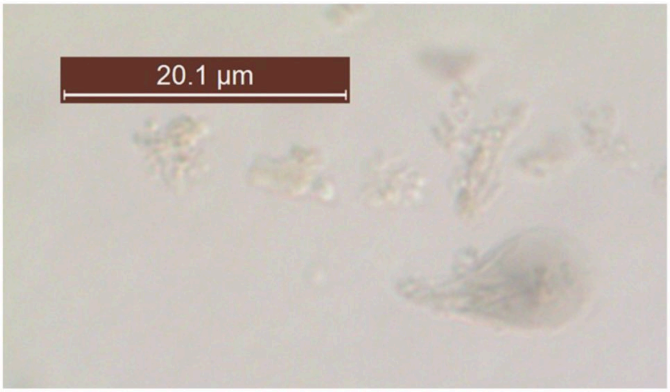


L2 Entry 7 *Iodamoeba buetschlii* cyst

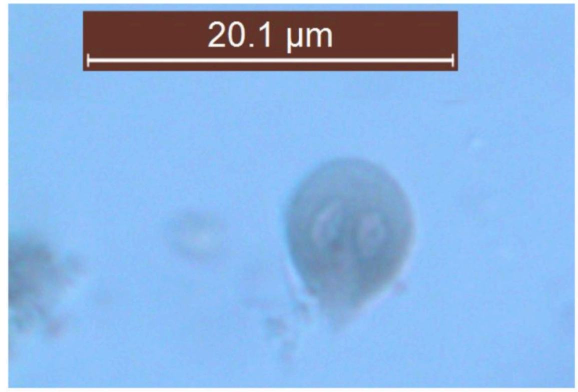


L2 Entry 8 *Naegleria fowleri* amoeboid trophozoites

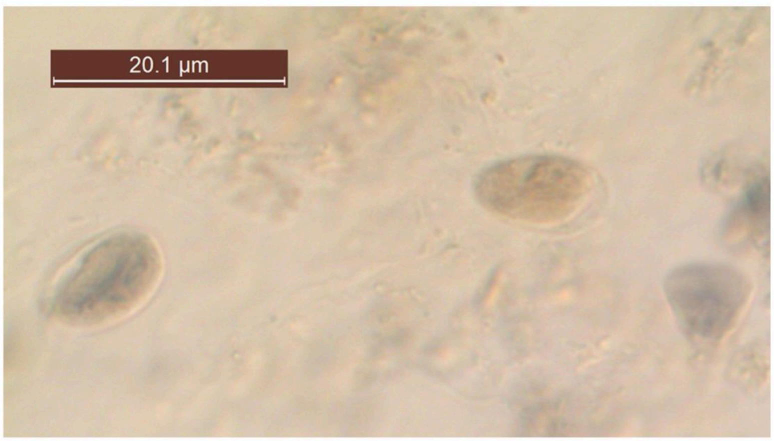
Lab 2



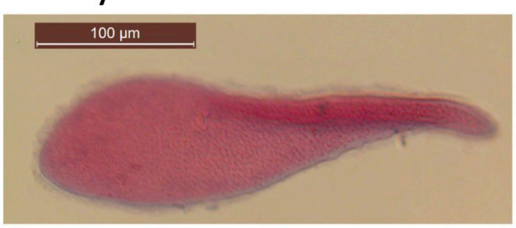
L2 entry 9 *Giardia lamblia* troph



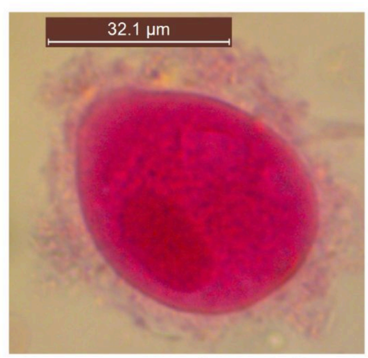
L2 Entry 9
Giardia lamblia 2nd troph



L2 Entry 10 *Giardia lamblia* 2 cysts 1 troph



L2 Entry 11 *Opalina* trophozoites



L2 Entry 12 *Balantidium coli* trophozoite

Laboratory #3: trypanosomes and their kin

Phylum Parabasalia

The members of this phylum possess a conspicuous axostyle or stout rod composed of microtubules that runs from the kinetosome to the posterior end of the trophozoite. Some members of this phylum lack mitochondria.

***1. *Trichomonas vaginalis*. Trophozoite. Vaginal smear.** *Slide box slide* 7 (see figure & text pg. 95 Fig. 6.12). Species of *Trichomonas* are part of a group of the phylum Parabasalida that lack mitochondria, do not form cysts, and typically possess 4 or 6 flagella. Recognize the following structures: nucleus, kinetosome, anterior flagella, undulating membrane, recurrent flagellum (note extent), costa, and axostyle. Note: for your drawing use a cell that is not in the midst of fission!

Where would you expect to find this species in its host? _______________________

What is the function of the flagella that you can see? ________________

Phylum Cnidaria – cnidarians including myxozoans

Cnidarians make up a diverse phylum of 1000s of species that are an important part of sealife and familiar animals to you, including jellyfish, Portuguese man o' war,

anenomes, and coral. But why are they mentioned in this lab exercise? Phylum Cnidaria is now known to include a group of single celled parasitic organisms known as the Myxozoa. Myxozoa, or myxozoans have traditionally been considered their own phylum. With the advent of the use of DNA sequence data to study patterns of evolutionary relatedness, however, it has come to light that myxozoans are actually cnidarians! In other words, the single-celled parasites known as myxozoans, are actually more closely related to jellyfish, than they are to other "protistan" parasites.

2. M***yxobolus* sp. Gill smear. Demonstration.** (text pg. 178 Fig. 11.3). This specimen was found attached to the gills of a Quilback (a type of fish in the sucker family) from western Nebraska. Examine the specimen and recognize the polar filaments. Polar filaments are basically modified cnidarian nematocysts. This thread-like structure was shot out of the cell and is used for attachment to the fish gill. The previous host in the life cycle would have been an aquatic worm (oligochaete annelid).

Consider the specific habitat of these parasites. What would be a possible pathological effect on the fish host, caused by the presence of *Myxobolus*?_______________________

Phylum Euglenozoa

Members of this phylum typically have one or two flagella and mitochondria with discoid (instead of tubular) cristae. This phylum includes many free living species, as well as various species that are parasites of plants or animals.

Subphylum Kinetoplasta

Members of this subphylum are very distinctive in their possession of a unique mitochondrion containing a large disc of DNA composed of both mini and maxi circles, commonly referred to as a kintoplast.

Order Trypanosomatida

The species in this group possess a single flagellum that is usually attached to the body via an undulating membrane. Most species in the group are heteroxenous; their life cycles include both an invertebrate and a vertebrate host. These are among the most pathogenic species of flagellates. You will see representatives of two trypanosomatid genera, *Trypanosoma* and *Leishmania*.

I. Trypanosomatida exhibiting a trypomastigote form

Be sure to use the accompanying figures in your textbook as you study the characteristics that help differentiate trypomastigotes of different species. Be sure to take measurements whenever possible. Remember that sketches are useful to refer to in the future.

3. *Trypanosoma lewisi*– Trypomastigotes. Rat blood smear. Slide tray. (text page 76 Fig. 5.15). These specimens are excellent examples of trypomastigote morphology. Locate the following: nucleus, kinetoplast, kinetosome (if visible), undulating membrane, and free flagellum. Note that the kinetoplast is posterior to the nucleus in position and the very long undulating membrane that are characteristic of this trypanosomatid body form.

***4. *Trypanosoma brucei gambiense* or *T. b. rhodesiense* or *T. b. brucei*– Trypomastigotes. Mammal blood smear.** *Slide box slide* 8 (text page 63 Fig. 5.3f). Although these three subspecies are the etiological agents of very different diseases, their trypomastigotes are morphologically indistinguishable. This is why some researchers consider all 3 taxa to be subspecies of the species *Trypanosoma brucei*. Note: in your drawing you should include 1-2 red blood cells for context.

Typomastigotes have two forms. Which form do you see on the slide? _____________

Fill in the table below with details on the hosts of the 3 subspecies of *Trypanosoma brucei*:

	Trypanosoma brucei brucei	*Trypanosoma brucei rhodesiense*	*Trypanosoma brucei gambiense*
Possible definitive host			
Intermediate host species (be specific)			

What other life cycle stage, besides the trypomastigote, is present in the life cycle of all of these subspecies?__________

***5. *Trypanosoma cruzi*– Trypomastigotes. Human blood smear.** *Slide box slide* 9 (text page 71 Fig. 5.8). Trypomastigotes of this species have a characteristic "C" shape. Find the nucleus, kinetoplast, kinetosome (if visible), undulating membrane, and free flagellum. Note: in your drawing you should include 1-2 red blood cells for context.

How does the geographic distribution of this species differ from that of the 3 subspecies of *T. brucei*?____

6. *Trypanosoma cruzi*– Amastigotes. Section of infected heart muscle. Demonstration (text page 73 Fig. 5.11). Concentrated pockets of amastigotes of *T. cruzi* are often referred to as "pseudocysts."

What other ontogenetic stage(s), besides amastigotes, of this species is found in humans?___________________

II. Trypanosomatida lacking a trypomastigote form

***7. *Leishmania donavani*– Amastigotes. Hamster spleen smear**. Slide tray. (text page 78 Fig. 5.18). Note the tiny size of these organisms; they are among the smallest known eukaryotic cells. Also note the shape of the cell, the conspicuous kinetoplast, and the very short flagellum (if visible) that is characteristic of the amastigote body form. Label in your drawing: nucleus, kinetoplast. See text page 84 Figs. 5.22 and 5.23 for examples of visceral leishmaniasis ("kala azar") and post-kala-azar dermal leishmanoid.

8. *Leishmania tropica*– Amastigotes and promastigotes. Culture smear. Slide tray. This species is virtually impossible to distinguish morphologically from *L. donovani* but the two species cause very different diseases. See text page 81 Fig. 5.19 for an example of "Oriental sore." Locate a promastigote. Note the position of the kinetoplast relative to the nucleus.

Does this form possess an undulating membrane?________________________

What life cycle stage of this species would you expect to find at the site of a cutaneous lesions?________________________________

III. Intermediate host

9. *Glossina* sp. (Tsetse fly). Whole mount. Demonstration. (text page 65 Fig. 5.5). There are many species in the genus *Glossina* that are known to act as an intermediate host for species of *Trypanosoma* in Africa.

What sorts of habitats could you find a species of *Glossina* in Africa?_______________

Provide an example of a wild animal on which a Tsetse fly could bloodfeed:__________

10. *Rhodnius* sp. or *Triatoma* sp. (Kissing bug or Assassin bug). Demonstration. See text pages 559-561. This insect belongs to the hemipteran family Reduvidae.

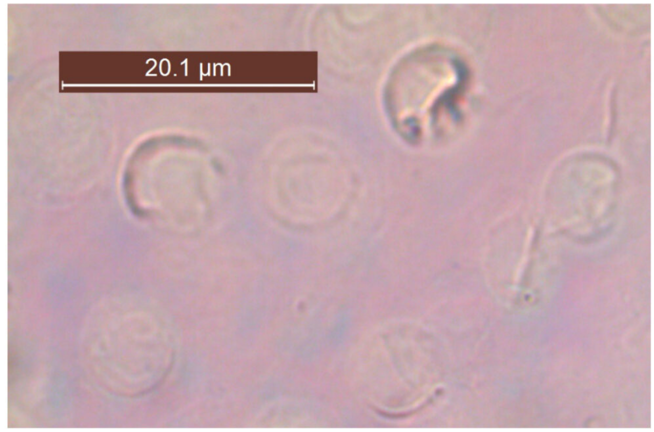
What does it eat?________________What ontogenetic stage of *T. cruzi* is infective to its intermediate host?____________ What ontogenetic stage of *T. cruzi* is infective to its definitive host?____________

11. *Lutzomyia* sp. Demonstration (if available). *Lutzomyia* is a biting dipteran referred to as a sandfly that occurs in the 'New World,' or Western Hemisphere. When these flies suck the blood of an individual infected with a species of *Leishmania* (such as *Leishmania panamensis*) they take in amastigotes of the parasite. The amastigotes develop and divide within the gut of the sandfly and eventually move to its pharynx where they morph into promastigotes. Promastigotes, in turn are the stage of the parasite within the sandfly that is infective to the next host (another mammal). Complete development in the fly takes about 10 days from the time the parasite was acquired to the time that it is infective to the next host.

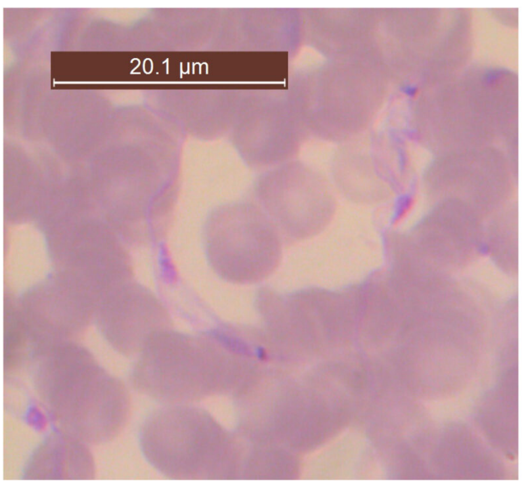
Provide another example of a bloodsucking dipteran—other than a sandfly—that transmits a species of protozoan parasite:____________

What is the name of the division process/type of asexual reproduction that is undergone by the parasite within the host *Lutzomyia*?_____________________

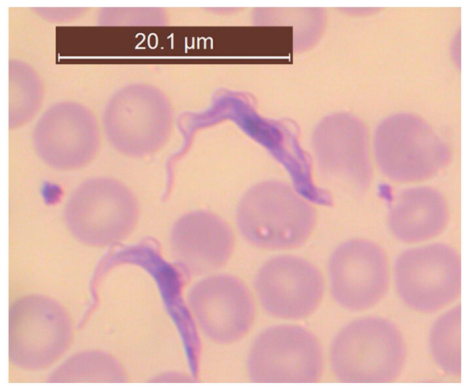
L3 Entry 1 *Trichomonas vaginalis* troph



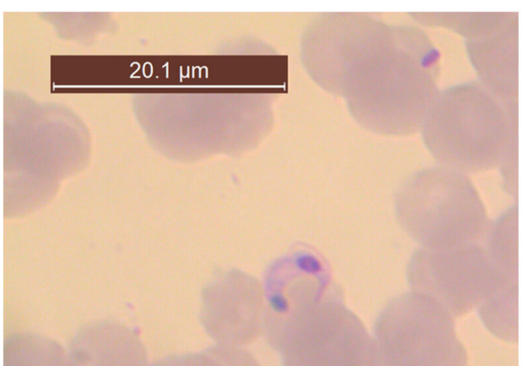
L3 entry 2 *Myxobolus* sp. cells in gill smear



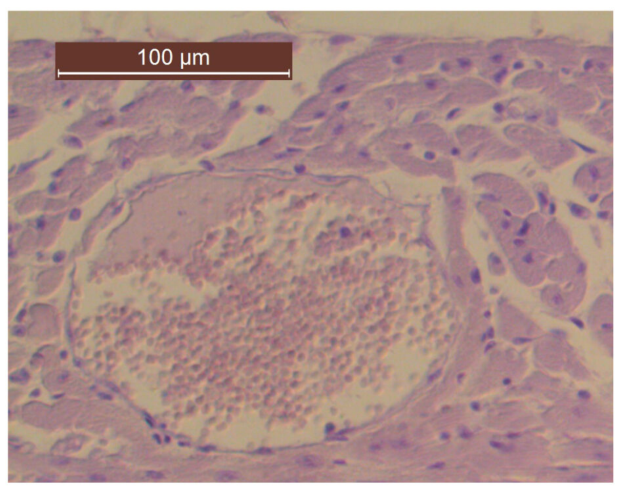
L3 Entry 3 *Trypanosoma lewisi* trypomastigote



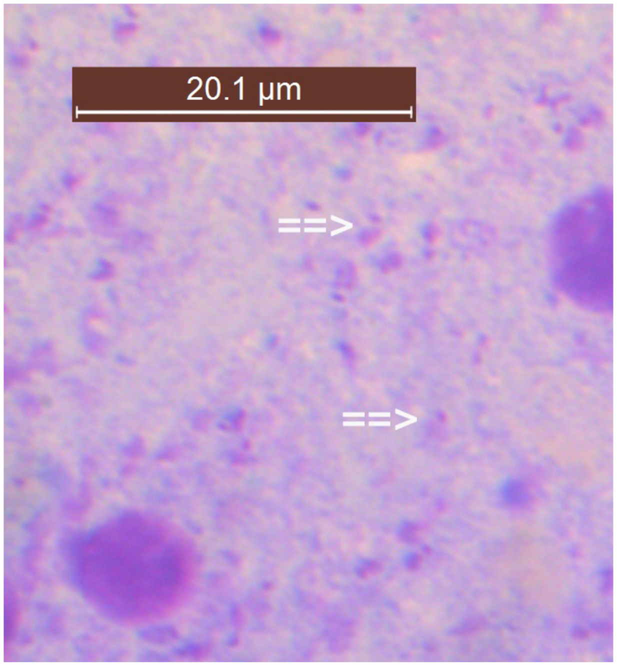
L3 entry 4 *Trypanosoma brucei* trypomastigote



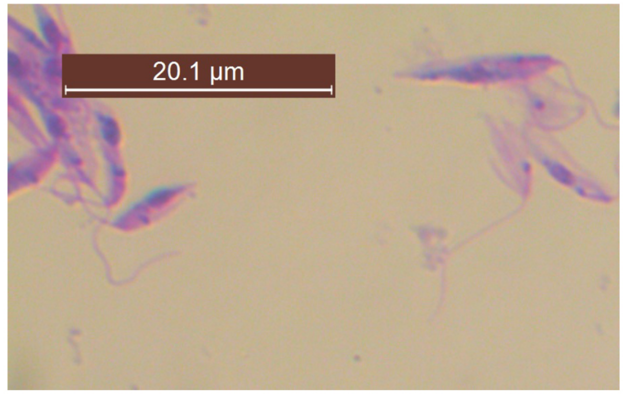
L3 entry 5 *Trypanosoma cruzi* trypomastigote



L3 Entry 6 *Trypanosoma cruzi* in heart muscle pseudocyst



L3 Entry 7 *Leishmania donavani* amastigote



L3 Entry 8 *Leishmania tropica* promastigote

L3 Entry 9 Tsetse fly *Glossina* adult mm ruler

L3 Entry 10 Assasin bug *Rhodnius* adult mm ruler

Laboratory #4: Apicomplexa 1: Plasmodium

Phylum Apicomplexa (Part I)

Members of this phylum possess an apically positioned group of organelles collectively known as the apical complex. The apical complex, which is only visible with electron microscopy, is involved with host cell penetration. All members of this phylum are parasitic. Most important to humans are the blood-dwelling Haemospororida, which include the species responsible for the various forms of malaria. We will deal with the blood dwelling apicomplexans in this lab. Those species that parasitize other tissues of their vertebrate hosts will be treated in Lab 5.

Class Aconoidasida

The life cycle of species in this group generally includes all 3 of the following reproductive processes: schizogony or merogony (production of merozoites), sporogony (production of spores), and gametogony (production of gametes). Species in this group generally do not undergo syzygy (i.e., there is no fusion of gamonts or gametocytes during sexual reproduction).

Order Haemospororida

In this group macro and microgametes develop independently of one another. The microgametocyte usually produces 6-12 flagellated microgametes through a process termed exflagellation. The zygote (termed an ookinete) is motile and the sporozoites are naked (i.e., they are not surrounded by a protective cyst wall). All species in this order are heteroxenous; one of the hosts is usually a vertebrate host and the other host is a blood-sucking insect such as a fly or a mosquito.

I. *Plasmodium* spp. Life cycle stages in the vertebrate host

The goal is for you to view and distinguish as many of the life cycle stages of *Plasmodium* as possible. **Use the color plates following page 141** in your text to help identify the various life cycle stages of *Plasmodium vivax*, *P. falciparum* and *P. malariae*. Also use the table **on page 146** that highlights distinguishing features. Consider the differentiating features of the parasites within the blood cells, and also their effects on the blood cells in which they are found. Note the effects on the red blood cells you observe in *P. vivax*. As you view specimens and refer to the color plates, consider what it would be like to be a clinician needing to make a species specific identification.

1. *Plasmodium vivax* preerythrocytic schizont in liver tissue. Demonstration. See text 147 Fig. 9.2. A mosquito infected with *Plasmodium* introduces sporozoites to the human bloodstream. After ~24 hours the sporozoites end up in the liver where they invade hepatocytes (liver cells). Within liver cells sporozoites undergo exoerythrocytic schizogony, i.e., they divide into multiple merozoites.

What ontogenetic stage came before the stage shown here in the liver? _______________

2. *Plasmodium vivax*. Various stages. Human blood smear. *Slide box slide* 10. See color plate 1. When merozoites leave liver cells they enter the bloodstream and begin the erythrocytic cycle. This begins with merozoites penetrating host erythrocytes. Upon entry, the merozoite becomes a trophozoite.

***2A.** Scan the slide for **trophozoites**. Refer to the color plate as you search.

Does the red blood cell you located infected with a trophozoite of *P. vivax* contain Schuffner's dots in its cytoplasm? ________________________

Note the characteristic "signet ring" form of this trophozoite resulting largely from the presence of a conspicuous vacuole in the center of the cell.

What do you suppose is contained in that vacuole? I.e., what is the parasite eating during this stage?_________

Be sure to illustrate both the host cell and the trophozoite. On your drawing label: red blood cell, Schuffner's dots, and vacuole, nucleus, and cytoplasm of the trophozoite. Note the most red blood cells are only infected with a single trophozoite.

Compare infected erythrocytes with uninfected ones. Do they differ in size? __________

If so, how?____________________

***2B.** Trophozoites grow and develop into **schizonts** with the red blood cells. If you find a good example of a schizont, please be sure to show the rest of us; they are harder to find than trophs! The schizont stage is recognized by having mutiple nuclei. You may observe dark brown pigmentation associated with the schizonts. These are hemozoin granules, waste products from the parasite's metabolism of the hemoglobin of the host cell.

Do you see evidence of vacuoles in the schizonts?____________

***2C.** Schizonts undergo schizogony resulting in multiple merozoites. Merozoites penetrate additional red blood cells and can develop as **macrogametocytes or microgametocytes**, or as trophozoites. Search for either type of gametocyte on your slide. The macrogametocyte fills most of the cytoplasm of the red blood cell and is generally round or oval. Microgametocytes are usually less common than macrogametocytes and are stained a lighter color. Look for Schuffner's dots, enlargement of infected red blood cells, and look for the nucleus, cytoplasm and hemozoin granules associated with the macrogametocyte.

Find a gametocyte and describe how you would distinguish it from the other stages:______________

3. *Plasmodium falciparum*. Various stages. Human blood smear. *Slide box slide* 11 See color plate 3.

***3A.** *Plasmodium falciparum* can be distinguished from other species of *Plasmodium* based on the relatively small size of its ring trophs and the frequent occurrence of multiple **trophozoites** per red blood cell. Scan your slide for **trophozoites**.

Do you see evidence of Maurer's clefts? __________Compare infected blood cells to uninfected ones; do the infected red blood cells look enlarged? ________Note the "signet ring" form of the trophs, a result of the presence of a conspicuous vacuole in the center of the cell.

3B. You may also encounter schizonts of *P. falciparum*, but these are generally rare in peripheral blood.

3C. Look for either **macrogametocytes or microgametocytes** of *P. falciparum*. (Or see **demonstration or example from another student**) Both of these stages have a characteristic banana-shape, but macrogametocytes tend to be more darkly stained.

Do you see evidence of Schuffner's dots or hemozoin?______________

What ontogenetic stage follows the macro- or microgametocyte stage?_______________

4. *Plasmodium falciparum* in brain tissue. Histological section. Demonstration. See text Fig. 9.7. This condition results in cerebral malaria, in which capillaries are filled with red blood cells infected with *Plasmodium falciparum*.

What is the source of pigment that is visible in this slide?_________________

5. *Plasmodium malariae*. Various stages. Human blood smear. *Slide box slide 12*

See color plate 5.

***5A.** Search for trophozoites of *P. malariae*. These closely resemble trophozoites of *P. vivax*. However, some *P. malariae* trophozoites are band-shaped, rather than ring-shaped. The trophozoites of *P. vivax* are never band-shaped.

Do you see evidence of Schuffner's dots or enlargement of infected red blood cells?_________________

II. *Plasmodium* spp. Life cycle stages in the invertebrate host

6. *Plasmodium vivax* ookinetes. Demonstration slide- smear taken from the stomach of a mosquito. Once ingested by the mosquito, a single macrogametocyte develops into a single macrogamete. However, a single microgametocyte will undergo a process known as exflagellation and form 6-12 microgametes. Microgametes swim around the mosquito gut until they find a macrogamete, which it penetrates and fertilizes. The product of that union is a zygote that is elongate in form, known as an ookinete.

Are ookinetes haploid or diploid?____

Based on the life cycle, would you consider the mosquito host the intermediate or definitive host? Explain your answer:____

____

7. *Plasmodium vivax* oocyst. Demonstration slide- sections of mosquito stomach infected with multiple oocysts. See text page 148 Fig. 9.3. The ookinete moves to the mosquito stomach wall, penetrates cells, and transforms into an oocyst. The first division within the oocyst is reductional, followed by repeated mitosis. After a series of divisions, the oocyst contains thousands of sporozoites.

What ontogenetic stage(s) in the *Plasmodium* life cycle are diploid?__________________

What type of process takes place within the oocyst (sporogony, gametogony, or schizogony)?____________________________

8. *Plasmodium vivax* sporozoite. Demonstration slide. Smear taken from the salivary gland of a mosquito. See text page 148 Fig. 9.4. Sporozoites emerge as the oocyst ruptures. They migrate from the mosquito body cavity to the salivary glands. Once a mosquito has sporozoites in its salivary glands, it is infective for the rest of its life, which is long enough to include multiple blood meals, unfortunately.

Describe the morphology of the sporozoite that you see:_____________________

III. *Plasmodium* and related species in other animals.

Species of P*lasmodium* infect many other animals besides humans.

9. *Plasmodium cathemerium* or *Plasmodium gallincaeum* trophs. Bird blood smear. Slide tray. Locate the small blue bodies in the cytoplasm of the red blood cells. These small bodies are trophs of *P. cathemerium*. Also, try to locate schizonts.

10. *Haemoproteus columbae* gametocyte. Pigeon blood smear. Demonstration. See text page 159 Fig. 9.11. Species of this genus can infect a variety of birds. Note how the gametocyte occupies the majority of the cytoplasm.

Is this stage infective to other pigeons? ______________________________________

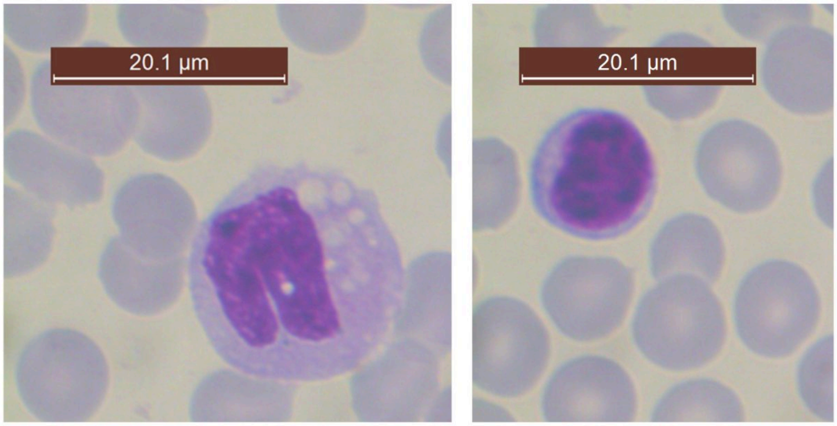
What is the infective stage of *H. columbae* to the pigeon?_______________________

IV. An invertebrate (or "definitive") host of species of *Plasmodium*

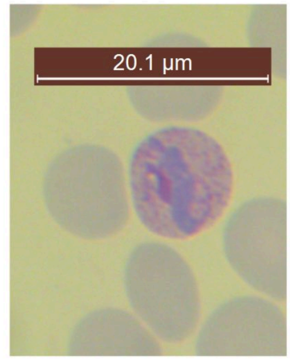
11. *Anopheles quadrimaculatus*. Whole mount. Demonstration. See page 582 Fig. 39.6. This is one of many species in the genus that P*lasmodium* species can infect. When this female mosquito takes a human bloodmeal containing gametocytes of P*lasmodium*, it becomes infected and the cycle continues in its gut. Non-suitable mosquito hosts would digest gametocytes along with the rest of the blood meal at this point.

List all of the ontogentic stages of the *Plasmodium* life cycle that occur within the lumen of the mosquito host gut/ intestine:____ and,

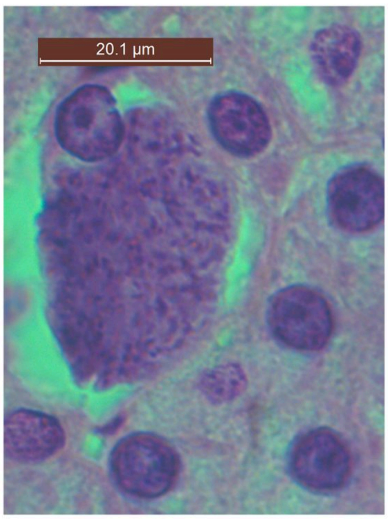
list all of the stages that occur in other mosquito body parts:________________________



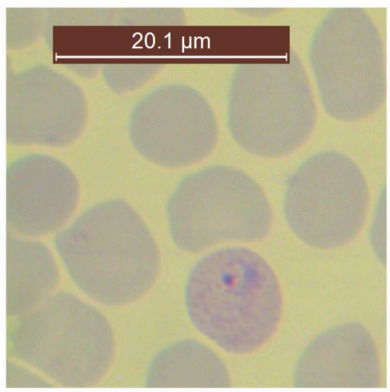
L4 blood smear beware the white blood cells; they are not *Plasmodium*!



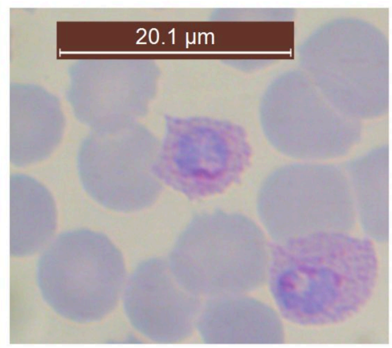
L4 Entry 2A *Plasmodium vivax* ameboid trophozoite



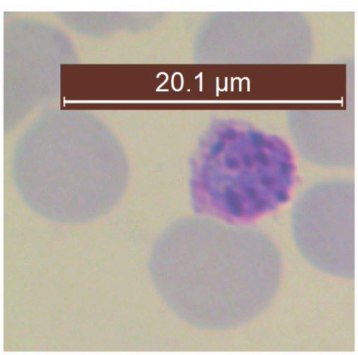
L4 Entry 1 *Plasmodium vivax* liver schizont



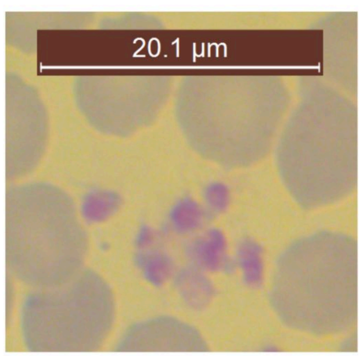
L4 Entry 2A *Plasmodium vivax* ring troph



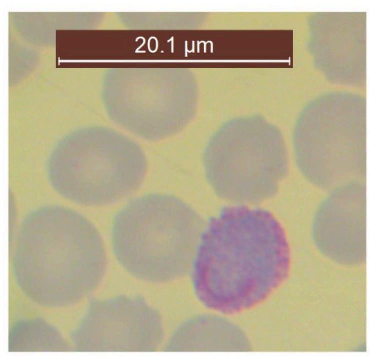
L4 Entry 2A *Plasmodium vivax* 2 large ring trophs



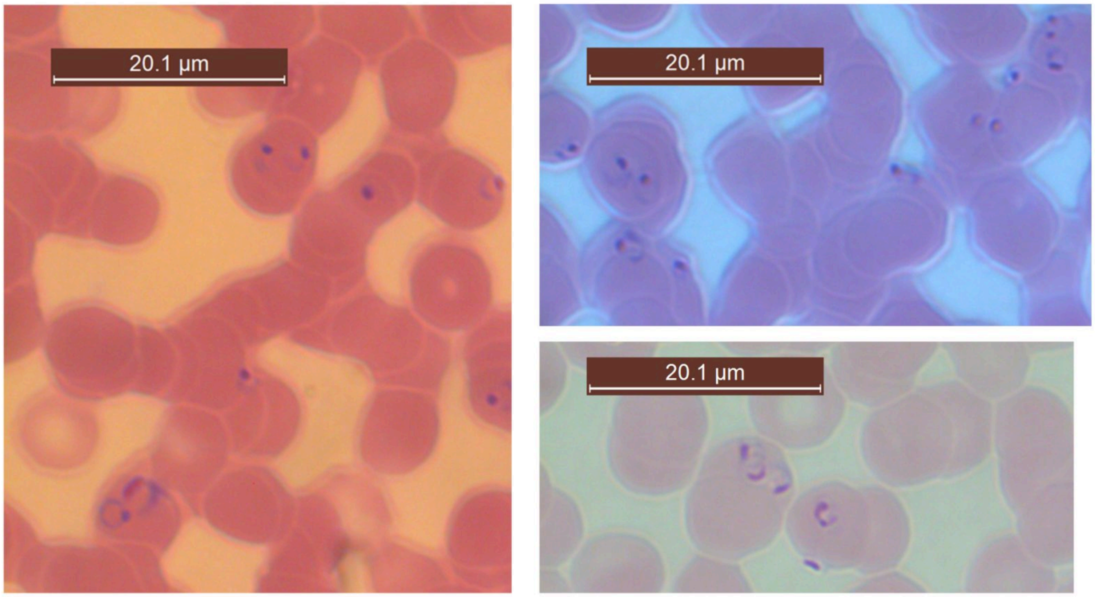
L4 Entry 2B *Plasmodium vivax* schizont intact



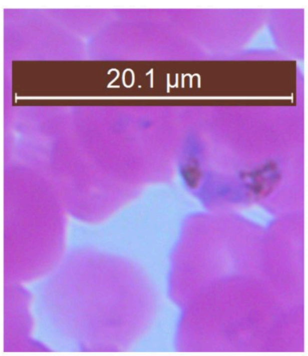
L4 Entry 2B *Plasmodium vivax* schizont breaking open



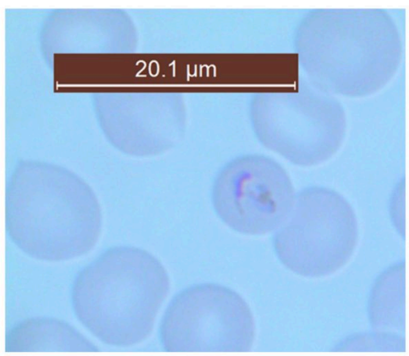
L4 Entry 2C *Plasmodium vivax* macrogametocyte



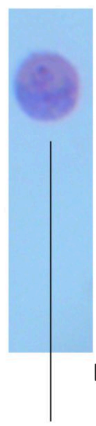
L4 Entry 3A *Plasmodium falciparum* ring trophs various (see erythrocytes with 1 or 2 ring trophs)



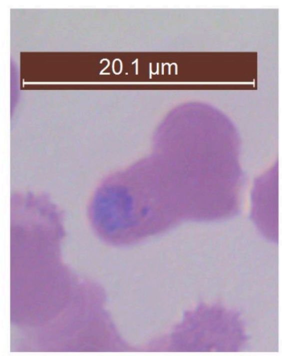
L4 Entry 3C *Plasmodium falciparum* crescent-shaped gametocyte



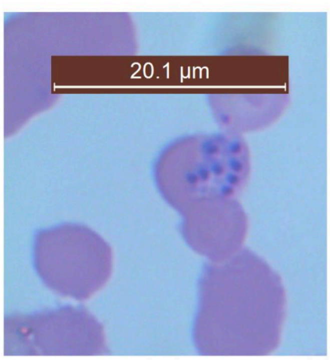
L4 Entry 5A *Plasmodium malariae* ring troph variant



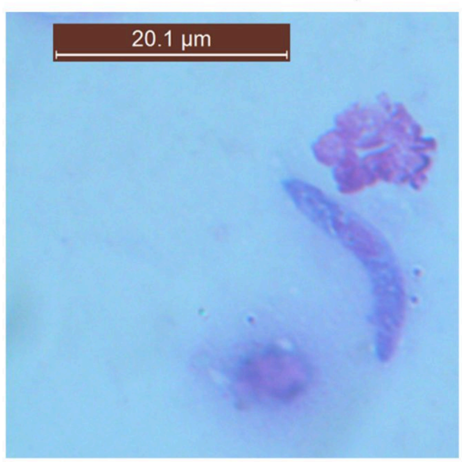
amoeboid *P. malariae* troph, nearly band-shaped



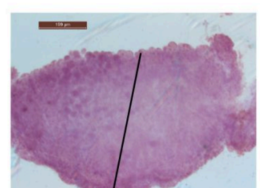
L4 Entry 5 *Plasmodium malariae* amoeboid troph



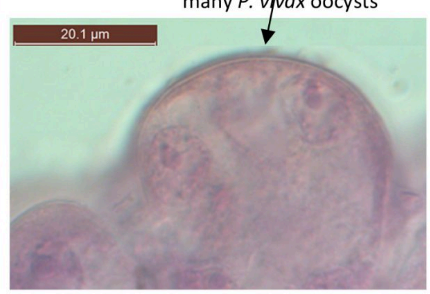
L4 Entry 5 *Plasmodium malariae* schizont



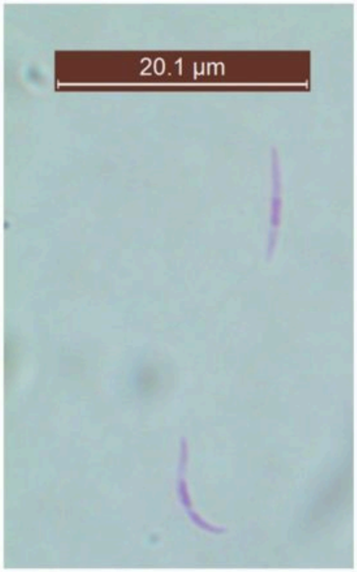
L4 Entry 6 *Plasmodium vivax* ookinete



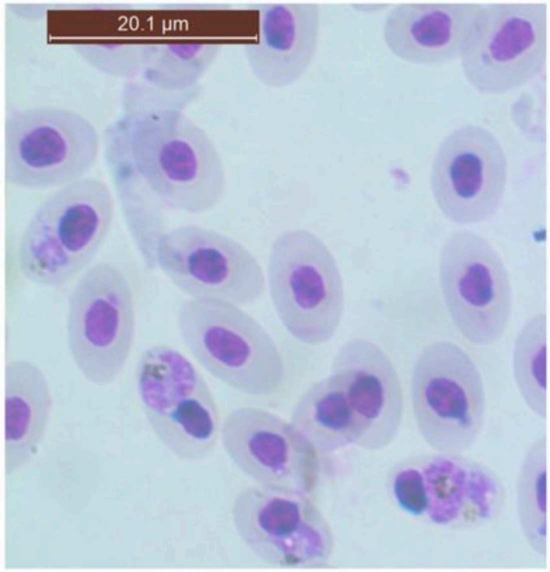
L4 Entry 7 mosquito gut with many *P. vivax* oocysts



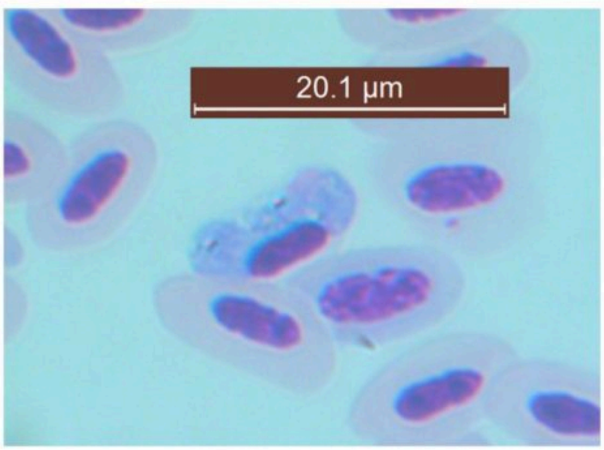
L4 Entry 7 *Plasmodium vivax* 1 oocyst



L4 Entry 8 *Plasmodium vivax* sporozoites



L4 Entry 9 *Plasmodium gallinarum* trophs



L4 Entry 10 *Haemaproteus columbae* gametocytes

L4 Entry 11 *Anopheles quadrimaculatus,* mm ruler

Laboratory #5: Apicomplexa 2

Phylum Apicomplexa (Part II)

In the first part of today's lab we will examine a species from another order in the Class Aconoidasida. The classification is shown here, with respect to the apicomplexans we saw last week:

Phylum Apicomplexa – possess an apical complex

Class Aconoidasida – no syzygy; intracellular reproduction

Order Haemospororida –heteroxenous; naked sporozoites

Plasmodium spp., *Leucocytozoon* sp., *Hemoproteus* sp.

Order Piroplasmorida – Members of this order are parasites of ticks and mammals. Trophozoites dwell within the mammal host erythrocytes. Unlike *Plasmodium*, there is no exoerythrocytic phase in the mammal host (e.g., see text page 163 Fig. 9.16). This order is of considerable veterinary importance.

1. *Babesia bigemina*. Blood smear. Demonstration of trophozoites (= "merozoites"). Text page 162 Fig. 9.15. This species parasitizes cattle, or other ruminants, and tick species of *Boophilus*. It is the etiological agent of a disease in cattle called "Texas cattle fever" or "red-water fever", which can result in death within a week of infection. Observe the merozoites, which are often found in pairs in red blood cells. Hence the species name.

Refer to the life cycle of another species of *Babesia*, shown on text page 163:

What stage is infective to the tick, from the mammal host?________________________

What stage is infective to the mammal host?___________________

Class Conoidasida

Unlike the previous apicomplexan class you studied, members of this group possess an apical complex with a conoid.

Subclass Coccidiasina – coccidians. These parasites typically occupy sites other than blood in their vertebrate hosts, and lack syzygy. Sporozoites are usually enclosed in sporocysts within an oocyst. Some members of this group are monoxenous while others are heteroxenous.

Oocyst morphology is important for species identification. For example, *Eimeria* species all possess sporulated oocysts that contain 4 sporocysts, each with 2 sporozoites. Whereas the sporulated oocysts of members of the genera *Toxoplasma* and *Sarcocystis* contain 2 sporocyts, each with 4 sporozoites.

Eimeria is monoxenous, while *Toxoplasma* and *Sarcocystis* are heteroxenous.

2. *Eimeria stiedae* in rabbit liver. Various life cycle stages. Demonstration. Text page 129 Fig. 8.9 & attached Fig. 1. This is very similar to the species *Eimeria tenella*, which infects chickens and causes a high level of mortality in young birds. *Eimeria stiedae* is also harmful and can cause lesions in the rabbit host liver.

It is possible to observe the following stages in the liver section: schizont, macrogametocyte or microgametocyte, and unsporulated oocyst. Of these, which do you see in this field of view (list them)?_______________________________________.

Answer the following two questions based on what you know about the life cycles of *Eimeria* species,:

Which stages of *E. stiedae* could occur inside liver epithelial cells?____________________

Which stages of *E. stiedae* could occur on the outside of the cells (i.e., free within the bile duct)?________________________

3. *Eimeria tenella.* Section through chicken cecum with various life cycle stages. *Slide box slide* 13. Text page 129 & attached Figs. 1 and 2. You should be able to locate each of the following stages. Note that you may draw these all together on a single page:

***3A. Schizont**. Find a schizont within an epithelial cell of the cecum section you are viewing. Identify the merozoites within the schizont as well as the epithelial cell and epithelial cell nucleus.

***3B. Macrogametocyte.** Locate a mature macrogametocyte within an epithelial cell. Identify the macrogametocyte, peripheral bodies, epithelial cell, and epithelial cell nucleus.

***3C. Microgametocyte.** Locate a mature microgametocyte within an epithelial cell. Identify the microgametocyte, epithelial cell, and epithelial cell nucleus.

***3D. Unsporulated oocyst.** Find a mature, unsporulated oocyst. Be sure to identify the oocyst and oocyst wall. Note that some oocysts are within epithelial cells, while some are free beyond the epithelial cells of the section you are viewing. Each oocyst breaks free from its epithelial cell, enters the lumen of the cecum and eventually pass to the outside via the feces of the host. Why wouldn't you expect to find sporulated oocysts on the slide of *E. tenella* in a chicken cecum?___________________________________

4. *Toxoplasma gondii.* Tachyzoites. Demonstration. See text page 134 Fig. 8.14 for examples of tachyzoites, and text page 133 for the life cycle. Tachyzoites are small, merozoite-like stages. Once known only from tissue stages in the brain and other organs, *Toxoplasma* was shown in 1970 to undergo typical coccidian merogony (=schizogony) and gametogony in the intestine of cats, and to produce oocysts in the feces. This demonstration slide is a tissue smear from the acute phase of an infection. Note the small size of these organisms.

***5. *Sarcocystis tenella.* Zoitocysts in sheep muscle.** *Slide box slide* 14. See text page 138 Fig. 8.18 for the life-cycle of another species of *Sarcocystis*. The life cycles of species of *Sarcocystis* are now known to include 2 vertebrates: a herbivore and a carnivore. These slides show zoitocysts typically known as "rice grain" cysts from the intermediate host. Note that this stage is large enough to see with the unaided eye, unlike other protist stages you have seen in lab to date! It should be drawn at your lowest magnification. The life cycle of members of this genus was not completely known until 1972.

What life cycle stage is found inside these zoitocysts?_____________________________

What sort of animal might ingest a zoitocyst?__________________________________

6. *Cryptosporidium parvum.* Smear. Demonstration. Text page 123 Fig. 8.4. Species in this genus parasitize the brush border of the epithelial cells of the gut. Note the tiny size of these organisms. This species infects humans, and is especially problematic in immune-compromised individuals, and in children. This slide contains oocysts.

Subclass Gregarinasina – gregarines. The mature gamonts (gametocytes) of this group dwell within extra-cellular sites within their hosts. They are generally large and most possess a conoid modified into a mucron or epimerite for attachment to host cells. Schizogony does not take place in the gregarine life cycle. Gregarines do typically undergo syzygy, or the pairing of gametocytes, during the course of sexual reproduction. The paired gametocytes of many gregarines are surrounded by a protective gametocyst. All species are monoxenous parasites of invertebrates, typically occupying the digestive tract, body cavity or seminal vesicles.

We should have an opportunity to observe living gregarines later in the course. There are potentially many undiscovered species of gregarines, even locally!

7. *Monocystis lumbricus*. Oocysts or spores. Demonstration slide-smear from earthworm seminal vesicle. See text page 121 Fig. 8.2 for the life cycle. This is an example of an acephaline gregarine. Its body consists of a single unit. This slide contains a gametocyst containing many oocysts, each of which contains 8 sporozoites.

What reproductive process is present in *Eimeria* but is lacking in *Monocystis*?

____

8. *Gregarina* sp. Trophozoites (=trophonts). *Slide box slide* 15-smears from damselfly gut. See attached Fig. 3 & text page 122 Fig. 8.3. This is a cephaline gregarine. Identify the epimerite, protomerite, and the deutomerite containing the nucleus in each individual. Why do you suppose gregarines are so diverse?_________________________

Practice Quiz:

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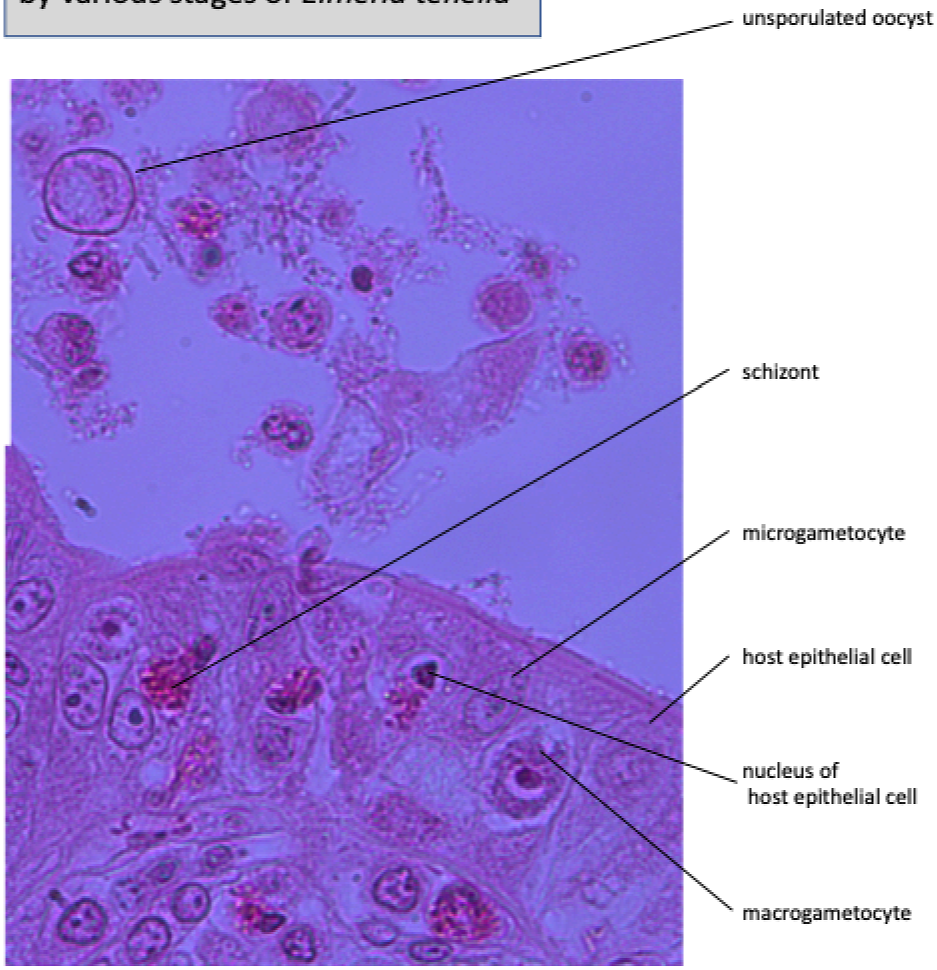
Figure 2. Histological section of chicken cecum showing cecum epithelial cells that are parasitized by various stages of *Eimeria tenella*



Laboratory #6: Cryptosporidium Outbreak

Today we will read a case study of an outbreak of *Cryptosporidium parvum* that occurred in Wisconsin in 1993. After reading the paper you need to discuss the following questions with your neighbors and complete this lab handout.

1. What specific events led the Milwaukee Department of Health to suspect there was a widespread health problem in Milwaukee?
2. Summarize the methods that were used in this study. **Be specific.**
3. What is the difference between laboratory-confirmed *Cryptosporidium* infection and clinical confirmed infections?
4. What specific information was obtained from the people interviewed by telephone?
5. What other disease-causing agents, besides *Cryptosporidium*, were identified in stool samples?
6. What other clinical characteristics, besides diarrhea, were associated with cryptosporidiosis? (Hint, look in Table I)
7. Which people were most likely to experience severe effects of cryptosporidiosis? In other words, who was especially vulnerable to infection?
8. What was the mean number of watery stools passed by individuals with clinically-determined Cryptosporidiosis each day? **See Table 1.**
9. How did the authors of this study estimate magnitude of outbreaks (total number of cases)? What was the estimated magnitude? Show the numbers and the parts of the calculation.
10. Why did it take so long for the community in Milwaukee to recognize *Cryptosporidium* infection as the cause of the outbreak?
11. What went wrong at the southern Milwaukee Water Works plant?
12. What may have been the source of this *Cryptosporidium* outbreak?

Laboratory #7: Platyhelminthes I

Phylum Platyhelminthes — flatworms

Members of this phylum are dorso-ventrally flattened. They are also bilaterally symmetrical and lack a body cavity (i.e., they are acoelomates). The majority of species lack an anus, rather, they have a blind gut and thus food does not flow uni-directionally through their body. Instead, food enters and solid wastes exit through the mouth (if present). Most species are hermaphroditic.

This phylum consists of both free-living and parasitic species. *Dugesia*, also known as planaria, is an example of a free-living species that is often used in introductory biology and zoology courses.

"turbellarians" Turbellarians are not a monophyletic group, rather, they represent several early branches in the evolutionary history (phylogeny) of Phylum Platyhelminthes. This assemblage consists of the mainly free-living predator (i.e., non-parasitic) species of the Phylum Platyhelminths. Some "tubellarians" live in symbiosis as commensals. Members of this assemblage lack a neodermis.

1. Temnocephala species. Adult. Demonstration. (See text pg. 197 and Fig. 13.5 on pg. 198). This specimen was collected in southeaster Peru, from the outside of an amphipod in a tributary of the Amazon river. It is a species with several tentacles around the oral end (and hence referred to by some as the "Hamburger helper worm"). This unidentified, and perhaps new, species most likely uses the crustacean simply as a substrate for attachment, and uses its mouth to feed on smaller organisms or detritus.

Subphylum Neodermata

The four major groups of platyhelminths that parasitize vertebrates (Aspidogastrea, Digenea, Monogenea, and Cestoda) form a cohesive group, which is considered more derived than free-living species of platyhelminths. These four groups are considered to be monophyletic, and are collectively known as the **Subphylum Neodermata**, because of their possession of a neodermis (see below).

All species of this subphylum are obligate parasites at some point in their life cycles. In early developmental stages, members of this group possess an outer body layer in the form of a normal epidermis, i.e., a single layer of ciliated cells. However, when their first host is encountered, the ciliated epidermis is shed and replaced with a syncytial layer called the **neodermis**. A neodermis is considered **syncytial** because the cytoplasm of the cells in this layer is not separated by cell membranes—rather, it is continuous throughout the entire surface of the animal. The nuclei of these cells are located below the outer muscle layers. **See texbook page 309**.

References:

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Rieger, R. M. 1998. 100 years of research on "Turbellaria" Hydrobiologia 383: 1-27.

Class Trematoda

There are 2 classes of neodermatans. Members of **Class Trematoda** generally possess a ventral, muscular sucker, which is sometimes subdivided into loculi.

Subclass Aspidogastrea

Species in this group parasitize molluscs, or occasionally fishes or turtles. The key feature is a large, ventral adhesive disk that is subdivided by muscular septa into individual chambers, known as loculi.

2. *Aspidogaster conchicola*. Adult. Demonstration. From pericardial cavity of a freshwater clam (Text pg. 204 Fig. 14.6). Be sure to recognize

the massive, ventrally located adhesive disk, as well as the buccal funnel, pharynx, tubular gut, and single ovary and testis.

Does this stage of this animal possess a neodermis?________________________

Is this animal monoecious or dioecious?_______________________________

Subclass Digenea

The Digenea are commonly known as **flukes**. They generally use two or more hosts in their life cycles. Almost all digeneans use a **mollusc** as their first intermediate host. Definitive hosts are generally vertebrates, but there are some exceptions in which invertebrates, such as arthropods, serve as definitive hosts.

Digeneans are characterized by their possession of a series of unique larval forms including: **miracidium, sporocyst, redia, cercaria, and metacercaria**. The digeneans consist of thousands of species and >2500 genera, all of which are parasitic. In the first portion of this lab you will be introduced to the various larval life cycle stages of the flukes.

1st part of lab: Larval forms

The following entries serve as examples of typical digenean life cycle stages, in order. There are, however, a number of exceptions to this generalized theme, in which certain stages have more than one generation, or, are lacking.

Do not worry about species identities for this portion of the lab; learn the stages themselves, and their features, so that you have a general understanding of each stage as part of the digenean life cycle:

***3. Egg (same as #13).** *Slide box slide 16*. (see text 259 Fig. 17.6). This is an egg of the species *Fasciola hepatica*. Note the operculum at one end of the egg. Be sure to label in your drawing: egg shell, operculum, and developing miracidium.

What function does the operculum serve?________________________________

What life cycle stage came before this one?_____________________

What life cycle stage follows this one?_____________________

***4. Miracidium.** *Slide box slide 17*. (see examples in Figures in text pg. 221). This is a miracidium of *Fasciola hepatica*. The miracidium is one of the digenean life cycle stages that are usually free swimming.

Do you see cilia? ___________Eyespots? _______________An apical organ?____________. Look in the posterior half of the specimen and locate individual germ balls. What will each germ ball become (hint, think of what stage comes *two stages* after this one)?____________________. Does this miracidium possess a neodermis?______________

***5. Sporocyst.** *Slide box slide 18*. (see figure in text pg. 222). These are sporocysts of an identified species of trematode collected from the snail *Physella heterostropha* as part of a student research project in my lab. Sporocysts are essentially sacs that contain many individuals of the next generation within.

What stage preceded (came before) this one?___________________

Examine the sporocysts with the microscope. What stage is visible inside each sporocyst?_____________________________

***6. Redia.** *Slide box slide 19*. (see figure in text pg. 222). These are redia of *Cryptocotyle lingua*. Note the mouth and obvious pharynx in this life cycle stage. Like sporocysts, redia are essentially sacs that contain many individuals of the next life cycle stage developing within.

What life cycle stage is developing within these redia?__________________________

In what kind of host animal would you seek redia?_____________________________

***7. Cercaria.** *Slide box slide 20*. (see text pg. 224 Fig. 15.24m). Cercaria are diverse in form and are therefore classified in terms of their body types. Examples of cercarial types are shown in Fig. 15.24 in your text. This is a cercaria of *Schistosoma mansoni*. Schistosome cercaria are termed apharyngeate furcocercous cercaria, i.e., they lack a pharynx, and have a forked tail.

Based on the morphology of this cercarial type, how do you think it encounters the next host in its life cycle (crawling or swimming)?_____________________________________

***8. Metacercaria.** *Slide box slide 21*. This is a metacercaria of *Clinostomum marginatum* (or "Yellow grub", see figure) from muscle of a Pumpkinseed sunfish. This is a metacercaria that is commonly found in local fish of economic importance (e.g., Largemouth bass). Metacercaria of *C. marginatum* can encyst but they are commonly found freely creeping about the body cavity of the fish, or in the muscle, or on the fins. Metacercaria of other digenean species are nearly always enclosed in a bag-like cyst (see attached Fig. 5). In any case, these specimens were mounted after having been removed from the cyst. For this species, fish are considered the second intermediate host. Herons serve as the definitive hosts, while certain snail species serve as the first intermediate host. This is essentially a juvenile adult, differing from the adult only in that its reproductive system is undeveloped.

How might metacercaria differ anatomically from adult specimens of *Clinostomum marginatum*?____

Examine the figure of Yellow grub, and the other metacercaria shown in the figure. Is your specimens of Yellow grub an encysted, or unencysted, metacercaria?____________

2nd part of lab: Liver flukes

In the remainder of this lab, and in lab next week, you will become familiar with adult specimens of some of the digenean species of veterinary or medical significance.

You need to be able to distinguish the adult specimens to species, as well as understand and recognize the major components of their morphology. Study the morphology of each adult example. Note that overall size and shape of the worm can be helpful. Bear in mind that many of the species of this highly diverse group are parasites of other animals, including local wildlife. Also remember that every species we discuss utilizes a **snail** as the first intermediate host.

The following group of digenean species you will examine in this lab are usually found associated with the **liver** (or gall bladder) of their definitive hosts. **You should be able to identify each of these species by the end of the lab.** The families to which these species belong are listed, but you are not required to learn them.

Family Opisthorchidae

9. *Clonorchis sinensis* adult.** *Slide box slide* 22. Note that this species is referred to as ***Opisthorchis sinensis in some texts. (see text Fig. 18.18 pg. 276). This species is commonly known as the **Chinese liver fluke**. It is the etiological agent of "Clonorchiasis."

Identification of digenean species rely on recognition of the morphological features of the adult worms, thus it is important that you understand the various structures of a typical digenean. See the morphology section of this species in your texbook for a helpful overview.

Make a full-page illustration of the adult of *C. sinensis*, and label the following structures: oral sucker, acetabulum (=ventral sucker), pharynx, esophagus, intestine, testes (there are 2, and are dendritic, or branched), seminal vesicle (hard to see and not required to draw), genital pore, uterus, eggs, ovary, seminal receptacle, vitelline glands, and Laurer's canal. This plate will serve as a reference for examination of other species of digeneans.

What is the function of the vitellaria?____

Is this specimen monoecious or dioecious?_______________________________________

How does this specimen void undigested matter?________________________________

Does this specimen possess a neodermis?____________________________________

Does this specimen possess cilia on its outside surface?___________________

10. *Clonorchis sinensis* eggs. Demonstration. (see text pg. 278 figure 18.20). Eggs of this species are distinguished based on their small size, and the presence of a small knob at the abopercular (i.e., end opposite the operculum)

How many microns long is the egg you viewed?___________________________________

11. *Clonorchis sinensis* section of adult worms in liver tissue. Demonstration. **(**see text figs. on pg. 279**)**. Note the tissue reaction associated with this parasite.

What do you suppose this specimen eats within its specific habitat?___________________

Family Fasciolidae

12. *Fasciola hepatica* adult. (*Slide box slide* 23). (see text pg. 256) Please view with a dissecting microscope **AND BE CAREFUL WITH THIS VALUABLE SLIDE**! This is the "sheep liver fluke." These specimens were collected from the liver of either a sheep or a cow. This species is the etiological agent of "Fascioliasis", which can result in gross pathology of the liver. Note the extensively branched ceca of this species. The two testes, arranged in tandem, are also branched. Also note the oral cone giving the animal an appearance of possessing "shoulders" and the anterior position of the acetabulum (ventral sucker).

Do you see evidence of spines on the surface of this species?__________Can you distinguish the two lateral fields of follicular vitellaria from the testes?________________ How do adults get to the liver?_______________How do adults of this species differ from those of *Clinorchis sinensis*?______________________

***13. *Fasciola hepatica* eggs.** (*Slide box slide 16 in boxes* 1-14). (see text pg. 259) View an example of an egg of *F. hepatica*. Note the operculum and lack of a conspicuous abopercular knob or thickening in this species.

How does this egg differ from that of *Clonorchis sinensis*?_______________________

How many microns long is the egg you viewed?_____

14. *Fasciola hepatica* cercaria. Demonstration. (see text pg. 257). This cercaria drops its tail and encysts on aquatic vegetation if encountered. If not, the cercaria merely encysts in the water. The definitive host is infected either by eating aquatic vegetation, or by drinking water containing metacercaria.

How does this aspect of the life cycle of this species differ from the life cycle of the oriental liver fluke, C. *sinensis*? (Hint compare text Figs. 17.5 and18.19):_____________

15. *Fasciolopsis buski* adult. Demonstration. (see text pg. 260). Although this species is closely related to *Fasciola hepatica*, it is NOT a liver fluke, rather it inhabits the small intestine of its definitive host. Note that the ventral sucker is larger than the oral sucker. Also note the unbranched ceca and dendritic (branched) testes in the posterior half of the worm.

How does this species differ from morphologically from *F. hepatica*? ___________

What is the definitive host of this species?___________________________________

Family Dicrocoelidae

16. *Dicrocoelium dendriticum* or (*D. lanceatum*) adult. Demonstration. (see text pg. 266). Compare the morphology of this species with that of other adult worms you have examined, and in particular with C. *sinensis* to which it is most similar in terms of size and shape. The natural definitive hosts of this species include sheep, goats, cattle, pigs, deer, marmots and rabbits.

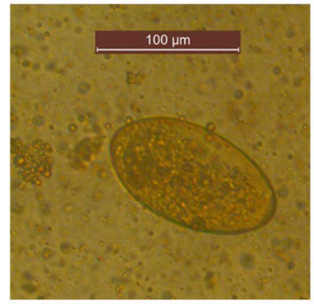
In what kind of first intermediate host would you expect to find this fluke? ___________

Do the cercaria of this species actively burrow out of the first intermediate host? ____

How does the life cycle of this species exemplify a parasite's ability to alter the behavior of its host to the parasite's advantage?____

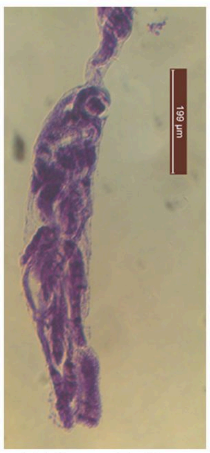
L7 Entry 1 Temnocephala species adult

L7 Entry 2 *Aspidogaster conchicola* adult



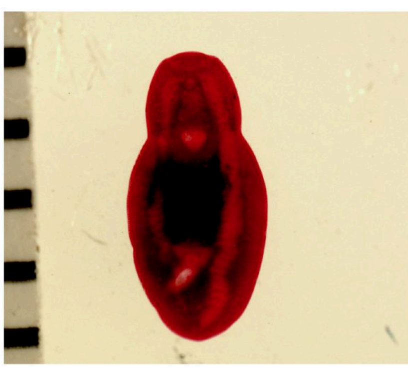
L7 Entry 3 digenean egg (it's *Fasciola hepatica*)

L7 Entry 4 digenean miracidium

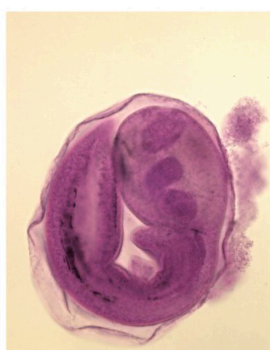


L7 Entry 5 digenean sporocyst

L7 Entry 6 digenean redia

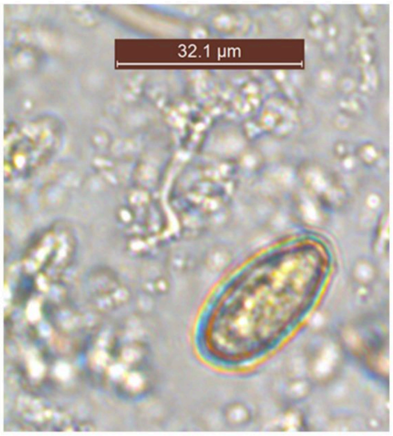


L7 Entry 8 digenean metacercaria, *Clinostomum marginatum,* or "yelllow grub"

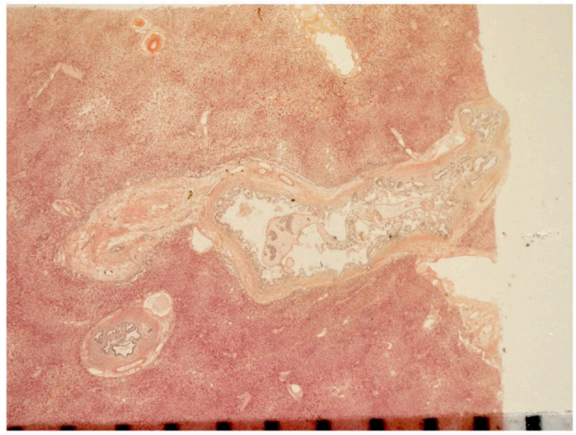


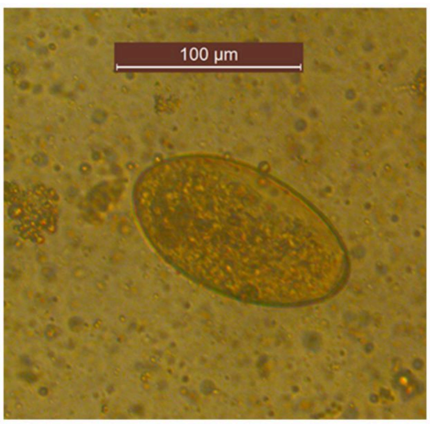
An encysted metacercaria of another digenean species, also from a fish

L7 Entry 9 *Clonorchis sinensis* adult, mm ruler



L7 Entry 10 *Clonorchis sinensis* egg


L7 Entry 11 *Clonorchis sinensis* in liver tissue, mm ruler

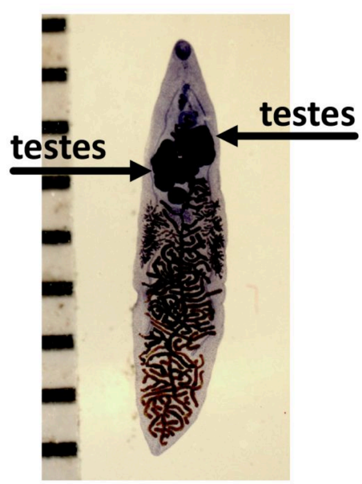


L7 Entry 13 *Fasciola hepatica* egg

L7 Entry 14 *Fasciola hepatica* cercaria

L7 Entry 12 *Fasciola hepatica* adult, mm ruler


L7 Entry 15 *Fasciolopsis buski* adult, mm ruler



L7 Entry 16 *Dicrocoelium lanceatum,* mm ruler

Laboratory #8: Platyhelminthes II (lung fluke, blood flukes, and flukes of wildlife)

Phylum Platyhelminthes — flatworms

Subphylum Neodermata

Class Trematoda

Subclass Aspidogastrea

Subclass Digenea

Last time you were introduced to digenean life cycle stages and the general morphology of an adult digene, and you also observed liver flukes. Now you will become familiar with lung flukes and blood flukes, which are some of the most detrimental parasites known. Bear in mind that many of the species of this highly diverse group are parasites of other animals. Also keep in mind that nearly every species we discuss utilizes a snail as the first intermediate host.

Lung fluke

The lung flukes are usually found associated with the lungs of their definitive hosts. Our lung fluke example is *Paragonimus westermani*. Lung flukes can be found in a variety of hosts, such as a species in bullfrogs that they acquire by consuming dragonflies.

1. *Paragonimus westermani* eggs. Demonstration. Fecal smear. (see text pg. 271). Note the distinctive shape of this egg as well as the operculum and characteristic "shoulders" at the junction of the operculum with the rest of the shell.

How large is this egg?______________________________
Recognize these structures: operculum, shell, and shoulders.

How do eggs of this species leave the body of the definitive host? ___________________

How would you distinguish eggs of this species from those of C. *sinensis* or F. *hepatica*?____

2. *Paragonimus westermani* in lung tissue section. Demonstration. (see life cycle on text pg. 270). Worms inside the lung stimulate an inflammatory response that ultimately results in the worms becoming enshrouded in a capsule of scar tissue. Study the section for eggs and worm fragments of P. *westermani*

Will eggs produced earlier or later be more likely to be transmitted to the next host in the life cycle? Explain:____

3. *Paragonimus westermani* adult. Demonstration. Whole mount. (see text pg. 269). **L**ung parasite is the etiological agent of paragonimiasis in humans. This is a good example of a zoonosis; P. *westermani* typically parasitizes wild animals—especially cats—but causes disease when it infects humans. Observe the lobed testes and ovary, which is slightly off the center of the body. There is a short uterus and suckers are inconspicuous. The overall size and shape of this specimen is similar to a coffee bean.

What are the intermediate hosts in the life cycle of *Paragonimus westermani*?____

Blood flukes

Family Schistosomatidae

These kinds of flukes parasitize the circulatory system of their definitive host. The schistosomatids parasitize warm-blooded definitive hosts while other families of blood flukes parasitize cold-blooded definitive hosts, such as fish, elasmobranchs, or reptiles.

All species in this family are unusual for digeneans in that they are dioecious. In addition, members of this family are unusual in that they lack a metacercarial stage in their life cycle, rather, cercaria locate, and subsequently penetrate, the definitive host directly. Three species in this family are notoriously important parasites of humans. Recent work suggests that several other species may also regularly parasitize humans, but their geographic distribution and prevalence is much lower than the 3 species we will view.

Many other species in this group parasitize birds or other groups of mammals. These schistosome species are responsible for a condition in humans known as "swimmer's itch" or "clam digger's itch," when cercaria of these species attempt to penetrate the skin of unsuitable (human) hosts.

Human schistosomes

Schistosoma mansoni adults Text pages 238-249 for full coverage; for today focus on the first three pages. Examine separate slides of males and females, as well as a slide of the 2 sexes together en copula. Make a plate of the worms en copula.

4A. Adult male. Demonstration. Locate oral sucker and acetabulum (ventral sucker).

Do you see an intestine?_____________________________________

4B. Adult female. Demonstration. Compare the female to the male and note the difference in morphology between the 2 sexes.

Which sex is more robust? _____________________________

Which is slighter?____________________________________

***4C. En copula.** *Slide box slide #24.* Note the oral suckers and acetabulum of both individuals. Also note the female's position in the **gynecophoral canal**. Label any other structures you can recognize, and take special care to label which worm is male and which is female.

Can you see a feature of the male's outer body that would enable it to maintain its position in its habitat? ____

If so, what is the feature? ____

Does the female also have this feature?____________________________________

5. *Schistosoma mansoni* adults and eggs in section of liver tissue. Demonstration. (see text pg. 246 fig. 16.16). It is important to keep in mind that there are two sources pathology associated with schistosomiasis, adult worms, and the eggs they produce. Look for evidence of **pseudotubercles**, a type of granuloma caused by *Schistosoma* eggs.

How can you recognize these eggs as *Schistosoma mansoni*? You may wish to wait until after completing entry #7 to answer this.____

6. *Schistosoma mansoni* cercaria. *Slide box slide 20*. (see text pg. 241). You examined these last week. This is a typical schistosome cercaria: it lacks a pharynx, and has a forked tail.

How would this specimen locate the next host in its life cycle?___________________

Look on page 242 for images of the following 3 egg species:

***7. *Schistosoma mansoni* eggs.** Fecal smear. Slide tray. (see text pg. 242). Note the characteristic lateral spine on this egg.

What is the size of the egg?________________________________

Make a drawing and label: miracidium (if one is present and visible), shell, and lateral spine.

Do you see evidence of an operculum?_________________________________

How do miracidia exit these eggs? (Hint, see fig. in text pg. 243)___________________

By what means do these eggs exit the definitive host?__________________________

***8. *Schistosoma haematobium* eggs.** Urine smear. Slide tray. (see text pg. 242). Note the characteristic terminal spine on this egg.

What is the size of the egg?________________________________

Do you see evidence of a miracidium in any of these eggs?___________________

Make a drawing and label: miracidium (if one is present and visible), shell, and terminal spine.

By what means do these eggs of this species exit the definitive host?__________________

How does this egg differ from an egg of S. *mansoni*?______________________________

***9. *Schistosoma japonicum* eggs.** Fecal smear. Slide tray. (see text pg. 242). Note the characteristic rudimentary lateral spine. It is less prominent than the spine of S. *mansoni*. on this egg.

What is the size of the egg?___________________

Make a drawing and label: miracidium (if one is present and visible), shell, and lateral spine.

How does this egg differ from the previous 2 species?_____________________________

How do eggs of this species exit the definitive host?______________________________

Flukes of other animals

10. *Crepidostomum cooperi* from Largemouth bass intestine. Demonstration. This digenean species was collected from the Thayer Farm Big Pond at the Biological Field Station—the location of our field trip later this semester. The first intermediate host in the life cycle is *C. cooperi* is a fingernail clam. Cercaria emerge from clams until they find mayfly larvae (also aquatic). The life cycle is completed when a fish consumes an infected mayfly, which serves as the definitive host. Look at the specimen on demonstration. This species is characterized by numerous papillae surrounding the oral sucker.

What is different about the hosts in the life cycle of *C. cooperi*, compared to other digeneans we have discussed?_____________________________

11. *Clinostomummarginatum* (or "Yellow grub") metacercaria from muscle of a Pumpkinseed sunfish. Demonstration. This is a metacercaria that is commonly found in local fish of economic importance (e.g., Largemouth bass). Metacercaria of *C. marginatum* do not encyst, rather, they can be found freely creeping about the body cavity of the fish, or in the muscle, or on the fins. Fish are considered the second intermediate host. Herons serve as the definitive hosts, while certain snail species serve as the first intermediate host.

How might metacercaria differ from adult specimens of *C. marginatum*?____

What do you suppose would happen if you consumed metacercaria (e.g., imagine having largemouth bass sushi)?____

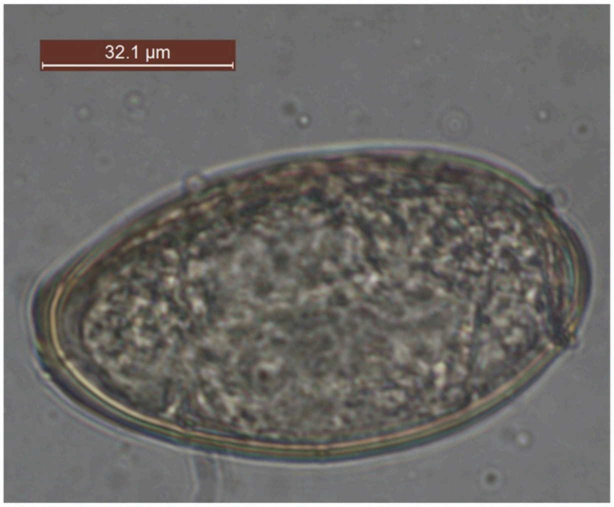
Can this parasite "travel" from lake to lake? How?_________________________________

12. "Black grub" on external surface of a minnow. The visible black spots are not actually grubs; they are dead metacercaria. We cannot know the species identity of these parasites because they are immature worms and because they are largely destroyed by the host immune response. That is, there are a variety of trematode species that could be responsible for "black grub" in fish.

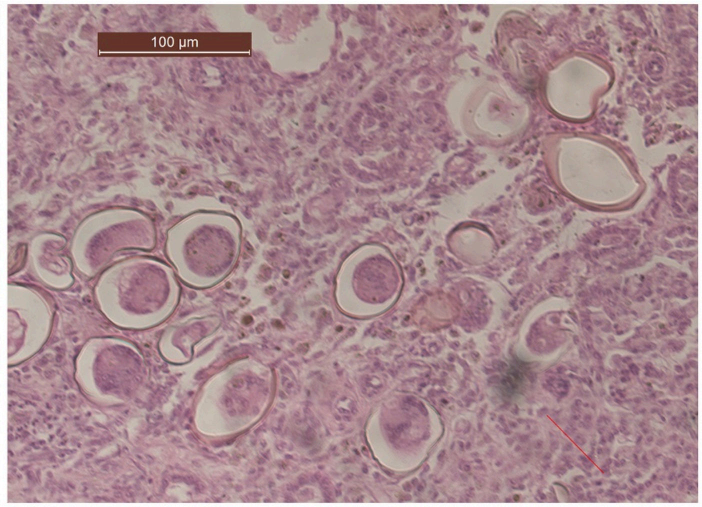
What is the term for the host role that this fish plays in the life cycle of the "black grubs" visible here?______________________________

13. *Haematoloechus longiplexus* from lung of a bullfrog. Demonstration. (see similar species in Fig. 18.4 in text pg. 268). This adult specimen was collected from a bullfrog lung, in Nebraska. *Haematoloechus* species are lung flukes, i.e., they live and mate as adults in the lung tissue of frogs. First and second intermediate hosts in the life cycle are snails and dragonflies, respectively. Dragonflies acquire cercaria via jet-like propulsion into their anal gills. Frogs acquire worms when preying on dragonflies. We have collected specimens of *Haematoloechus* from all three hosts in the life cycle, at a tiny mucky pond at the Biological Field Station.

What might be the food item of adult *H. longiplexus*?_____________________________

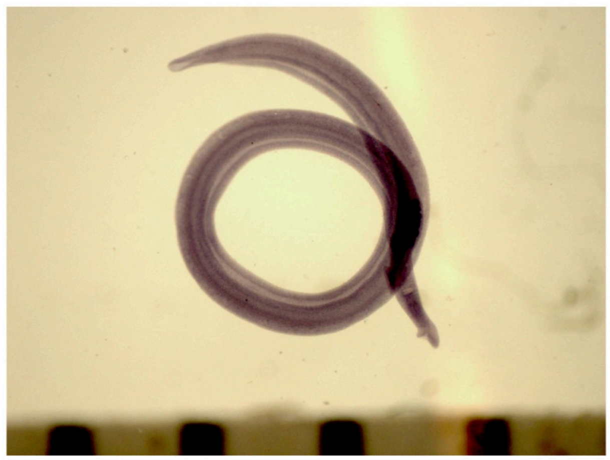


L8 Entry 1 *Paragonimus westermani* egg

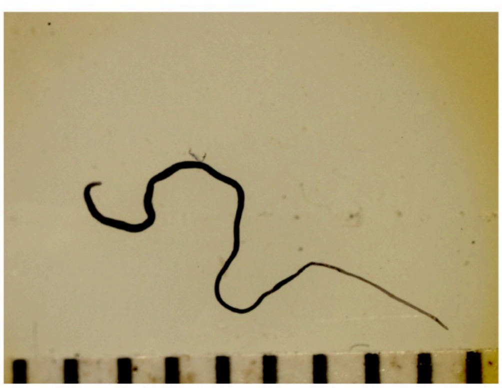


L8 Entry 2 *Paragonimus westermani* eggs, lung tissue

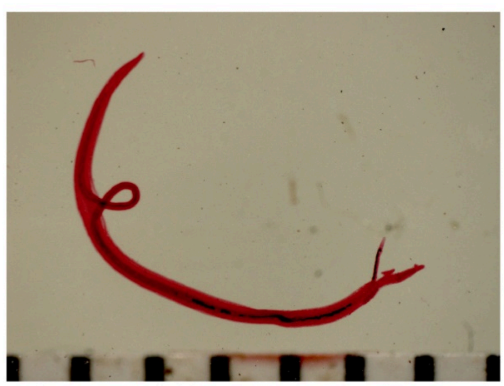
L8 Entry 3 *Paragonimus westermani* adult, mm ruler



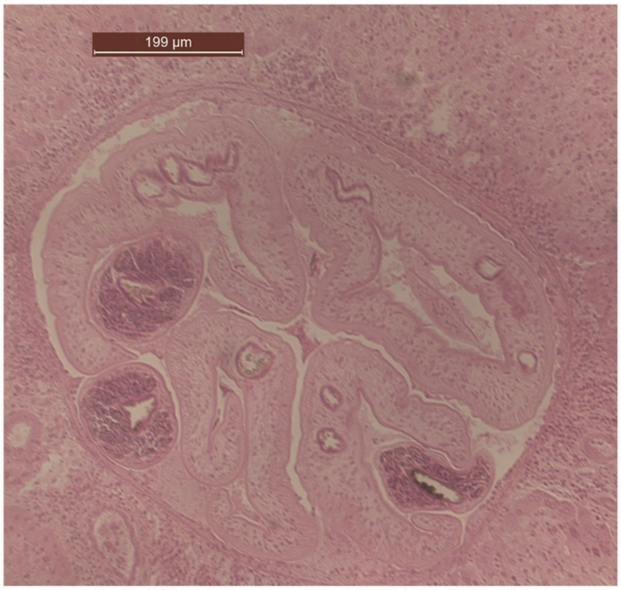
L8 Entry 4a *Schistosoma mansoni* adult, male, mm ruler



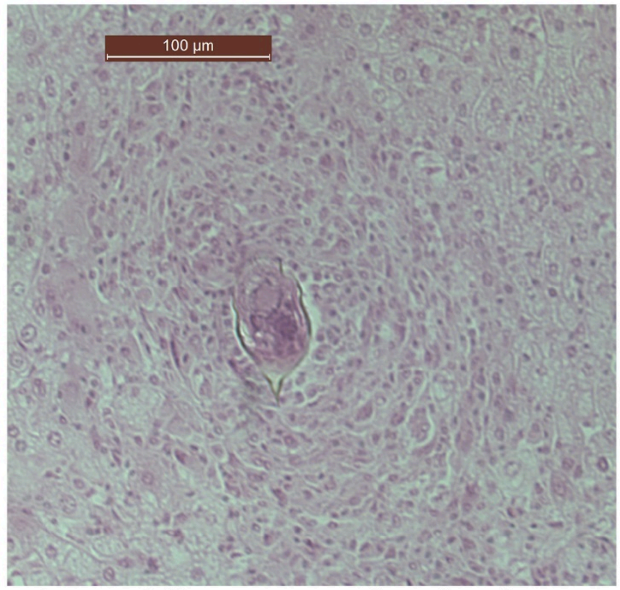
L8 Entry 4b *Schistosoma mansoni* adult female, mm ruler



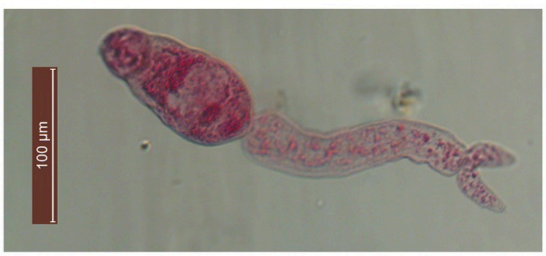
L8 Entry 4c *Schistosoma mansoni* en copula, mm ruler



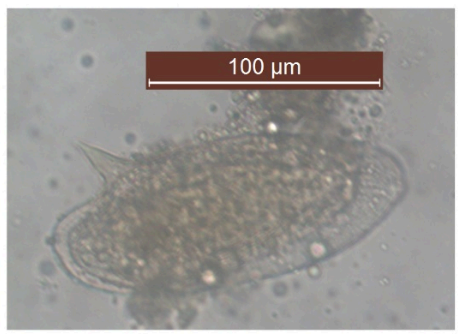
L8 Entry 5 *Schistosoma mansoni* adults, liver tissue



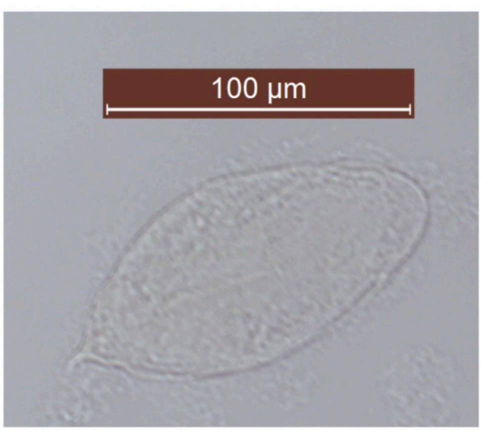
L8 Entry 5 *Schistosoma mansoni* egg, liver tissue



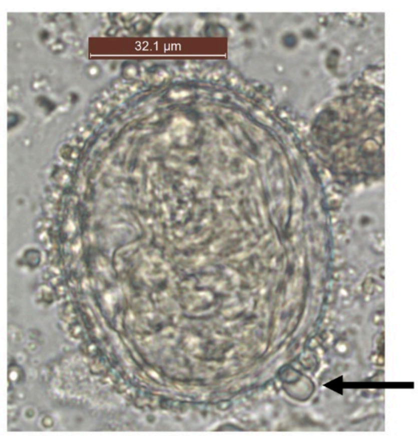
L8 Entry 6 *Schistosoma mansoni* cercaria



L8 Entry 7 *Schistosoma mansoni* egg

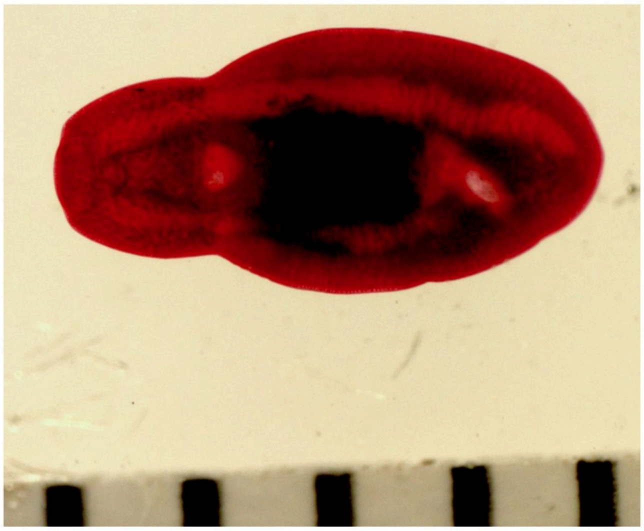


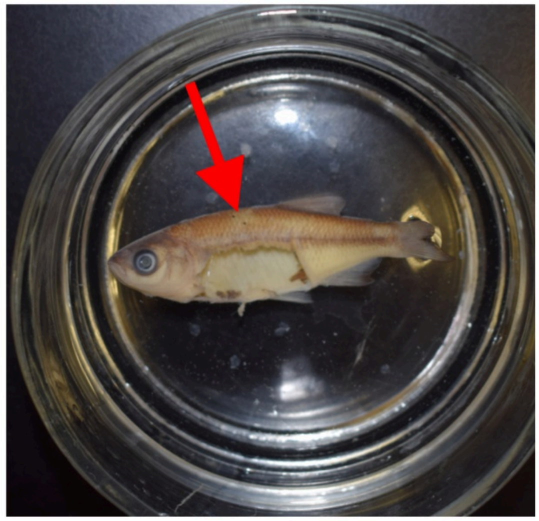
L8 Entry 8 *Schistosoma haematobium* egg



L8 Entry 9 *Schistosoma japonicum* egg

L8 Entry 10 *Crepidostomum cooperi* adult


L8 Entry 11 yellow grub metacercaria, mm ruler


L8 Entry 12 Black grub metacercaria, near tip of arrow


L8 Entry 13 *Haematoloechus longiplexus* adult, mm ruler

Laboratory #9: Platyhelminthes III (Monogeneans and cestodes)

Phylum Platyhelminthes — flatworms

Subphylum Neodermata

Class Trematoda

Class Cercomeromorpha

Subclass Monogenea

Subclass Cestoda

Class Cercomeromorpha

Species belonging to this class possess an extension of the body known as the "cercomer". The cercomer typically bears 3 or more pairs of hooks. This feature is present in both larval and adult stages of monogeneans, but only in larval cestodes.

I. Subclass Monogenea

Species of this group are typically ectoparasitic, and have monoxenous life cycles. They are typically found parasitizing the skin and gills of fishes. A few species parasitize amphibians and reptiles, and one species, O*culatrema hippopotami*, is a parasite on the eyes of hippopotamuses. The monogeneans have a cercomer (as a haptor) in the adult stage, and eyespots in the larval stage, known as the oncomiracidium.

1. *Actinocleidus* sp. whole mount. Demonstration. See textbook page 292. This serves as an example of a locally collected monogenean species. This specimen was obtained from the gills of a Smallmouth bass in Otsego Lake, New York. Compare this specimen to Figure 2.

What part of the fish do you suppose serve as attachment points for the hooks that are visible in the haptor of this specimen?____

How many hosts are in the life cycle of *Actinocleidus* sp.?___________________________

2. *Microcotyle* sp. whole mount. Slide tray. **MUSEUM SPECIMENS: TREAT WITH A HIGH LEVEL OF RESPECT AND CAUTION!** This worm was collected from the gills or skin of a Quillback rockfish (*Sebastes maliger*), from the North Atlantic coast. This monogenean species belongs to a group within the Subclass Monogenea known as the Polyopisthocotylea. Polyopisthocotyleans possess a haptor that is subdivided into one or more pairs of suckers, and in this example, sclerotized attachment structures called clamps. Label the following in your drawing: haptor, clamps, testes, vitellaria, attachment organ, pharynx, gut, ovary, egg capsule, and vitelline duct.

II. Subclass Cestoda

Cestodes, or tapeworms, are endoparasites that typically occur in the gut (i.e., small intestine) of vertebrates during the adult stage. Cestodes have been reported from all classes of vertebrates but are most in common in fishes, birds, and mammals. Perhaps the most striking morphological feature of cestodes is the scolex, a holdfast organ at the anterior portion of the body. The scolex is highly variable among cestode species, and can include specialized suckers, and hooks in many cases. The cestode body consists of a chain of segments or proglottids, which each contain a complete set of male and female reproductive organs. Most cestodes produce proglottids near the posterior margin of the scolex, in a region often called the neck. The proglottids nearest the neck are immature (newest formed), and the proglottids mature as they are pushed away from the neck by the more newly formed proglottids. Mature proglottids (i.e., proglottids containing fully formed and functioning reproductive organs) are found towards the middle of the strobila. Gravid proglottids (proglottids filled with eggs) are found towards the posterior end of the strobila in tapeworms, except in species that drop proglottids. Cestodes drop their proglottids from their strobila at different points of maturity and it is not uncommon to find large numbers of individual free proglottids in a host. In general, cestodes require at least two host in order to complete their life cycles.

Cestodes also differ from other platyhelminths in that they lack a digestive system. Instead, tapeworms absorb nutrients across their neodermis, or tegument. In cestodes, the neodermis is covered with variable structures called microtriches (see textbook page 309).

You will learn some general features of the cestode body. While the proglottids of cestodes are very similar to the bodies of digeneans (i.e., a complete set of male and female reproductive structures are present), they do differ in several ways.

3. *Acanthobothrium larsoni* whole mount of adult. Demonstration. See figures in this manual and research article. This specimen was collected from the spiral intestine of a stingray off the coast of the Malaysian portion of the Island of Borneo. Observe the 4 bothridia of the scolex, which have muscular edges and septa that form distinct loculi. Each bothridium bears a pair of bipronged hooks. Note the relatively small size of this species. Look at the copy of the research article included in the lab. You'll see that in it I named this species after my college parasitology instructor, Dr. Ingemar Larson.

How many proglottids are present on this worm?__________________________________

Are all the proglottids mature? How can you tell?__________________________________

4. *Schizocotyle acheilognathi* whole mount of adult. Demonstration. This specimen was collected from a type of minnow called a golden shiner, from near Otsego Lake here in New York. This species is well-enough known to have a common name, the **Asian fish tapeworm**. Asian fish tapeworm has been reported from >200 species of fish hosts from around the world. It's originally from Asia–probably Japan–but has since been introduced to other continents by way of fish farming of food fish such as grass carp. This species is considered an invasive species because of its ability to switch to other fish hosts wherever it gets introduced. It has been reported from 20 states in the USA, including New York (see copy of published paper on its report in New York).

Describe as best you can the morphology of the scolex of this specimen:____

5. *Diphyllobothrium latum* whole mount of gravid proglottid of adult. *Slide box slide* # 25. See text Fig. 20.17 pg. 310 and Fig. 21.3 pg. 326. This species is commonly known as the broad fish tapeworm. The definitive host is usually a fish-eating carnivore, such as a polar bear or human. Note the lateral follicular vitellaria. In this species, eggs are released from the uterine pore of gravid proglottids while still attached to the stobila. Examine a gravid proglottid and locate the uterine pore.

How would you distinguish this proglottid from a proglottid of T. *pisiformis*?____

6. *Diphyllobothrium latum* whole mount of eggs. Demonstration. (See text pg. 326 Fig. 21.4). Study the representative egg. Recognize the following structures: hexacanth, shell, operculum, abopercular knob. The mature oncosphere of *Diphyllobothrium* possesses a ciliated layer and is thus called a coracidium larva.

Is this egg ready to "hatch" in the water? _________________________________

Why or why not?____

How would you distinguish eggs of this species from the eggs of digenean species you already examined?____

****7. *Taenia pisiformis.* Composite slide of adult worm.** *Slide box slide #26.* See textbook page 310 for diagram of mature proglottid. Adults of *T. pisiformis* occur primarily in dogs. Dogs become infected with *T. pisiformis* when they consume cottontail rabbits that are infected with the larval stage of the worm. Examine the scolex with its 4 suckers and rostellum bearing numerous hooks (see scolex of similar species on text pg. 333). Make an illustration of the scolex and label hooks and suckers. Adult worms consist of 100s of proglottids and can be quite long, too long to fit on a single slide. This slide therefore includes representative immature, mature, and gravid proglottids. Try and find each of the structures labeled in attached Figure 3, and make a full-page plate of a mature proglottid—**check to be certain you have chosen a mature proglottid before you invest time drawing!** Also compare the uterus in mature and gravid proglottids.

What is the function of the uterus?____

What is the function of the vitellarium?____

8. *Taenia pisiformis.* Composite slide of various portions of strobili & intact worm in dish. Demonstration. This partial worm was obtained from the vomit of "Popcorn", a domestic cat in Otego, New York. Observe the specimen with the dissecting microscope, as well as without the aid of a microscope, to gain a sense of its actual size. How do you suppose "Popcorn" acquired infection with this specimen?____

9. *Taenia solium* and *Taenia saginata* gravid proglottids. Demonstration. (See life cycle text pg. 334). Both of these species parasitize humans and possess eggs that are nearly identical. Both of these species also release detached gravid proglottids into the feces of their hosts. Thus, it is common to find gravid proglottids, rather than eggs, in the feces of individuals infected with either species of tapeworm. One of the most important features for distinguishing between infections of these two species is the number of uterine branches in the gravid proglottid. Compare the gravid proglottids of each of the two species.

Count the number of branches on the gravid uterus and specify below:

Taenia solium has _______ uterine branches.

Taenia saginata has _______ uterine branches.

10. *Taenia solium* or *T. saginata* egg. Demonstration. (See text pg. 331). This slide demonstrates an eff of a species of *Taenia* in which the outer envelope has been lost. While eggs of genus *Taenia* have a distinctive appearance, it is very difficult to distinguish between *Taenia solium* and *Taenia saginata* based only on the egg. This is why it's very important to use gravid proglottids to distinguish between these 2 species.

Imagine you are a clinician examining a patient who has either *Taenia solium* or *Taenia saginata*. Why would you want to be sure to correctly identify the *Taenia* to species?____

11. *Taenia solium* cysticercus. Whole mount. Demonstration. (See text figs. on pgs. 314 & 335). Humans can also host this ontogenetic stage by incidental consumption of eggs of *T. solium*.

What ontogenetic stage precedes this one in the life cycle of *T. solium*?_____________

What ontogenetic stage follows this one in the life cycle of *T. solium*?_____________

12. *Taenia solium* cysticercus-section in pig muscle. Demonstration. (See text figs. on pgs. 314 & 335). Cysticerci of this species are normally found in pork and are often referred to as "*Cysticercus cellulosae*". Humans who ingest this larval stage will serve as definitive host to the adult stage of this worm.

What practice, with regards to pork, would minimize risk of infection?_______________

13. *Dipylidium caninum* adult. Composite slide of adult worm. Demonstration. (see fig. in text pg. 343). Observe the proglottids, which are diagnostic in that they each possess two sets of male and female reproductive organs and two genital pores. The adults of *D. caninum* are common parasites of domestic dogs and cats and have occasionally been found in children. The larvae are simple cysticercoids that develop in fleas.

How do you suppose dogs become infected with *D. caninum*?____

14. *Monoecocestus* sp. from area porcupine. Demonstration. The cestode specimens in the bottle and on the slide were obtained from 2 roadkill porcupines from Otsego County, NY, near Oneonta. Both had dozens, if not hundreds of these cestodes in the small intestine. The intermediate host is a species of orobatid mite, a tiny free-living mite that lives in the grass. Mites become infected by ingesting eggs left in the grass when the porcupine defecates. This likely happens in summer, when ground vegetation is the predominant part of the porcupine diet. Porcupines are arboreal at other times.

How do you suppose porcupines become infected with these cestodes?____

***15. *Echinococcus granulosus* adult from intestine of dog or other carnivore.** Whole mount. *Slide box slide #27.* (See text pgs. 337-339). Adult individuals of this species are very small, consisting solely of a scolex, a short neck, and a total of only three proglottids. Adults are parasites of the intestinal tract of canids and felids.

How many proglottids are present in this specimen?________________________________

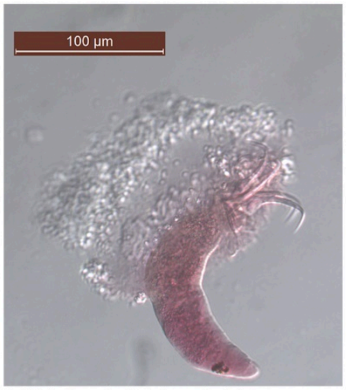
Does the scolex of this specimen more closely resemble *Acanthobothrium larsoni*, or *Taenia pisiformis*?_______________________________________

16. *Echinococcus granulosus* histological section of sheep muscle showing wall of unilocular hydatid cyst. Demonstration. See text pgs. 337-339. Larvae, in the form of unilocular hydatid cysts, are generally found in mammals such as sheep and caribou. Unilocular hydatid cysts are generally significantly larger than the adult worm; one record exists of a unilocular hydatid cyst with up to 40 liters of fluid! Humans become infected with unilocular hydatid cysts by consuming eggs of *E. granulosus*. The cyst consists of a fluid-filled bladder surrounded by a cyst wall that is lined with a germinative epithelium. This germinitive layer can produce several million prtoscoleces in a single common bladder. Budding within daughter cysts producing more protoscoleces is also possible. Protoscoleces that break free and sink to the bottom of a cyst are called hydatid sand. These scoleces are still infective to the intermediate or definitive host.

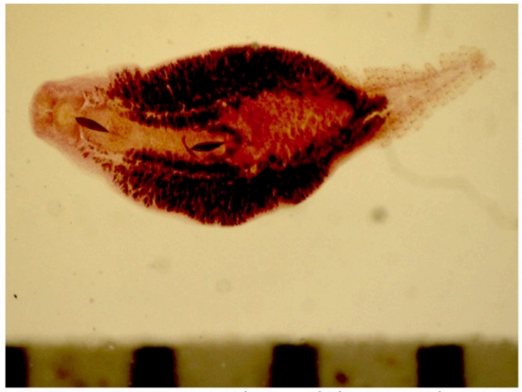
How does this larval stage differ from the cysticercus you saw of *T. solium*?____

17. Sheep liver with unilocular hydatid cysts of *Echinococcus granulosus*. Demonstration. This sheep liver is laden with many cysts of *E. granulosus*. Each of these contain thousands, or potentially millions, of protoscoleces that each could potentially develop into an adult worm, had this sheep (and its liver) been consumed by a carnivore such as a dog or a wolf. This demonstration should help you put into context your observations of #15, a section of one of these unilocular hydatid cysts. Remember that humans can serve as the intermediate host of *E. granulosus*. I.e., infected organs in humans, such as the liver or lungs, could take on a similar appearance as this example. Remember that humans cannot serve as the definitive host of this tapeworm species.

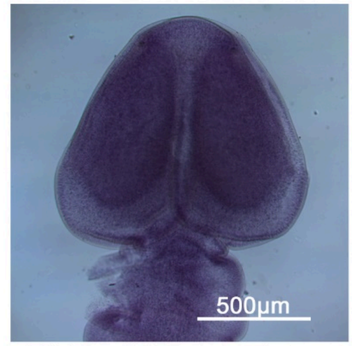
Based on diet, what type of animal do you think would consume the host of a unilocular hydatid cyst?_______________________________________



L9 Entry 1 *Actinocleidus* sp adult

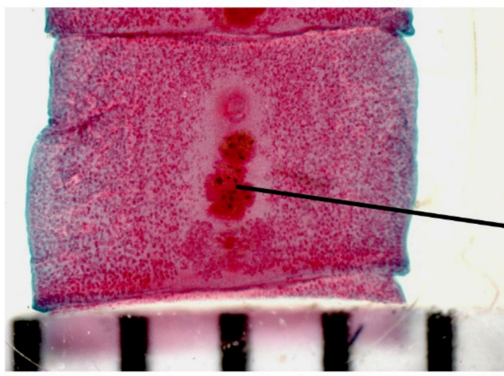


L9 Entry 2 *Microcotyle* sp adult, mm ruler

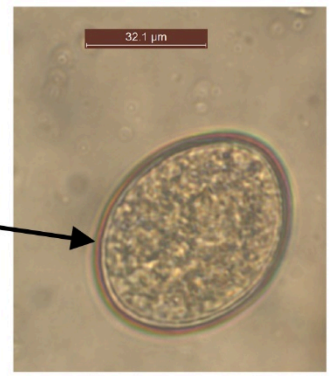


L9 Entry 4 *Schyzocotyle acheilognathi* (Asian fish tapeworm) scolex

L9 Entry 3 *Acanthobothrium larsoni* adult

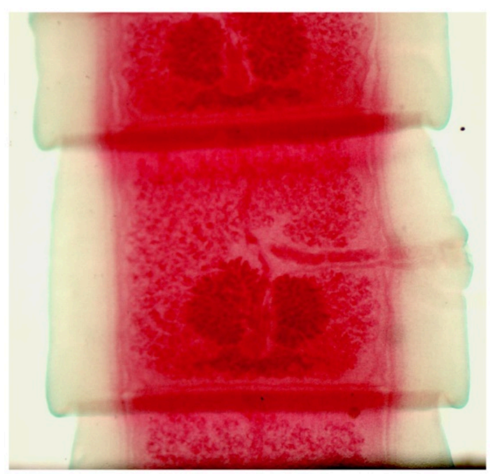


L9 Entry 5 Di*phyllobothrium latum* gravid proglottid

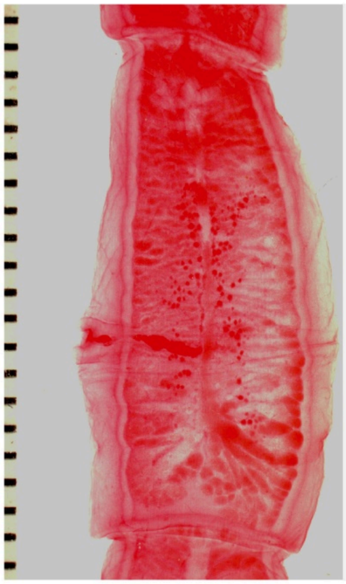


L9 Entry 6 *Diphyllobothrium latum* egg

L9 Entry 8 *Taenia pisiformis* scolex

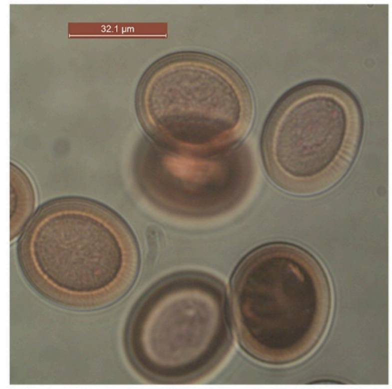


L9 Entry 8 *Taenia pisiformis* mature proglottid



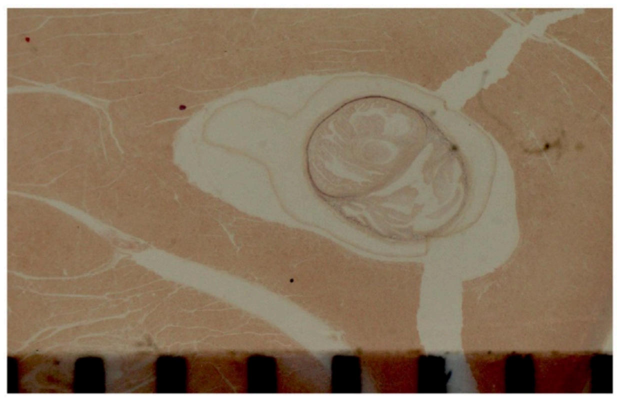
L9 Entry 9 *Taenia saginata* gravid proglottid, mm ruler

L9 Entry 9 *Taenia solium* gravid proglottid, mm ruler



L9 Entry 10 *Taenia* sp. egg, without outer membranes

L9 Entry 11 *Taenia solium* cysticercus, mm ruler

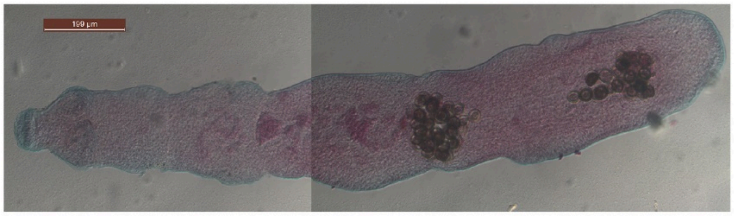


L9 Entry 12 *Taenia solium* cysticercus, pig muscle section, mm ruler

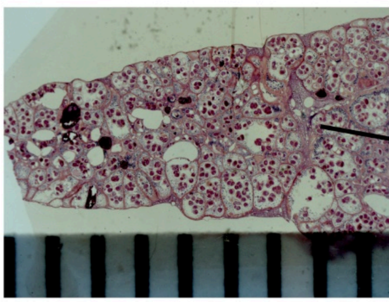
L9 Entry 13 *Dipylidium caninum* scolex

L9 Entry 13 *Dipylidium caninum* mature proglottid

L9 Entry 14 *Monoecocestus* sp. adult mm ruler



L9 Entry 15 *Echinococcus granulosus* adult



L9 Entry 16 *Echinococcus granulosus* hydatid cyst in sheep muscle section, mm ruler

L9 Entry 16 *Echinococcus granulosus* hydatid cyst sheep muscle section, high magnification

L9 Entry 17 *Echinococcus granulosus* hydatid cysts visible in sheep liver

Laboratory #10: Nematoda I

Phylum Nematoda

Many nematodes occur as free-living animals in the sea, freshwater, and soil, but there are thousands of species that are parasitic in plants and animals. Human diseases caused by nematodes include Onchocerciasis, Filariasis, Dracunculiasis, Ascariasis and hookworm disease. These are more common in tropical countries, especially in overpopulated areas where sanitation is a problem. But, even in countries like the United States, pinworms flourish among children of rich and poor alike, and Trichinosis (acquired by eating undercooked pork) is not uncommon. Nematodes also cause numerous plant diseases (e.g. root knot nematodes), and others serve as vectors for plant viruses. Our focus here is on nematodes of vertebrates, especially those species that infect humans.

Most nematodes are dioecious. Most species possess four juvenile stages prior to the adult stage. These five life cycle stages are separated by molts. Nematodes possess a fluid-filled body cavity known as a blastocoel (or pseudocoel). Male nematodes have one or two spicules, which aid in copulation. Nematodes possess a thick cuticle and move in a serpentine fashion. Nematodes lack a circulatory system, and the excretory system may consist of a pair of specialized cells, or in the case of *Ascaris*, consist of a pair of lateral excretory canals. Nematodes possess a prominent digestive system, and are said to have a "tube within a tube" body plan. The digestive system includes a long straight intestine, a rectum, and a subterminal anus. Thus the gut is considered complete.

The two nematode classes are distinguished on the basis of sensory organs, which are difficult to see without the aid of an electron microscope. Both classes include free-living and parasitic species:

- **Class Chromadorea / Order Rhabditea (=Secernentia =Phasmidea)** – possess cephalic amphids and caudal phasmids
- **Class Enoplea (=Adenophorea)** – only possess cephalic amphids

I. Class Chromadorea / Order Rhabditea (=Secernentia =Phasmidea)

Most species in this group are soil-dwelling forms. It seems likely that the parasitic species in this group evolved from free-living ancestors.

Infraorder Ascaridomorpha

These kinds of nematodes are typically large, stout, intestinal parasites with three lips, but there are a few exceptions. The esophagus is usually muscular, and simple (without a conspicuous bulb). The life cycle is usually monoxenous.

CROSS SECTIONS AS EXAMPLES OF GENERAL NEMATODE STRUCTURE:

***1. *Ascaris lumbricoides*. Cross section of adult female.** *Slide box slide* #28. See text page 355. Fig. 22.11b. These cross sections illustrate some of the key features of nematodes. Make a full-page plate of a section through an adult female *A. lumbricoides*. The outer body layer is the cuticle, which surrounds the epidermis. Inside the epidermis is the hypodermis, the layer that contains the lateral excretory canals. Muscle cells are seated on the hypodermis and have a striated contractile portion and a non-contractile portion that has the nucleus and processes that extend to the dorsal or ventral nerve cords. Neuromuscular junctions of nematodes are unique; rather than possessing nerves that run to muscles, as in most other animals, the muscles of nematodes possess non-contractile portions that extend to either the dorsal or ventral nerve cord.

Locate the following structures on your plate: cuticle, hypodermis, lateral excretory canals, dorsal nerve cord, ventral nerve cord, uterus, egg, ovary, oviduct, blastocoel, intestine, and muscle cells.

What features are useful for distinguishing between sexes:____

List 3 of the differences between this nematode and species of platyhelminths:____

Because the reproductive tract is coiled, you may see several sections of the uterus, ovaries and the oviducts (in the section of a female) or portions of the testes and vas deferens (in the section of the male).

2. Cross section of adult male. *Slide box slide* #28. See text page 355, Fig. 22.11a. Compare the cross section of the male worm to the female worm that you just drew. Note that the testes and vas deferens are present instead of the female reproductive structures. All the remaining structures should be familiar to you based on the examination of the female.

3. *Ascaris lumbricoides.* Demonstration of adult male and female worm. See text page 412, Fig. 26.1. Note the size of these worms. If a dissected male is available, recognize: Testes, intestine, lateral lines. In females, recognize: Intestine, vagina, uterine branches, oviducts, ovaries, and lateral lines. Be able to distinguish males from females based on external appearance.

How could you distinguish the sexes of this species from an external view?____

Note the lateral lines (=lateral epidermal cord) running the length of individuals of both sexes. What structures do lateral lines contain?__________________________________

Look for evidence of muscle cells. Also locate the dorsal and ventral nerve cords.

***4. *Ascaris lumbricoides.* Eggs.** *Slide box slide* #29. See text page 412 Fig. 26.2 & 26.3. Note the rough, mammillated layer laid down by the uterus. Make a drawing of an egg and label the following: developing embryo and mammillated layer.

How do fertilized and unfertilized eggs differ? ____________________Refer to page 412.

5. *Toxocara cati.* Adult worms. Demonstration. See text Fig. 26.10 for image, and Fig. 26.7 for life cycle of similar species, *Toxocara canis*. This specimen was collected a cat that was dissected during an Anatomy & Physiology lab in this department. The worms were collected from the stomach, but their actual location in the living cat would have been the small intestine. These worms look similar to *Ascaris* species, but are much smaller, and have a pair of lateral projections at the anterior end known as cervical alae. *Toxocara canis* and *Taxocara cati* are nematodes of our domestic household pets. However, these species can infect humans, an improper host, and cause a condition known as **visceral larval migrans**, in which juvenile worms migrate throughout tissues, causing a variety of problems.

Why do you suppose these worms were actually encountered in the cat's stomach, rather than the small intestine?________________________

Infraorder Oxyuridomorpha

Members of this order are commonly known as "pinworms" because the females, in particular, have sharp, pointed tails. Males in this group possess a single spicule. Male pinworms are haploid and females are diploid, this condition is known as haplodiploidy. Pinworms are further characterized in having a prominent muscular bulb at the posterior end of the esophagus. Life cycles of oxyurids are usually direct, i.e., single-host.

6. *Enterobius vermicularis* adult female. Demonstration. See text page 446 Fig. 27.1 and 27.4. Locate the anterior lips (3), cervical alae (anterior cuticular inflations) esophageal bulb, pseudocoelom, uterus, vagina, egg, cuticle, and anus.

How are the eggs of this species transmitted?___________________________________

7. *Enterobius vermicularis* adult male. Demonstration. See text page 427 Fig. 27.3. Note the mouth surrounded by 3 lips, anterior cuticular inflations (cervical alae), and the presence of a spicule. Identify the structures in attached Figure 3.

How would you distinguish between individuals of the two sexes of this species?

____

8. *Enterobius vermicularis* eggs. Demonstration. See text page 427 Fig. 27.5. Eggs of this species can be recognized in that they are flattened on one side. Sketch this egg in your notes and label the shell, and developing JI inside the shell.

Infraorder Rhabditomorpha

Male individuals belonging to this group generally have a broad copulatory bursa (i.e., a bag) supported by a number of rays. See Text 418 Fig. 25.2 to become familiar with the morphology of a copulatory bursa.

Most species of this order are intestinal parasites of vertebrates as adults, and are monoxenous.

Family Ancylostomidae

Members of this group are known as the "hookworms". They typically have a sclerotized buccal capsule that is armed with cutting plates or teeth. They attach to the intestinal mucosa with this capsule and feed on blood and tissue fluids sucked from the mucosa.

***9. *Necatoramericanus*– Adult male and female.** *Slide box slide* #30. Text page 400 Figs. 25.6 & 25.7. This species parasitizes humans. It is characterized by the pairs of dorsal and ventral cutting plates at the anterior margin of the buccal capsule. It also has a pair of subdorsal and subventral teeth at the rear of the capsule. Compare the morphological differences between male and female N. *americanus* specimens. Note the copulatory bursa in the male; the spicules in this species are elongate and are fused distally.

10. *Necator americanus*– eggs. Demonstration. Text page 399 Fig. 25.4. The eggs of hookworms share the same general features. The eggs are laid containing embryos in the 2, 4, or several cell stage of development, which pass to the outside with the feces of the host. Note the egg shell and cells of a developing embryo. Make a drawing of an egg of this species. Label the egg shell and cells of the developing embryo.

On average, in what cell stage of cleavage are the embryos on your slide?_________

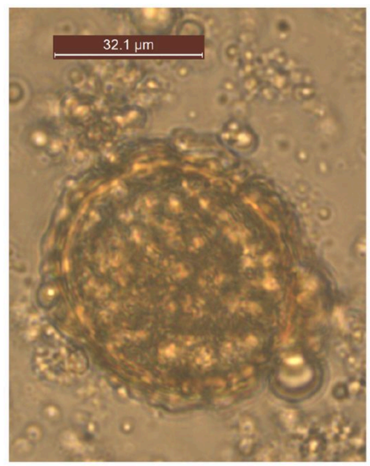
11. *Ancylostomaduodenale*– Adult male. Demonstration. Text page 401 Figs. 25.8 & 25.9. This species also parasitizes humans. It has a buccal capsule with anterior plates, each two bearing two large teeth. The spicules in male A. *duodenale* are elongate, but are **not** fused distally.

Do you see evidence of a copulatory bursa in this species?__________________________

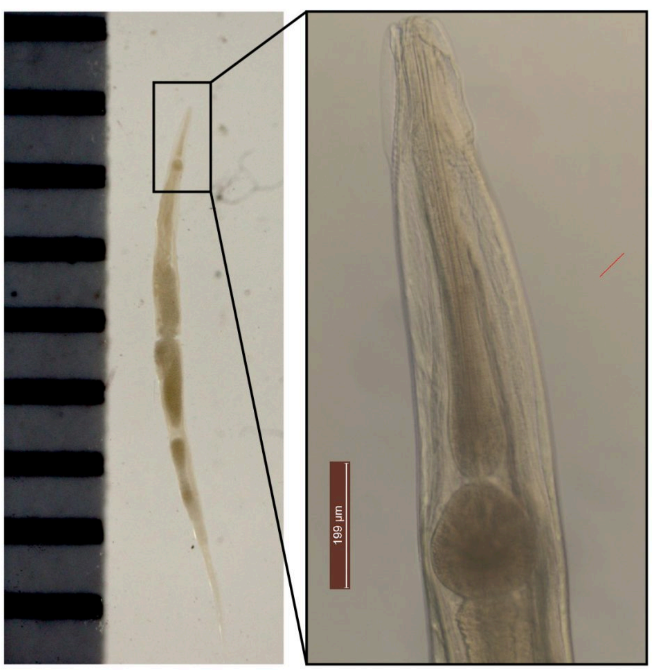
How could you distinguish this species from N. *americanus*?________________________

L10 Entry 1 *Ascaris lumbricoides* female cross section, mm ruler

L10 Entry 2 *Ascaris lumbricoides* male cross section, mm ruler



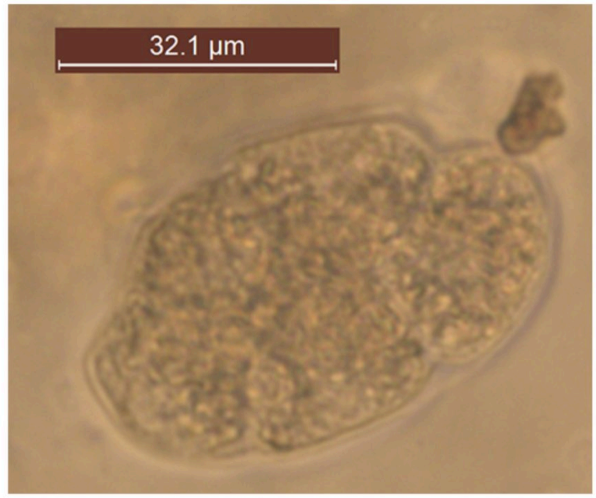
L10 Entry 4 *Ascaris lumbricoides* egg



L10 Entry 6 *Enterobius vermicularis* female, mm ruler

L11 Entry 6 *Enterobius vermicularis* male mm ruler

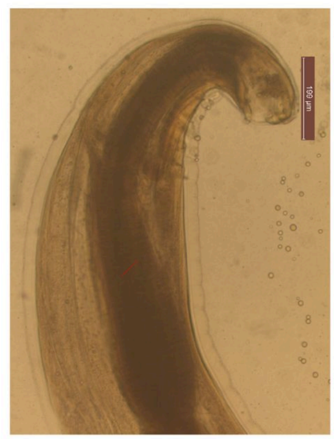
L10 Entry 8 *Enterobius vermicularis* egg



L10 Entry 10 *Necator americanus* egg

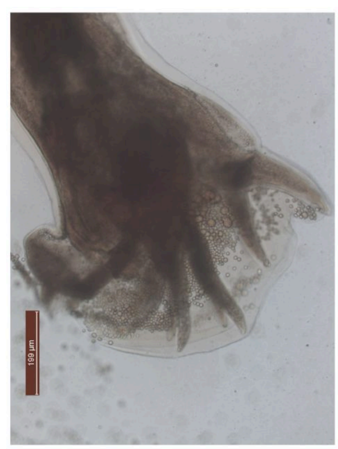

L10 Entry 9 *Necator americanus*
Female, anterior end


L10 Entry 9 *Necator americanus*
male anterior, end


L10 Entry 11 *Ancylostoma duodenale* male, anterior end


L10 Entry 9 *Necator americanus*
Female, posterior end


L10 Entry 9 *Necator americanus*
Male, posterior end


L10 Entry 11 *Ancylostoma duodenale* male, posterior end

Laboratory #11: Nematoda II

Phylum Nematoda (continued)

In the previous lab (#10) you learned the general features of the Phylum Nematoda, and you examined representatives of 3 orders of the Class Chromadorea. Today you will examine additional members of Class Class Chromadorea, as well a couple species of the Class Enoplea.

I. Class Chromadorea / Order Rhabditea

Most species in this group are soil-dwelling forms. It seems likely that the parasitic species in this group evolved from free-living ancestors.

Infraorder Ascaridomorpha – see Lab #10

Infraorder Oxyuridomorpha – see Lab #10

I**nfraorder Rhabditomorpha** – see Lab #10

Suborder Tylenchina

Members of this group seem to bridge a gap between free-living and parasitic modes of life because several species alternate between free-living and parasitic generations. Most members of this group live in decaying organic matter and are common in the soil, water, or rotting fruit and because of this end up in the bodies of larger animals, and in these cases they can become facultative parasites.

1. *Strongyloides stercoralis* free-living. Whole mount. Demonstration. See textbook Figure 24.2 page 393. Species of *Strongylus* are among the smallest nematode parasites of humans, and are especially interesting because of their ability to maintain parasitic life cycles, or to repeat free-living generations indefinitely. The slide contains adult free-living male and female worms; a rhabditiform esophagus can be seen. When these reproduce, their offspring will develop into rhabditiform–free-living and feeding– individuals, or into filariform individuals that will only develop further if they colonize a host via skin or orally and will subsequently develop in the intestinal submucosa. Development into the rhabditiform (free-living) form vs. filariform (parasitic) form seems to be determined by temperature.

List another species that can live either as a parasite, or free-living:_______________

Suborder Spiruromorpha

The esophagus of spirurids is usually divided into an anterior muscular, and a posterior glandular portion, but spirurids never possess an esophageal bulb. All members of this group are heteroxenous. Their life cycles involve an arthropod intermediate host in which they undergo development to the J3 stage.

2. *Spinitectis gracilis.* Demonstration. This specimen was collected from the intestine of a Rock bass in Otsego Lake at Cooperstown, New York. A distinctive feature of this genus is the presence of rows of backwards-pointed spines on the cuticle. Be sure to recognize the spines. Species of *Spinitectis* utilize mayfly larvae as intermediate hosts.

How do you think the rock bass acquired this local nematode species?________________

Can you see the spines (you may have to increase magnification)?_________What is the function of the spines?

Filarial worms

The next 4 species belong are known as the **filarial worms**, a group within Suborder Spiruromorpha. As adults, filarial worms are tissue dwelling parasites in the definitive host. All species use arthropods as intermediate hosts, most of which deposit third stage juveniles on the skin of the definitive host with their bite. Most are parasites of wild animals (particularly birds) but several are important in humans and domestic animals. In general, filarial worms are long, and slender with reduced or no lips and a buccal capsule. The filarial worms possess vermiform embryos called microfilariae. These are pre-J1 stage that, depending on the species, may or may not retain the vitelline membrane as a sheath around the body. This stage is infective to the intermediate host. The key to microfilariae found in the peripheral blood of humans in your text (page 443) is useful for distinguishing among human infections.

3. *Onchocercavolvulus*– Adults in sections of fibrotic nodule from skin. Demonstration slide-skin tumor section. See text page 447 Fig. 29.5. This species is the etiological agent of "river blindness". Adult worms are characteristically knotted together in groups in nodules in the subcutaneous tissues of the definitive host. Note the relationship between the worms and host encapsulation reaction. Females release **unsheathed microfilariae** that wander in the connective tissues of the host until they are picked up by an appropriate intermediate host.

What type of organism would serve the role of intermediate host?____________________

***4. *Wuchereria bancrofti*– microfilariae.** *Slide box slide* #31. human blood smear. See text page 443 Figs. 29.3 & 29.4. This species is the etiological agent of "elephantiasis" in humans. Microfilariae of this species characteristically **possess a sheath** (vitelline membrane). The nuclei in the body of the microfilaria do not extend to the very posterior tip of the tail. Adults live in the lymphatic ducts of humans; the microfilariae are found in the circulatory system of the definitive host. A life cycle diagram is found page 462.

What stage of this species is infective to the intermediate host?______________________

What stage of this species is infective to the definitive host?________________________

***5. *Dirofilaria immitis*– microfilariae.** Slide tray- dog blood smear. See attached Figure 2. Also see text page 454 Fig. 29.17 for pathology photo. Heartworm can be a severe and even fatal disease in domestic dogs throughout most of the world. Adult worms locate in the right side of the heart and pulmonary artery and can result in heart failure owing to vessel blockage. Adult worms mate and produce microfilariae, which then move to the peripheral circulation of the host. Microfilariae are transmitted to the mosquito host when a mosquito feeds.

The microfilariae of *D. immitis* are **unsheathed** and the nuclei of the body do not extend into the tip of the tail. Clinicians must be able to distinguish microfilariae of *D. immitis*, from microfilariae of the much less severe *Dipetalonema* sp., also found in dogs.

Does this species possess an egg stage during its life cycle?________________________

What is the name of the life cycle stage the follows microfilaria?____________________

6. *Loa loa* microfilariae. Demonstration. See text page 452 Fig. 29.14 & 29.15. This is the "eye worm" of Africa. Adult worms of this species have a tendency to wander through subcutaneous tissue and stimulate an immune inflammatory response. On occasion adults wander through the conjunctiva and cornea of the eye. Adult worms mate and produce microfilaria, the stage shown here. Microfilaria are found in the peripheral circulation of humans, and microfilaria are transmitted when a deerfly takes a blood meal.

What stages of *Loa loa* would occur within a deerfly? List them all.__________________

II. Class Enoplea

Most members of this class are free living species, but some are parasites of plants or animals. Members of this group possess five or more esophageal glands.

Order Trichinellida

Members of this order are usually more slender anteriorly than posteriorly and possess a buccal capsule that is absent or very reduced. In addition, all species have an esophagus that is embedded within one or more rows of large, glandular cells called stichocytes along most of its posterior portion. As a consequence, this region of the esophagus is referred to as the stichosome. Trichurid eggs are distinctive in their possession of 2 polar plugs. Males in this group either possess only a single spicule, or lack spicules entirely.

***7. *Trichuris trichiura*– adult male.** Slide tray. See text page 378 Fig. 23.1. Note the whip-like shape of the body, the single spicule, and the presence of a stichosome. This worm parasitizes the large intestine of humans, where they can cause extensive damage. Make a drawing of this specimen and label the following structures on your illustration: spicule, stichosome, anus, weak buccal cavity, intestine, and esophagus. Heavy infections of *T. trichiura* can cause rectal prolapse (see text page 379 Fig. 23.4).

Is the anterior or posterior end of this worm the thin end?___________________________

What is the function of the spicule?_______________________________

8. *Trichuris trichiura*– adult female. Slide tray. Note the very long stichosome. This and other worms in the same family are commonly known as "whipworms" because they are threadlike along most of the length of their body and then abruptly thicken at the posterior end, resembling the handle of a whip.

What feature definitively allows one to distinguish between the sexes of this species?____

Which sex of this species is larger? _______________________________

***9. *Trichuris trichiura*– eggs.** Slide tray. See text page 378 Fig. 23.2. Note the distinct lemon shape and the two prominent polar plugs of these eggs. This egg morphology is characteristic of many of the members of the whipworm family. Make a drawing of an egg of this species. Label: polar plugs, egg shell, developing embryo.

List a feature that would help you distinguish this egg from that of other species:_______

***10. *Trichinella spiralis*– intracellular juveniles.** *Slide box slide #32*. See text page 384 Figs. 23.10-23.12. This is the smallest nematode parasite of humans. It is the etiological agent of the disease known as "trichinosis" (or, "trichiniasis", and “trichinelliasis”). Note how numerous juveniles are present in this small amount of muscle tissue. Be sure to label the juvenile (J1) worm, collagen capsule, and nurse cell in the drawing.

What aspect of the life cycle of *T. spiralis* accounts for the common occurrence of huge numbers of juveniles in a single host? _______________________________________

How would a person acquire this type of infection?___________________________

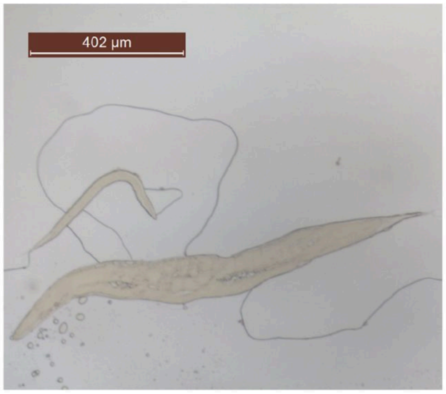
What stage of the parasite is found in this intracellular infection?_______________

Order Dioctophymatida

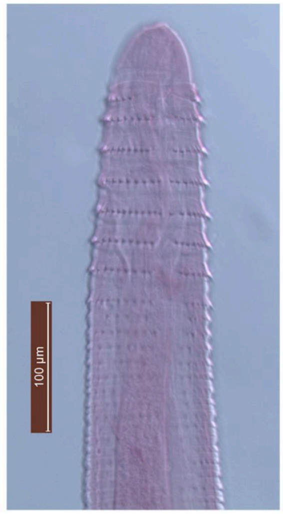
Members of this group are parasites of terrestrial mammals or, in the case of the example below, aquatic birds. This is not a medically important group, though human infections do sometimes occur.

11. *Eustrongylidestubifex.* Juvenile. Demonstration. This is included because it occurs locally. The specimen in the dish was collected from the body cavity of a Yellow perch in Otsego Lake at Cooperstown, New York. This species is also commonly found in sunfish, such as the Pumpkinseed and the Red-breasted Sunfish, and Rock bass. The life cycle nicely exemplifies one branch in the food web; First-stage juveniles occur in aquatic oligochaete worms called *Tubifex*, which are then ingested by a fish in which further juvenile development takes place. The adult stage is reached in the intestine of a piscivorous (fish-eating) bird such as a Kingfisher or a heron.

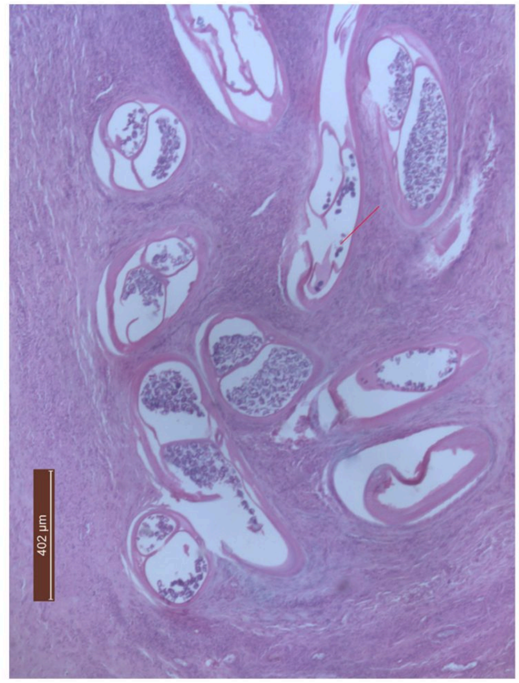
Look at the size of this specimen. Provide an example of a pathological effect that this worm would have on its fish host.__________________________________



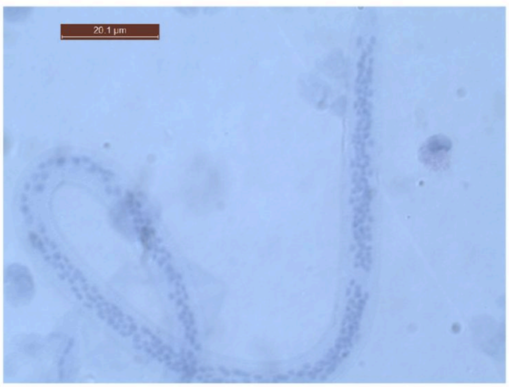
L11 Entry 1 *Strongyloides stercoralis,* free-living



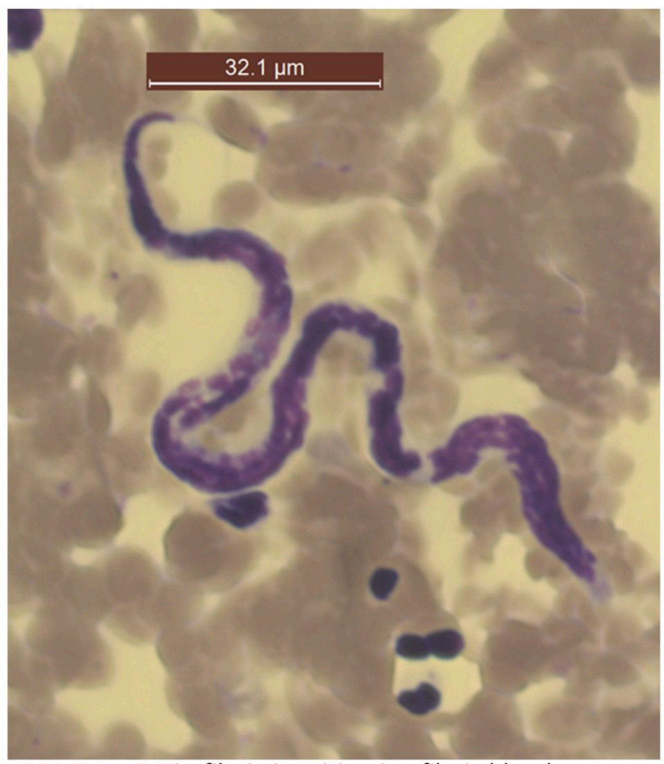
L11 Entry 2 *Spinitectus gracilis* anterior, adult



L11 Entry 3 *Onchocerca volvulus* adults in skin nodule



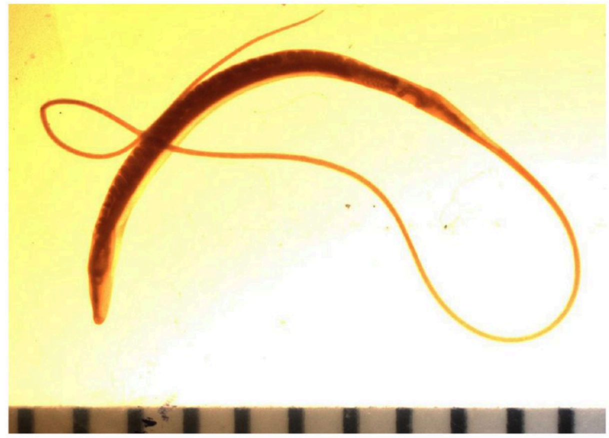
L11 Entry 4 *Wuchereria bancrofti* microfilaria blood smear



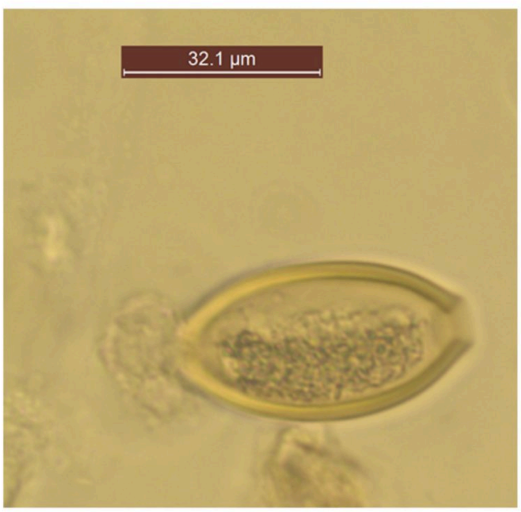
L11 Entry 5 *Dirofilaria immitis* microfilaria blood smear

L11 Entry 6 *Loa loa* microfilaria blood smear

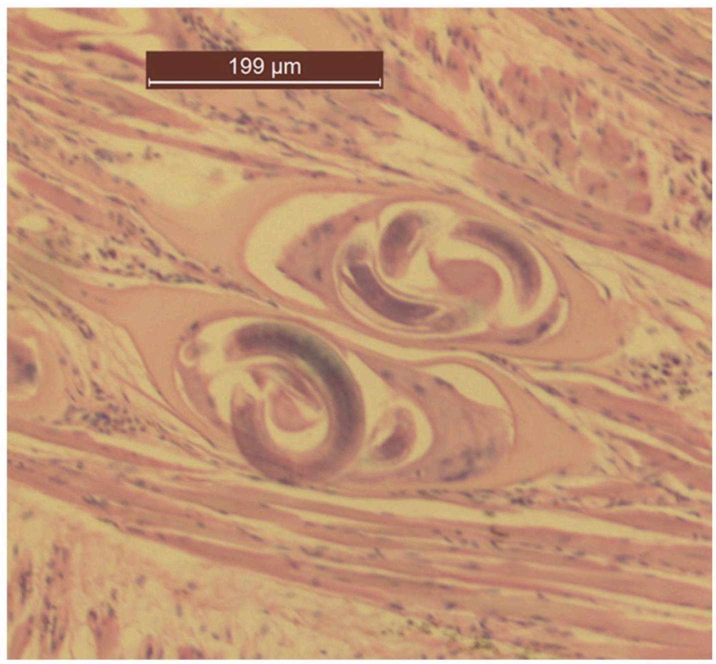
L11 Entry 7 *Trichuris trichiura* adult male, mm ruler



L11 Entry 8 *Trichuris trichiura* adult female, mm ruler



L11 Entry 9 *Trichuris trichiura* egg



L11 Entry 10 *Trichinella spiralis* intracellular juveniles

L11 Entry 11 *Eustrongylides tubifex* juveniles, mm ruler

Laboratory #12: Miscellaneous parasitic phyla

Phylum Acanthocephala (now in Phylum Rotifera)

Acanthocephalans, or "thorny-headed worms", inhabit the intestines of fishes, mammals, birds, and rarely amphibians and reptiles, and use their hooked proboscis to attach to the host intestinal wall. The acanthocephalans resemble nematodes in their possession of a fluid-filled body cavity referred to as a pseudocoelom (or blastocoelom). Acanthocephalans lack a digestive system, as was the case with cestodes. The muscular, nervous and excretory systems of acanthocephalans are poorly developed. Acanthocephalans are called thorny-headed worms because of their most prominent feature, a thorny eversible proboscis.

***1. *Leptorhynchoides thecatus* adult worm ex Largemouth bass**. *Slide box slide #1.* View the anatomy of your specimen in comparison to the one depicted in the anatomical diagram in your textbook (See page 476). Note the armed anterior proboscis with its well-developed muscles attached to the posterior end of the proboscis sheath. The lemnisci, which appear as sac-like structures extending into the blastocoel, are thought to serve a hydrostatic function in the protrusion and retraction of the proboscis. **For your drawing, only draw the proboscis, which should be viewed at 400x magnification. In addition, provide a detailed drawing of one hook, at oil immersion.** You need to decide whether your specimen is a male or female, and then be sure to look at an example of the other sex from one of your lab partners. **Females:** Observe the female egg masses, if present, floating free in the blastocoel where fertilization and early development has taken place. Eggs pass though a funnel-like uterine bell to the uterus and vagina. The genital pore is terminal. **Males:** Recognize the 2 rounded testes at the anterior end of the ligament. Posterior to the testes are 8 cement glands. These secrete a substance that prevents the female from accepting sperm from other males post-copulation. There is an eversible copulatory bursa, and also a penis, which is used to transfer sperm to the female. Be sure to recognize key structures, and to determine whether your specimen is male or female.

How does this organism acquire its nutrients?____________What other kind of parasite acquires its nutrients in a similar way?_____________

2. *Moniliformis moniliformis* adult specimen ex rat. Demonstration. This specimen was collected from the small intestine of a Norway rat in a wharf just a few blocks from the French Quarter of New Orleans. Can you see the anterior proboscis? It is small. Using the highest magnification available on the dissecting microscope, observe the individual hooks on the proboscis. This rat was infected with *Moniliformis* by consuming a cockroach that was infected with a larval stage known as a cystacanth, shown below. Once the cystacanth entered the rat, it grew into the female adult worm shown here. If a male was available, there would be mating and later the female would pass her eggs which would leave the rat via its feces.

What did the rat do to become infected with this parasite? ________________________________

3. *Moniliformis moniliformis* cystacanth stage. Demonstration. This specimen was collected from the body cavity of a cockroach. It has a proboscis that is invaginated. Once transmitted to rats, this stage grows into an adult worm. This stage of this parasite is known to change the behavior of its cockroach host, making it more likely that a rat would be able to capture a cockroach and eat it.

How do you suppose cockroaches become infected with *Moniliformis*? ____

4. ***Pomphorhynchus bulbocolli* adults attached to intestine of white sucker fish.** Demonstration. This acanthocephalan species can be found locally, in white suckers. White suckers are fish with ventrally-oriented mouths that feed on a variety

of invertebrates in the bottom layer, including amphipods, the intermediate host of *P. bulbocolli*. *Pomphorhynchus bulbocolli* is unusual among acanthocephalans in that it pokes its proboscis entirely through the intestinal wall. This can result in extensive pathology (damage) to the fish intestine–a topic that was the focus of one research project in my lab (see included publication on display).

Provide an estimate of the number of parasites visible at this demonstration:_____________

Phylum Nematomorpha

The nematomorphs, or "horsehair worms" are fairly common in freshwater. A few species are marine. This parasitic phylum is unusual in that the adult stage is actually free-living but the larvae are parasitic in the haemocoel of insects that are associated with water. Free-living adult worms mate and produce large quantities of eggs that grow into larvae that find and penetrate the appropriate host. Once in the host, larvae feed until ready to exit the host, which is done when the host goes near water.

5. Unidentified adult nematomorph emerging from grasshopper. Demonstration. See text page 465 Figs. 31.1a-b. Note the resemblance of this specimen to nematodes. They can be distinguished from nematodes on the base of their thick, often brown cuticle, and especially by lack of a mouth and possession of a bluntly rounded anterior end.

What stage in the life cycle stage of nematomorphs is parasitic?____________________

Phylum Mollusca

Molluscs are a hugely diverse phylum (~90,000 species; 2nd only to arthropods) that includes clams, snails, slugs, chitons, tusk shells, squid, octopuses, cuttlefish and nautiluses, among others. Most mollusks are free-living species, but there are several examples of parasitism within the phylum. Today we will focus on members of the freshwater clam family Unionidae. Unionids are free-living as adults, but possess a larval stage called the glochidia larva, that is an obligate parasite of freshwater fishes.

***6. Glochidia larvae.** Slide tray. This larval type is restricted to members of the freshwater clams belonging to either the unionid or margaritiferid families. Note the byssal thread if visible (increase contrast on your microscope), and hooks that are used to attach to the freshwater fish host. Glochidia larvae tend to be fairly host specific. These clams cannot develop in the absence of fish because the glochidia are obligate parasites on the gills and skin of fish.

What structures are visible that would not be present in the adult stage of this animal?________________________________

Which stage in the life cycle of the freshwater mussels travels/disperses a greater distance, the adult (free-living) stage, or the glochidia (parasitic) stage?_______________________________

Phylum Annelida

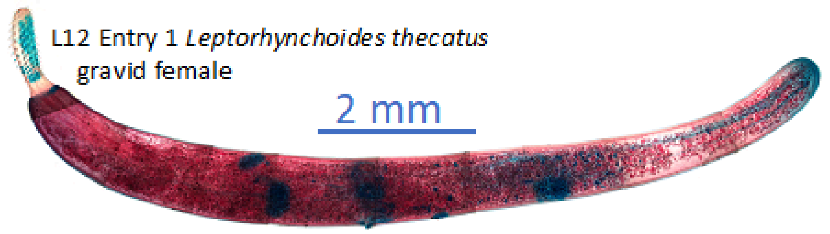
Annelids include earthworms, other segmented worms (including many marine forms, e.g., the feather duster worm in Dr.

Lentz's tank), and the leeches. The most common parasitic annelids are members of the Hirudinea group, also known as the leeches. Leeches are monoecious.

7. *Helobdella* sp– adult. Demonstration. See attached Figure 3 for an illustration of a similar species. Although many are free-living scavengers, many are blood-feeding parasites. For example, in our fish-parasite research we frequently see leeches attached to yellow perch. The leech shown here is actually considered an opportunistic feeder rather than obligately parasitic. It can feed on other leeches for their blood meal, or on soft tissues and body fluids of other invertebrates such as insect larvae, snails, aquatic oligochaetes, crustaceans and bivalves. It also uses open wounds of vertebrates to feed on the lymph.

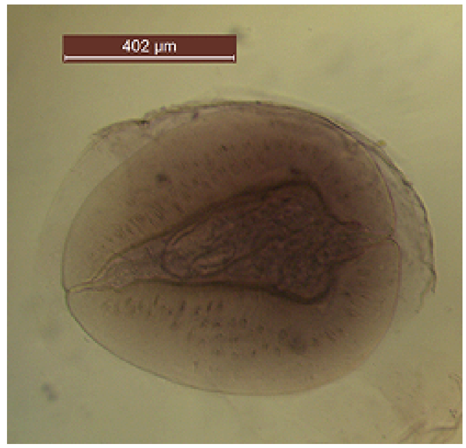
Note the general features of this specimen by comparing it to Figure 3. Recognize the posterior sucker, mouth, and extensive cecum.

How would you distinguish a leech from a monogene?_____________________________



L12 Entry 1 *Leptorhynchoides thecatus* gravid female

L12 Entry 2 *Moniliformis* adult, mm ruler



L12 Entry 3 *Moniliformis* cystacanth

L12 Entry 5 Nematomorph sp ex cricket, mm ruler

L12 Entry 6 glochidia larva

L12 Entry 7 *Helobdella* sp , mm ruler

L12 Entry 4 *Pomphorhynchus bulbocolli* in situ (white sucker Intestine)

Laboratory #13: Parasitic arthropoda

Phylum Arthropoda

This is the most diverse phylum of animals. It includes over 1 million described species, as well as a multitude of species that remain to be named. Examples of arthropods include lobsters, beetles, spiders, millipedes, ticks, and horseshoe crabs. Parasitism has evolved many times within this group. Here we focus on a few representative species of parasitic arthropods that can cause problems for animal or human populations.

Subphylum Crustacea

The 2 species we will examine should exemplify the extent that parasitic crustaceans are different than their free-living counterparts. Parasitic copepods and branchiurans (fish lice) can be a problem for fish hatcheries.

Class Copepoda – copepods

1. *Salmincola californiensis* female ex steelhead salmon. Demonstration. This specimen was collected from the gill of a salmon at a fish hatchery off a tributary of Lake Ontario. Both S. *californiensis* and its fish host are native to the Pacific Northwest, but have been introduced to the Great Lakes Note that this crustacean has lost some segmentation and essentially all appendages. Recognize the following structures: the bulla, a small amorphous anchoring process that is usually embedded in host tissue and is connected to the anterior region of the body of S. *californiensis*, termed the cephalothorax; the egg sacs, visible at the posterior end of the body.

Given that S. *californiensis* is monoxenous, make a prediction about what events will occur after the eggs in the egg sacs shown here hatch. Hint, the life cycle of this copepod is similar to that of monogeneans you studied previously:_________________________

2. *Ergasilis* sp. adult ex Smallmouth bass. Demonstration. See text page 516 Fig. 34.4. This specimen was collected from its site of attachment—the gill of a Smallmouth bass from Otsego Lake, New York. Ergasilids are common copepod parasites of fishes. Notice that in this species, the antennae are modified as organs of attachment; they are used to cling on to fish gill filaments. These ectoparasites feed on the epithelial cells and other cells of the gills, a behavior that results in damaged gills, leading to secondary infections that can ultimately kill the fish host.

Provide an example of a adaptation for parasitism exemplified by this copepod specimen:___________________________

Class Branchiura – fish lice

3. *Argulus* sp. adult ex white sucker fish. Demonstration. See text page 527 [551 in 8^{th} *Ed.*] Fig. 34.26. Fish lice are ectoparasitic on the skin of freshwater and marine fishes. The body is flattened and the head and thorax are covered by a large carapace. Both the antennules and the antennae are small; the former bears a large claw for attachment to the host. The bases of the maxillules have been modified into suckers; these structures are also used in attachment to the host. There is also a sucking cone present. It is associated with a large spine used for piercing the skin of the host.

What structures of *Argulus* may be harmful to the fish host?_________________________

Class Pentastomida – tongue worms or pentastomes

This unusual group of parasites, the pentastomes, was until recently considered to be its own phylum. Recent studies utilizing DNA sequence data have, however, demonstrated that pentastomes are actually crustaceans! Although adults look nothing like most crustaceans the larval stages do bear some resemblance. Adults of this phylum generally are parasites in the respiratory system of vertebrates, in particular reptiles, as definitive hosts. Intermediate hosts include mammals that would be prey to such reptiles.

4. ***Porocephalus* sp. ex *fer de lance*.** Demonstration. See text pages 535-540. This pentastome specimen were obtained from the lung of a pit viper commonly known as a *fer de lance* (*Bothrops atrox*) that was collected in the Peruvian rain forest. Note that there are four sclerotized hooks visible in this specimen.

Although this adult specimen has no obvious resemblance, it *is* a crustacean. What stage of this organism resembles other crustaceans?_____________________

How do you suppose the eggs of this pentastome species would exit the body of its snake host?______________________

Subphylum Hexapoda – insects and their kin

Class Insecta

Order Psocodea – lice; "chewing lice" and "sucking lice" and relatives

"Chewing lice"

5. *Trichodectes scalaris*. Whole mount. Demonstration. See text page 547 Fig. 36.6 for a similar species. Chewing lice feed primarily on feathers and hair (depending on their house) as well as sloughed epidermal cells. This "chewing louse" was collected from cattle, but is similar to *Trichodectes canis*, which is a common parasite of domestic dogs. *Trichodectes canis* can cause severe skin irritation, especially in puppies.

What do "chewing lice" eat?_______________________________________

"Sucking lice"

6. *Pediculus capitus* (= *P. humanus*) adult. *Slide box slide* #33. See text page 549 Fig. 36.10. Sucking lice feed on blood. This louse is found on the head in humans. In addition to irritating the host, this species transmits at last three important human diseases: epidemic typhus, trench fever, and relapsing fever. In your drawing, recognize and label: legs, head, thorax, abdomen, antennae, tarsal segment with large claw, eyes.

How does the head of this specimen differ from the head of a chewing louse?__________

Explain why P. *capitus* is more likely to transmit bacterial diseases between hosts than would a chewing louse such as T. *scalaris*:___________________________________

7. *Phthirius pubis*, human crab louse Adult specimen. Demonstration. See Ch. 36 page 551 Figs. 36.12, 36.13, & 36.14. This species is another "sucking louse", and is notable because of its site specificity for pubic hairs, though it can sometimes be found in the hairs of beards, armpits, etc. It causes pruritis, itchy, inflammed skin. This crab louse is often referred to as "crabs" which is of course misleading because it is not a "crab" (or crustacean) at all! Infection with this species is sometimes used as a predictor of other venereal infections, such as gonorrhea or chlamydia. How many legs are visible?_______________

Order Siphonaptera – fleas

8. *Xenopsilla cheopis* adult. Demonstration. See text page 568 Fig. 38.10. This is the tropical rat flea. Is a primary vector of the bacterium *Yersinia pestis*, which is the etiological agent of the plague, but apparently has no effect on rats. Note how this flea is flattened from the sides (laterally) rather than dorsoventrally flattened.

List two structures visible on this specimen that would facilitate its parasitic habit:__________________________

9. *Pulex irritans* adult. Demonstration. See page 567 Figs. 38.6 & 38.7. The human flea. This species is similar to X. *cheopis* in that it lacks genal and pronotal combs. However, in X. *cheopis* the ocular bristle originates in front of the eye, whereas in P. *irritans* it originates beneath the eye.

***10. *Ctenocephalides felis* adult**, from Reyda household, Otego, NY. *Slide box slide* #34. See text page 568 Fig. 38.9 for example of a similar species, *Ctenocephalides canis*. This species can be a nuisance to pets such as cats and dogs, and you should be aware that they are also transmissible to humans. The closely related C. *canis* is less common than C. *felis*. For your drawing structure labels, see generalized flea diagram on Fig. 38.1 page 564!

What long structure(s) would this specimen use to jump to its host?_________________

Order Hemiptera – bed bugs

Most species in this group are free-living. However, some species are parasitically associated with hosts, which are usually birds or mammals.

11. *Cimex lectularis*. Adult. Demonstration. See page 557 Fig. 37.5. This organism is commonly known as the "bed bug". Note the oval, dorsoventrally flattened body with many spines or setae, conspicuous compound eyes, and the absence of wings.

What do bed bugs eat?_____________________________

Order Diptera – Flies, sandflies, mosquitoes, etc.

Throughout this course you have learned about parasitic diseases that are transmitted via **adult** dipterans, such as malaria transmitted by mosquitos, or leishmaniasis transmitted by sandflies. **Larval dipterans** can also cause medical problems. The term "myiasis" is given for infections by fly maggots (larvae). Several dipteran families include species that cause myiasis. The following examples are from humans and cats.

12. *Dermatobia hominis.* Larval stage. Demonstration of specimen dissected from human buttocks. See text page 596 Ch. 39. Fig. 39.20. This organism is commonly known as the "human skin bot" or "botfly". Adult flies do not lay eggs on human skin; instead, adult flies capture mosquitoes and glue their eggs onto the legs of the moquito. When a mosquito with eggs of *D. hominis* lands on warm skin, eggs hatch and the larvae drop onto the host and bore into the dermis. Development from the larval stage to the pupal stage takes about 6 weeks, at which point the pupa emerges and further develops in soil. I removed this specimen from a friend using a pair of wooden matchstcks in 2000.

How did this larvae get into the human?______________________________

Subphylum Chelicerata

Class Arachnida – spiders, ticks and mites

While many arachnids are free-living species, many others are parasitic.

Order Acari – ticks and mites

These creatures play a major role in the transmission of a variety of viral, bacterial, and protozoan diseases of man and animals. When numerous, the blood feeders may also affect the health of hosts directly. Some mites are endoparasitic; many others are ectoparasitic associated with diseases such as mange. Still others are intermediate hosts in the life cycles of tapeworms and nematodes.

"Ticks"

13. *Ixodes scapularis* (= *I. dammini*) adult female and one of the bacteria it can transmit, ***Borrelia burgdorferi*** (the etiological agent of Lyme disease**).** Demonstration. Also known as the Black-legged tick. See page 614 Fig. 41.3. Adult *I. scapularis* generally feed on white-tailed deer. Female ticks survive through winter on their deer host, feeding on blood. The female will die the following spring, but only after mating, becoming extremely engorged, and laying up to 2,000 eggs. Larvae hatch out of the eggs and feed on white-footed mice, where it is suspected that they acquire *Borrelia burgdorferi*, the etiological agent of Lyme disease. The next stage is the nymph, feeds on a second host (usually a white-footed mouse, but could be some other mammal or a bird) the following year. It is the nymphal stage that most commonly bites humans, potentially infecting them with *B. burgdorferi*, other species of bacteria, or possibly the apicomplexan *Babesia microti*. At the end of the second summer the nymph molts to the adult stage.

What features of this tick might be helpful for species recognition?________________

How many legs are present on the tick?___________________________

Is it possible to acquire babesiosis and lyme disease from the same tick host?____

14. *Dermacentor variabilis* or the American dog tick. Adult male. *Slide box slide* #35. See page 615 Fig. 41.5 for an example of *D. andersoni*, a similar species found throughout the western USA. Species in this genus are among the most medically important of all ticks. *Dermacentor variabilis* is found in the eastern USA and is the main vector of Rocky Mountain spotted fever, though like many ticks, it can carry several different species of bacteria.

How, based on what morphological feature, can you distinguish this species from *Ixodes scapularis*?______________________

"Mites"

Mites have more setae on the surface of the body than do ticks. Parasitic mites themselves often cause some form of irritation or disease. Keep in mind that there are also many free living species of mites.

15. S*arcoptes scabei* adult. Demonstration. Fig. 41.26. Refer to text page 628 to answer the questions. This species is commonly known as the itch mite.

What disease is this species responsible for in humans?_______________________

What disease is this species responsible for in other animals?_______________________

How many legs are present?____________________

16. *Demodex canis* in dog skin. Histological section of skin. Demonstration. Humans often have a closely related species, *Demodex folliculorum* within hair follicles, but infections are usually benign. However, *Demodex canis*, also known as the dog follicle mite, can be very pathogenic. Infections, known as "red mange" are potentially fatal, especially in young puppies. In this slide you can see that the mites have penetrated the skin. Although it is difficult to recognize mites here, the slide demonstrates how extensive an infection and the resulting irritation can be.

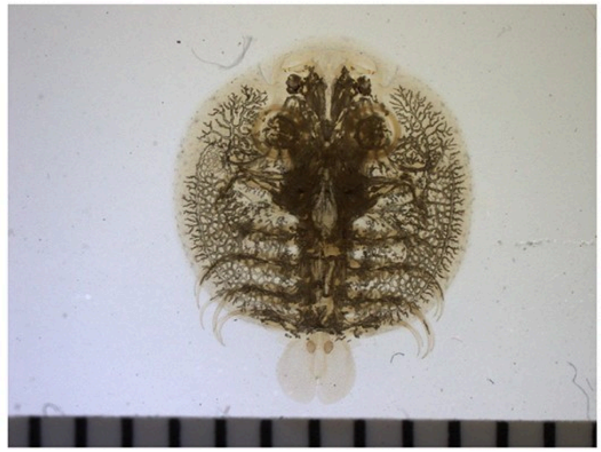
Is this an ectoparasite? __________

Why or why not?_____________________________

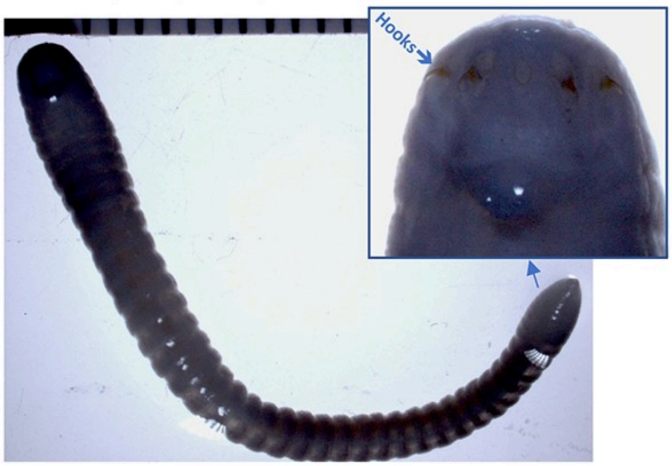
L13 Entry 1 Salmincola californiensis on steelhead gill

L13 Entry 1 Salmincola californiensis

L13 Entry 2 *Ergasilis* sp.



L13 Entry 3 *Argulus* sp. mm ruler



L13 Entry 4 *Porocephalus* sp, a pentastome, mm ruler

L13 Entry 5 *Trichodectes scalaris*

L13 Entry 6 *Pediculus capitus* mm ruler

L13 Entry 7 *Phthirius pubis* mm ruler

L13 Entry 8 *Xenopsilla cheopis* mm ruler

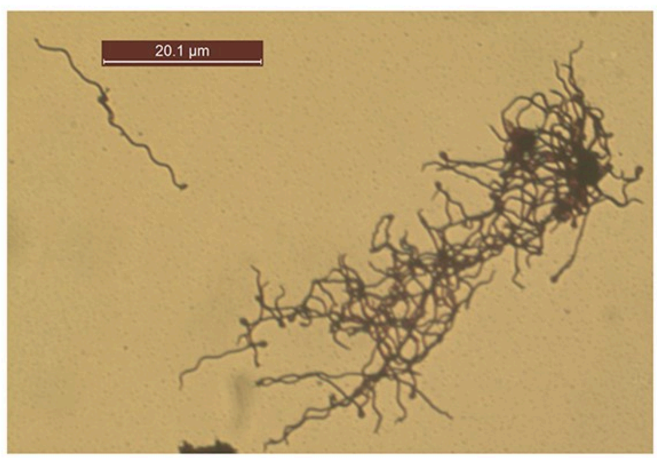
L13 Entry 9 *Pulex irritans* mm ruler

L13 Entry 10 *Ctenocephalides felis* mm ruler

L13 Entry 11 *Cimex lectularis* mm ruler

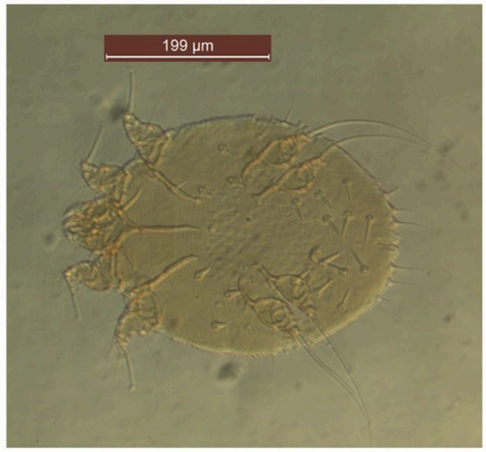
L13 Entry 12 *Dermatobia hominis* mm ruler

L13 Entry 13 *Ixodes scapularis* mm ruler

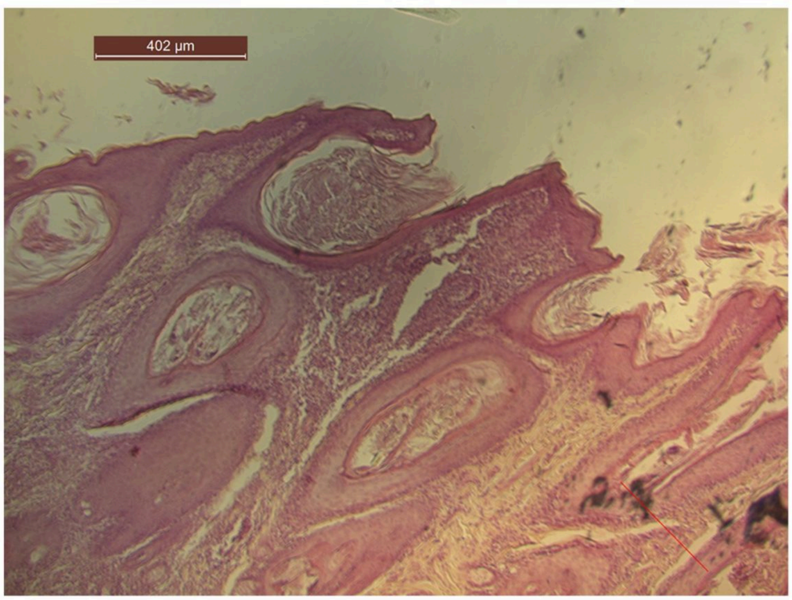


L13 Entry 13 *Borrelia burgdorferi* culture smear

L13 Entry 14 *Dermacentor variabilis* mm ruler



L13 Entry 15 *Sarcoptes scabei*



L13 Entry 16 *Demodex canis* dog skin section

cover page


Biol 346: Parasitology
Lab Manual & Atlas
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