# Arthropods of Domestic Animals

## (A Laboratory Guide to Veterinary Entomology)

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(A Laboratory Guide to Veterinary Entomology)

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# INTRODUCTION

Although molecular techniques have revolutionized the diagnostic approaches of ectoparasites, nevertheless, one cannot deny the importance of microscopic identification. To date, microscopy is a commonly used method not only in resource poor countries but also in technological advanced countries like the United States of America. The primary objectives of this manual is to introduce veterinary students to those aspects of knowledge of the arthropods that they will find useful in their careers and the diagnostic skills necessary to deal effectively with ectoparasite disease problems of domestic animals.

The first half of the Veterinary Entomology course (PARA 2505) is a systematic introduction to the identification, life history, and pathogenesis of parasitic arthropods. The relationship of these parasites to animal is emphasized. The second half of the course is devoted to treatment and control of parasitisms of dogs, cats, ruminants and horses, taking into consideration the influences of parasite life history and epidemiology, host behavior, and contemporary animal management practices. The techniques of antemortem, postmortem, and microscopic diagnosis that are presented exploit host and site specificity as adjuncts to parasite morphology. Experience has shown that this approach develops the greatest scope and precision of diagnostic skill with the least investment of specialized parasitologic training.

# BIOHAZARD LEVEL 2 PRECAUTIONS

While you are working with any fresh feces, be aware that such samples may contain "Biohazard Level 2" pathogens, for example, *Salmonella* or parasites such as *Toxoplasma*. These are pathogens that could potentially infect humans and cause illness. As part of our laboratory procedure on fecal techniques, please observe these precautions:

1. No eating or drinking in the lab.
2. Wear a lab coat in the laboratory, and keep it in the laboratory until it is laundered. (DO NOT bring it outside the laboratory, as this might spread contamination.)
3. Wear gloves while handling stool samples, including slides; wash your gloved hands with Betadine scrub before touching the microscope, books, pen, etc.
4. Wear a face mask while mixing or centrifuging feces to prevent contamination by aerosol.
5. Dispose of all fecal-contaminated material in a lined waste bucket, the contents will be incinerated later. (Slides, coverslips, and centrifuge tubes are disposable.)
6. When you have finished working with feces for the day, swab down your counter with Betadine solution, and wash your hands with Betadine scrub.

# DIPTERA

## MICROSCOPY AND FLIES

To begin, working as a group and without the aid of either a compound or a dissecting microscope, sort the specimens into sets conforming to the following rough categories: "gnats and mosquitoes, " "horse flies and deer flies," "house flies and their allies, " and "maggots and bots." It is important that you gain an impression of the relative sizes and general appearance of the specimens before the microscopes bring you so close to them that you lose the advantage of perspective.

Use the house fly, *Musca domestica*, as a size reference. Most horse flies, deer flies, flesh flies, blue-bottle flies, and bot flies are larger than house flies. All gnats and mosquitoes are smaller. Stable flies are the same size but have a stabbing beak instead of the sort of wet mop that house flies use to soak up liquids from pools. Horn flies look just like stable flies but are only half as large. Blue-bottle flies have a brilliant metallic luster provided the light strikes the specimen at an opportune angle.

After you have done your best to identify the specimens by inspection, study each carefully under the stereoscopic microscope and, in the case of very small specimens, the compound microscope. Remember that your objective is to learn how to identify flies and avoid getting too involved in terminology and structural detail. For example, the term "proboscis" has been applied to such diverse structures as the mouthparts of a mosquito and the trunk of an elephant. Therefore, it is pointless to concern oneself excessively as to whether "proboscis" includes the maxillary palps or not. **On laboratory exams, we will do no more than your future clients will very likely do. We will present you with a specimen and ask, "What is it?" On lecture exams we will also be interested in the answers to: "What does it do?" and "How can I get rid of it?"**

Please, DO NOT USE oil immersion lens with whole mounts of parasite specimens, because you may damage both the lens and the specimen. Also please DO NOT USE the oil immersion lens with wet preparations, i.e., coverslips placed over a drop of water, because the working distance between the lens and the coverslip is so small that the pressure from the lens causes the material that is being examined to move about under the coverslip.

**Points to be noted:**

1. Take "Musca fly" as reference for size of other important flies
2. Don't go into details of morphological feature until you become familiar with their apparent features
3. Always use the microscope with care due to avoid damage to lenses and specimens

# NEMATOCERA

**1. Culicidae: Mosquito**

The mosquitos in your samples were probably grown in a mass rearing system. The reason that this is probably the case is that some of you have male mosquitos and some have female mosquitos; the difference is that the males have more hirsute (hairy) antennae and for this species labial palps that are as long as the proboscis. Males do not feed on blood, rather they live by feeding on sugar containing plant exudates. Thus, male mosquitoes are seldom found around mammalian hosts.

The characteristics that should be noted on this specimen are the relatively long abdomen, wings, and proboscis. Mosquitos feed by inserting parts of this proboscis through the skin and into a venule or capillary. The long proboscis is the characteristic that can be used to recognize a mosquito as a mosquito. It is best not to rely on size; some mosquitos are much smaller than the one presented on this slide, and some such as *Psorophora* or *Toxorhynchites* reach body lengths of near a 1.5 cm or more. Thus, recognition should be based on determining whether or not the fly you are confronted with has a long proboscis or not.

Mosquitos are most commonly confused with specimens of heleids or psychoidids. They can be differentiated from these forms by the long proboscis. Compare this specimen with the heleid, *Culicoides* (slide #3) and note the difference in the proportional size of the mouthparts.
Mosquitos are the vectors of the canine heartworm, *Dirofilaria immitis*, and the malarias of rodents and primates.

**Points to be noted:**

- Long mouthparts should be noted to be a differential diagnosis of mosquitoes with other flies
- Male mosquito's antennae are hairier than female's, just like man has a mustache but woman do not

**Exercise/Notes:**
Draw and label male and female mouthparts of mosquitoes and highlights the differential points;

**2. *Simulium*: Black flies, or "buffalo gnats**

Black flies, or "buffalo gnats," are characterized by their short stubby appearance, short mouthparts, and short, non-hairy antennae that are formed of 9 to 12 similar segments. Again, only the female black flies take blood meals. The short mouthparts of the flies are designed for feeding by rasping a small hole in the surface of the skin. They are most commonly confused with heleids, but can be differentiated by their antennae. Thus, it is a good idea to compare this specimen also with slide #3. Some blackflies, such as the vectors of *Onchocerca cervicalis* in South America, members of the *Simulium metallicum*, complex have brilliantly metallic bodies as colorful as the calliphorid, or "bluebottle," flies. Those found in the United States tend to be more drab shades of brown and black.

Black flies are the vectors of various *Onchocerca* spp. and the malaria-like *Leucocytozoon* parasites of birds.

**3. *Culicoides*: A heleid or biting midge.**

These flies superficially resemble mosquitos and blackflies.
A *Culicoides* can be differentiated from a mosquito by its much shorter mouthparts. These flies can be differentiated from blackflies by their relatively longer antennae and the wings that tend to have various patches that are more darkly colored than the rest of the wing. Again, only females take blood meals, and like blackflies, they feed by rasping a small hole through the surface of the skin. Heleids are weak fliers and tend to have activities that are crepuscular, i.e. they feed in the early morning and evening when the day is quiet and not much wind is blowing.

Motorbike riders in evenings during summer usually have midges on their dresses and glasses, particularly, in the plains area of Pakistan.

Species of heleids are the vectors of various *Onchocerca* spp. and protozoan parasites, *Hepatocystis* of old world primates and various avian malarias, *Leucocytozoon* and *Haemoproteus*.

**Point to be noted:**

- Don't confuse Culicidae with *Culicoides*. The former is composed of the mosquitoes and latter f the biting midges.

**Exercise/Notes:**

Write down preventive measure for biting midges

## 4. Tabanidae: Horse fly

These flies are strong fliers and voracious feeders; again, however, only the females take blood meals. They tend to bother animals and people alike in the middle of the day. They are not all as large as this specimen, some being closer to the deer fly, *Chrysops*, in size. Horse fly species in the genus *Tabanus* can be differentiated from species of *Chrysops* by the shape of the antennae and the appearance of the wings; in *Tabanus*, the antennae are shaped like an old-fashioned can opener. The antennae of *Chrysops* are relatively longer and straighter and the wings are mottled. It should be noted that there is a third genus of Tabanids, *Hybomitra*, in the United States, Pakistan, India and China that is a pest of man and cattle, especially in the mountainous western areas.

Tabanids are vectors of various trypanosome species throughout the world; in some cases they only serve as mechanical vectors and in other cases they act as cyclopropagative hosts of these parasites. They also serve as vectors of *Elaeophora schneideri*, a filarial parasite of deer, elk, and sheep.

## 5. Tabanidae: deer fly

This is a local genus. *Chrysops* has eyes that in life are a brilliant metallic green. The mouthparts are similar to those of horse flies, but the antennae are proportionately longer. Compare the antennae to those of the horse fly, specimen #4. Deer fly wings have a definite band of brown pigment along the outer edge and extending towards the body at the widest part of the wing.

**Exercise/Notes:**
Draw antennae and wings of horse fly and deer fly

## 6. *Musca*:

The house fly or face fly (On the basis of morphology alone, only true experts can distinguish the house fly, *Musca domestica*, from the face fly, *Musca autumnalis*; the difference in these two species is a single tuft of hair on the thorax. Thus, it is best to differentiate them on the basis of their behavior. However, for the purposes of differentiating them from the other biting flies that have an apparently similar body structure, it will not be necessary to make a specific determination.

Species of *Musca* feed using their sponge-like mouthparts. They regurgitate saliva onto the material on which they are feeding (Vomit drop) and then sponge-up the partially digested meal with their spongiform proboscis. If a number of flies are captured and placed in a jar, 2 types of "fly specks" will collect on the wall of the container: the light colored specks are the regurgitated saliva and the dark colored specks are feces.

House files and face flies are most commonly confused with specimens of *Stomoxys* and *Haemotobia*, and they should, therefore, be compared with these specimens. Note that the mouthparts of *Musca* look like a sponge on a stick, while the mouthparts of these other two genera are straight and designed to penetrate the skin.

*Musca autumnalis* acts as a vector of the spirurid eye worm, *Thelazia*. *Musca domestica* serves as a biological vector of two spirurid nematode parasites of the horse, *Draschia megastoma* and *Habronema muscae*.

**Points to be noted:**

- The fly usually drops into cup of tea/coffee that is mostly likely face fly
- *Musca*, *Stomoxys* and *Haematobia* similar in size, but differ in the shape of their mouth parts

**Exercise/Notes:**

Write a brief note on "vomit drop"

**7. *Stomoxys calcitrans*: This is the stable fly**

The stable fly has a long pointed proboscis that is used to take a blood meal from the host. Both sexes of stable flies take blood meals. *S. calcitrans* can be differentiated from *Haematobia irritans* by the relative difference in the lengths of their respective labial palps. Those of *Stomoxys* are very short relative to the proboscis, those of *Haematobia* are almost as long as the proboscis.

*S. calcitrans* is a vector of the spirurid nematode parasite of the horse, *Habronema microstoma*.

**8. *Haematobia irritans*:**

This is the horn fly. The horn fly resembles the stable fly except that it tends to be smaller and to have labial palps that are almost as long as the proboscis. This fly serves as a biological vector of the filarial nematode parasite of the horse, *Stephanofilaria stilesi.*

**Exercise/Notes:**
Describe the reason of their common names

**9. *Glossina morsitans*: This is the Tsetse fly.**

This fly occurs only in Africa. It is the vector of African trypanosomiasis, the scourge and protector of the African continent that has kept European breeds of cattle and horses from being cultivated in the sub-Saharan portions of this continent. It is characterized by its protruding proboscis and the "meat cleaver" design produced by the wing venation. Fortunately for the rest of the world, this genus has never been introduced, or at least, has never become established on any of the other continents of this planet. The developmental biology of the tsetse fly is interesting because the female fly harbors the developing larva in her body until it reaches the pupal stage. Thus, this fly lays a puparium rather than an egg.

**Exercise/Notes:**
Draw the wing of the tsetse and focus on the "meat cleaver" like appearance of the wing

**10. *Melophagus*: This is a sheep ked.**

This fly has no wings and is characterized by its dorsoventrally flattened body. The usual mistake in identification is to call this fly a tick because of its lack of wings. As will be seen in a week or two, it can be differentiated from the ticks by its possession of only 6 legs and by its body which is obviously divided into three segments, a head, a thorax, and an abdomen. The mouthparts can also be seen to resemble those of the deer fly in their general construction. Related genera that are found on deer and birds have wings during part or all of their adult life. Hippoboscids are similar to tsetse flies in that they lay larvae that are almost ready to pupate rather than eggs.

Sheep keds are the vectors of trypanosome parasite of sheep. The hippoboscids of birds transmit several species of avian malaria of the genus *Haemoproteus*.

**Points to be noted:**

- This fly does not fly
- This is the only fly which is permanent parasite, from feeding to breeding, spend its whole life on the body of their host

**Exercise/Notes:**

What are differential points between ked fly and ticks

**11. Calliphorids: This is a blow fly or blue-bottle fly.**

These flies are similar to house flies in their general appearance and in the appearance of their mouthparts. The more common species, however, differ from the house fly in that their bodies have a brightly metallic hue that is often blue, green, or copper. These flies are mainly of importance to veterinary medicine in the pathology produced by their maggots in cases of myiasis.

One species of calliphorid, *Cochliomyia hominivorax*, the American screw-worm fly has a life cycle that required the development of larval stages in living tissue. This fly, unlike many calliphorids, has its superficial metallic luster confined to only two very small areas of its body. Fortunately, this parasite has been virtually eradicated from the United States by an avid use of a sterile-male release program.

**12. Sarcophagids: This is a flesh fly.**

Sarcophagids, like calliphorids, are of importance because their larvae cause myiasis. They can be differentiated from house flies by the gray and black stripes or checkerboard appearance of the dorsal side of their thorax. Also, the ovipositors on the posterior end of the female flies tend to be red in color. These flies, like calliphorids, tend to show up at barbecues where they can be seen to be depositing larvae on the hamburgers or steaks that are awaiting the grill. Compare this specimen to the *Musca*.

**13. *Oestrus*: This is the sheep nasal bot fly.**

Like the Calliphorids and Sarcophagids, this fly is important because of the myiasis caused by the maggots. The adult fly has vestigial mouthparts and appears superficially similar to a honey bee.
Note on your specimen that the mouthparts are almost invisible even when the stereomicroscope is used.

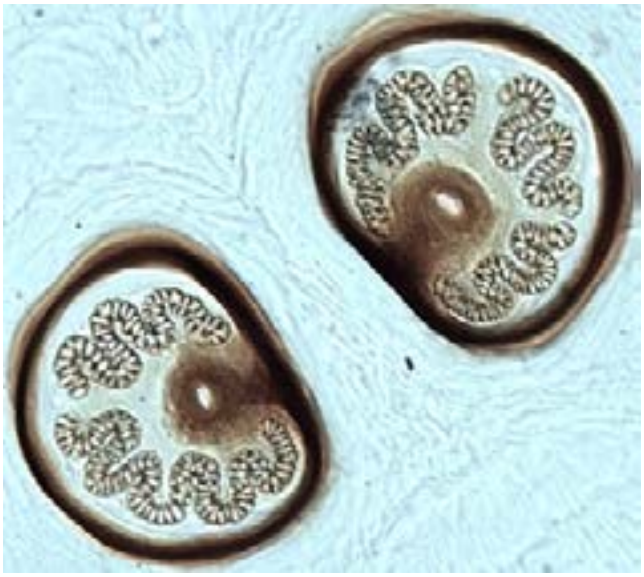
## MYIASIS

The specimens in this set represent those larval flies that are important to veterinary parasitology because they cause disease or might be confused with those larvae that do cause disease. In many cases, e.g., *Gasterophilus* and *Hypoderma*, the host and the location in or on the host will be an aid to the identification of the organism. However, caution should be exercised in relying too rigorously on the host for the purpose of identification, not because it will often lead one astray, but rather, because by learning the basics needed for identification, one will be prepared for the unexpected location or host that may occasionally be presented to a practicing veterinarian.

### 14. *Musca* larva

These are examples of the larva of the common house fly. First examine, the larva itself. Note the apparent segmentation of the body. On the anterior of the larva, can be seen the two dark mouth hooks. On the posterior of the body, can be seen the two spiracles. Compare the general morphology of this fly maggot with that of the maggot *Sarcophaga*.

### 15. *Musca* spiracles



The spiracles are two large openings of the respiratory system of the fly maggot. They can be thought of as vent coverings similar to the various grids and covers on heater and air conditioning ducts. Fortunately for taxonomists and others who would like to identify flies, the spiracles have different constant morphologies for the different species. This makes them a useful tool for fly identification. Although the diagnoses that will be made in practice often will not make use of spiracular morphology, it is important that the skills be known so that strange or refractory cases can be properly diagnosed and treated.

Compare the morphology of these spiracles with those of the other flies. Notice how under the microscope, the spiracles look exactly like the photograph in the book. This is the beauty of using the spiracles to make an identification, they look just like the published information. The trouble comes when you are dealing with an odd species or a strange instar. When this happens, you may discover that the spiracles do not look like the picture, or what could be worse, the spiracles of a early instar (different instars stages sometimes have spiracles that differ with every stage) may look like those of a completely different genus. Then, you might be led down a path of false enlightenment and make a mistaken diagnosis. Fortunately, however, most of the time you will not even need to examine the spiracular morphology.

## 16. *Stomoxys* spiracles

Again, the spiracles look just like the picture. The maggots of *Stomoxys* and *Musca* are not normally considered to be agents of disease. They need to be recognized for what they are usually, accidental findings. These may occur when dogs or other animals have ingested meat or other material containing maggots, they will then be passed in the feces and a proper identification can do a great deal to set an owner's mind at ease about the health of his or her pet.

## 17. Calliphoridae spiracles

Note that in calliphorid spiracles, the bottom of the innermost slit is pointed at the ventral midline.

## 18. Calliphoridae larva

Calliphorid maggots cause cutaneous myiasis when the eggs are laid in wounds or damaged tissue on the surface of an animal. The disease can become quite serious, especially in debilitated dogs. Other species, such as *Cochliomyia hominivorax* and *Lucilia cuprina*, are even more important because of their tendencies to develop on otherwise healthy animals. Usually, it will not be necessary to distinguish calliphorid larvae from sarcophagid larvae for the purposes of treatment, but in some cases, it may be desired.

## 19. Sarcophagidae spiracles

Note that in the sarcophagid spiracles, the bottom of the innermost slit points away from the ventral midline.

**20. Sarcophagidae larva**

Sarcophagid maggots, like calliphorid maggots, are involved in cases of cutaneous myiasis. Again, it is usually not necessary to identify the family involved before beginning treatment, but it is possible. Examine the spiracles and note that they can be differentiated from those of the calliphorid larvae.

**21. *Oestrus* spiracles**

**22. *Oestrus* larva**

These are obligate internal parasites. Usually, a diagnosis can be made using the criteria of host and where in the host (the nasal sinuses) that they are found. In rare instances, they have been found as parasites in other hosts in other sites. Also, in rare instances, sheep may be parasitized by other genera of nasal bots. If nasal bots are found in other hosts such as deer or antelope, there is a good chance that they belong to other genera of the family Oestridae , and if the spiracles are examined, they will look different than those of *Oestrus*. Again, compare the spiracles with the figure in the text and note that they look just like the picture.

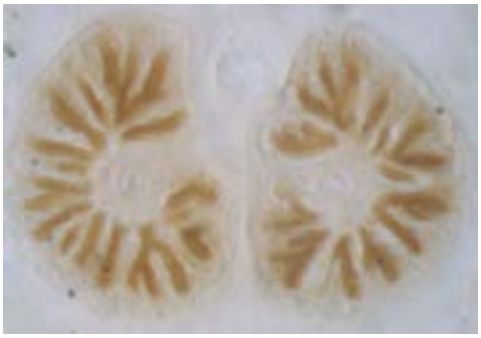
**23. *Gasterophilus* spiracles**

**24. *Gasterophilus intestinalis* larva**

**25. *Gasterophilus nasalis* larva**

Again, a diagnosis will usually be made on the basis of host and location within the host. The examination of the spiracles is basically an academic one for the sake of completeness. The species can be differentiated by the spines on the body. *G. nasalis* has one row of spines on each body segment; *G. intestinalis* has two rows of spines on each body segment. Compare the two specimens and note that you can tell them apart. If for some reason, one wanted to differentiate G. *intestinalis* from *G. hemorrhoidalis*, it could be done by comparing the morphology of the spines. The spines of *G. intestinalis* appear to have blunted tips; the spines of *G. hemorrhoidalis* come to a fine point.

**26. *Hypoderma* spiracles**



**27. *Hypoderma* larva**

This is another maggot that will usually be diagnosed by the site of the lesion within the host. Spiracular morphology is again mainly an academic pursuit; compare the specimen with the figure in the text. Occasionally, however, this genus causes encephalitis in other hosts, and it then becomes necessary to identify the maggot from the tissue for the purposes of determining the genus of parasite involved. This is an instance where the morphology of the spiracles becomes important to those trying to make a diagnosis. Usually, however, as Dr. Georgi has stated in the text, "when found in their accustomed locations in their normal hosts, bots present very little in the way of a diagnostic challenge."

**Exercise/Notes:**

Draw and label spiracles of *Musca*, *Stomoxys*, Calliphoridae, Sacrophagidae, *Gasterophilus*, Oes*t*rus and *Hypoderma*

# LICE AND FLEAS

Begin by examining specimen *Haematopinus* (Louse) with *Ctenocephalides* (Flea). Note that lice are dorsoventrally compressed while fleas are laterally compressed. Also note, that both have 3 pair of legs as is typical of insects.

The behavior of these two groups of insects is much different. Lice are slow and spend a good deal of their time slowly moving from hair to hair; fleas move rapidly about their hosts and are laterally compressed to allow easy movement between the hairs of the host's body.

Next compare an anopluran louse *Haematopinus* with a mallophagan louse *Damalinia*. Note that the head of *Haematopinus* is relatively narrow in relation to the thorax while the head of *Damalinia* is as wide as or wider than the thorax. Although, this difference between anopluran and mallophagan lice may not hold in all cases, it is a good rule of thumb for the separation of these two groups of lice.

# THE ANOPLURAN LICE

***28. Haematopinus-.***

Cattle have 4 lice, 3 anoplurans (*Haematopinus*), *Linognathus*, and *Solenopotes*) and 1 mallophagan (*Damalinia*). Fortunately, for those who want to tell the different genera apart, the task is not too difficult. Look at the tarsal claws on the end of the legs of this specimen of *Haernatopinus* and note that they are all of about equal size. This is the only genus of anoplurans of cattle where this is the case.

**29. *Linognathus***

*Linognathus*, another anopluran louse, has a first pair of tarsal claws that is smaller than the second and third pair. However, *Solenopotes*, also is similar in this respect, so if the louse was removed from a cow, attention must be paid to the number of setae per abdominal segment and the spiracles on each abdominal segment.

*Linognathus* has several rows of setae per segment and spiracles that are flush with the outer edges of the abdominal segments. Compare this specimen with *Solenopotes* that has 1 row of setae per abdominal segment and protruding spiracles.

If the louse was removed from a dog, it has to be differentiated from *Trichodectes*. This is rather easy because *Linognathus* is an anopluran and *Trichodectes* a mallophagan.

**30. *Solenopotes***

This is an anopluran louse that occurs on cattle. As stated above, the first pair of tarsal claws are smaller than the second and third pair, there is only one row of setae per abdominal segment, and the spiracles on each abdominal segment protrude from the body.

**31. *Pediculus* . This is an anopluran parasite of man and other higher primates.**

In recent years the subspecies, *Pediculus humanus capitis*, has appeared in numerous epidemic outbreaks of head lice in public schools. The reasons for these outbreaks are still not well understood. The body louse, *Pediculus_humanus humanus*, which lives by clinging to clothing rather than hair, tends to appear in large numbers only when people are crowded into area s with poor sanitation.

Veterinarians have to know about human lice to protect the pet from being mistaken as the host. If confronted with one of these lice supposedly from a dog, the specimen would have to be differentiated from *Linognathus*. Fortunately, however, due to the fact that outbreaks of head lice have become more common, it is now easier due to client awareness to make clients understand that the pet is not at fault.

**32. *Pthirus pubis***

The anopluran crab louse, so named because of its shortened abdomen which gives it a round appearance, is a parasite of the course hairs of humans, i.e. pubic, armpit, beard, mustache, and eyebrow hair. It moves between hosts during extended close contact such as coitus.
Again, the only importance of this louse to veterinarians is that dogs are sometimes presented as the offending source. This would be a most unlikely occurrence; usually the owner or owners are to blame.

# THE MALLOPHAGAN LICE

**33. *Damalinia***

This is a mallophagan louse that is found on cattle, horses, sheep, and goats. The mandibles rather than the legs are modified for hair clasping. By now it should be easy to differentiate this genus from the anopluran lice of large animals. Their females have potential to produce eggs parthenogenetically.

**34. *Felicola*** This is a mallophagan louse of the cat.

Again, note that the mouth parts have been modified for hair clasping. The width of the head should help in the recognition of this louse as a mallophagan.

Do not make it too hard however. This is the only louse that is typically found on cats, and the identification is usually that simple. Of course, cats, like dogs, will sometimes be blamed at the host of a human louse, but you should be able to tell the difference between this mallophagan louse and the 2 anopluran lice of people.

**35. *Trichodectes***

This is the mallophagan louse of the dog.

Lice are becoming rarer and rarer on dogs in the United States, probably due to better hygiene being practiced by owners. Thus, in summary, the lice that might be found (or reportedly found) on a dog include *Trichodectes*, *Linognathus*, *Pediculus*, and *Pthirus*. Determining whether the organism in question is a dog louse or a human louse will be an important part of your decision of how to treat the presented case.

**36. *Amblycera* This is a chicken louse.**

Free-ranging chickens will often have large numbers of lice that usually seem to cause no ill effects. There are a lot of different genera and species of lice that occur on chickens and other poultry; and all the species found are mallophagan lice.

This is another mallophagan bird louse. Note the elongate body. Pigeon owners often encounter these lice in Pakistan.

**Points to be noted:**

- Anopluran lice are only found on placental mammals, mallophagan lice are found on birds and mammals.
- Anopluran needs piercing and sucking type mouth parts so their head is narrow while mallophagan needs chewing type mouth parts so their head is broader and wider to support strong mandibles.
-

**Exercise/Notes:**

Fill the following table;

| Host | Anoplura | Mallophaga |
|---|---|---|
| Dog and cat | 1.<br>2. | 1. |
| Cow | 1.<br>2.<br>3.<br>4.<br>5. | 1. |
| Horse | 1. | 1. |
| Sheep and Goat | 1.<br>2.<br>3.<br>4.<br>5. | 1.<br>2.<br>3. |
| Human | 1.<br>2.<br>3. | None |

# THE FLEAS

**38. *Ctenocephalides***

This is the Siphonaptera genus that is the usual cause of flea problems throughout the United States.

Examine the anterior end of the flea and note the extra thick spines that look like a mustache and a collar, these are the genal and pronotal combs, respectively.

The presence, absence, and orientation of these combs are characters that are used in the identification of the various flea genera. Usually, a diagnosis of flea will be sufficient for your treatment to be effective. Occasions may arise, however, when the major offending genus involved is a rodent flea; then, for control efforts to be successful other methods will have to be employed than those usually used for the dog and cat flea. Thus, when control situations arise that do not seem to respond to normal regimens, it may be worthwhile to determine the genus and species of flea involved.

**39. *Echidnophaga***

This is the "sticktight flea" of poultry.

On rare occasions it can be found with its anterior end embedded into the skin of canine or feline hosts.

Note that there are no genal or pronotal combs present on these fleas.

**40. *Xenopsylla***

This is the rat flea which is famous because of its potential to transmit bubonic plague, the black death caused by *Yersinia pestis*. If the flea is examined closely, the lack of pronotal and genal combs will be noticed as well as a characteristic vertical rod that is present on the mesothorax. These are the characters that are used in identification.In every large port city in the United States, and most of the world, there are groups of people who collect the fleas from rats that have been trapped in the wharves.

The fleas are identified and attempts are made to isolate the plague organism. The ultimate goal is preventing an outbreak of bubonic plague. The problem is that if plague is identified in a rat or flea population, by international law the port will have to be closed. Thus, great pressure is applied to rodent control operations in these cities. They are supposed to find and isolate the plague organism, but they dare not report it if they do.

Inside this flea can be seen a group of bristles or spines at the proventricular entrance into the stomach. These bristles are used

to break down the erythrocytes that have been ingested. When Xenopsylla becomes infected with plague, the bacteria multiply in the area of these bristles and plug the entrance to the stomach. Then, the flea keeps feeding, but because it cannot fill its stomach, it stays hungry. This constant state of hunger and the plague induced death of the normal rat host leads these fleas to seek out other hosts, including humans.

Finally, however, the plague infected flea will die a slow death of starvation. The other common genera of fleas, e.g. *Ctenocephalides* and *Pulex*, do not appear to be good natural vectors of plague perhaps because the proventriculus does not become blocked as easily. Several other fleas do transmit plague rather well. Two such genera are *Diamanus* and *Hoplopsyllus* which transmit the plague in a sylvatic cycle between ground squirrels in the western United States. Note the "rod" in the basal segment of the second leg. *Pulex* lacks this rod.

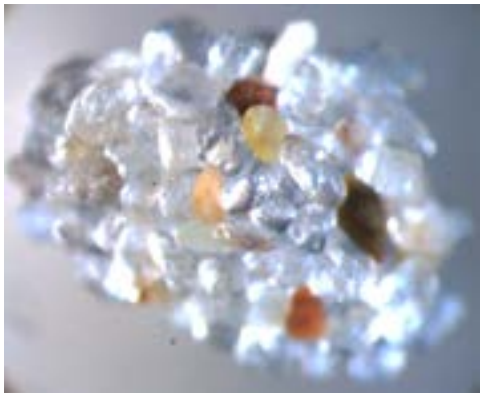
***41. Pulex***

This is the human flea. However, the most common hosts for this flea are probably usually the dog and pig. In a study on the fleas of dogs from Georgia and Mississippi, it was found that over 80% of the fleas were *P. irritans*. Thus, it may be that in certain areas of the country that this flea is more prevalent than *Ctenocephalides*, the flea that would usually be expected. Compare the basal segment of the second leg to the same structure in *Xenopsylla*, which has a "rod".

### *42. Rodent flea*

Rodents host a variety of flea species, some of which have both a genal and a pronotal combs that are not at right angles to each other; some species have only one comb, and some have none. There are lots of fleas on many different animals, and with care they can usually be identified. Fortunately, this task is not usually required.

### *43. Flea pupa*



Fleas undergo complete (or complex) metamorphosis. Thus, at one stage of their life cycle they look like worms, then they spin a cocoon and pupate, and then they look like fleas. Flea larvae are fleas too, and it is important for the purpose of control that you be able to recognize them for what they are when they are presented in the context of a flea problem or a dog and cat problem. Thus, note that they are small caterpillar- like organisms, and remember that they are usually found in an animals bedding, although sometimes they will be found on the animal itself.

Flea pupae look like small oval masses of debris, because they are encased in a cocoon. While the cocoon is being spun, it is very sticky, and it becomes covered with small particles from the environment. These fleas were reared in sand.

### 44. Flea larva (cat bed)

Locate the caterpillar-like specimen with your 4x objective. With your l0x objective, note that the anterior end of the larva has few "hairs," or setae, and that the setae are longer and more numerous at the posterior end. With your 40x objective, observe the chewing mouthparts at the anterior end. The pair of shiny mandibles are amber colored and are serrated on one side. They may be extended, or may be retracted into the head. These enable the larva to ingest solid food. The head is also equipped with 2 pairs of segmented sensory structures. The antennae are the longer pair, with a long spine and several short ones at the apex. In some specimens the long spine may be broken off. Antennae are cylindrical or slightly cone-shaped but with a flat or domed apex. Palps are shorter, with a number of short spines at the apex, which may appear slanted. At the extreme posterior end of the larva are a pair of short, pointed prolegs that are used in locomotion.

**Exercise/Notes:**
Collect flea from dog and domestic poultry and draw their pics;

# THE BUGS

## 45. Reduviidae - kissing bugs

This reduviid bug is the vector of American trypanosomiasis or Chagas' disease which is caused by the organism *Trypanosoma cruzi*. These bugs, especially those in the genera *Triatoma* and *Rhodnius*, have adapted to life in human habitation, and in South and Central America are household plagues that are often as hard to eradicate as cockroaches. Fortunately for those of us in North America, the genera of reduviid bugs here prefer to live in animal burrows or warrens and to feed on animals in the wild. Thus, although *T cruzi* is present in sylvatic animal populations from Texas to areas as far north as Maryland, there have only been a few cases of disease in domestic animals or man in North America.

These bugs feed at night. They come out with stealth from cracks where they have been hiding and feed rather slowly. Each bug, however, can take up to almost 0.5 ml of blood at a meal. While feeding, the bug extrudes excess moisture from the blood meal through its anus so that it will have room for more food. The infective trypanosome that causes Chagas's disease is also shed in this fecal material, and when the host scratches the wound site, it rubs the infectious fecal material into the bite wound to initiate the trypanosome infection.

Note the large size of this bug and the large proboscis with which it feeds. When not feeding, these bugs fold the proboscis under their bodies.

## 46. *Cimex* - the bed bug

The bed bug has a flattened body for hiding in small cracks. They come out of the cracks only to feed. They are very common in caves and burrows, where they feed on bats and other animals. They can become 'domesticated' and move into kennels and. houses. Once within an establishment, they are difficult to remove. They have not been incriminated as vectors of any disease with the possible exception being the spread of Hepatitis B in Africa.

Bed bugs have flattened bodies and three pairs of legs. The mouthparts are similar to those of the reduviid bug. They should not be confused with ticks.

**Exercise/Note:**
Draw and label mouth parts beg bugs

# OTHER INSECTS

## 47. Cockroach

Cockroaches have mouthparts that are not designed for the taking of blood meals, rather the domesticated species live off of the waste of those with whom they share habitation. They are vectors of several animal parasites, and in situations of animal containment such as primate colonies, their existence can become a bane to the control of several spirurid nematode and acanthocephalan parasites.

## 48. A beetle

Beetles, like cockroaches, have mouthparts that are not designed for taking blood meals. They are also, like cockroaches, vectors of several spirurid nematodes, some acanthocephalans, and some cestodes.

**Points to be noted:**

- Dung beetles are intermediate host for *Spirocerca lupi* (Oesophageal worm of dog) that is found in every single dog post-mortem in our college (CVAS, Jhang).

**Exercise/Notes:**

Collect the dung beetles from the livestock farms and dissect them to get the larvae of *Spirocerca lupi*.

# TICKS

Begin by comparing an argasid or soft tick with an ixodid or hard tick, the very first thing, count the legs and note that adult ticks have 8 legs. Next, note that the mouthparts of the soft tick are under the body while those of the hard tick protrude anteriorly in front of the body. Also, note that the hard tick has a scutum or shield on its dorsal surface. On the male hard tick, the scutum covers almost the entire dorsal surface; whereas on the female and nymph of hard ticks, the scutum only covers the anterior portion of the body. The scutum of *Dermacentor* is said to be ornate, i.e., it has varied colored markings on its surface. Soft ticks do not have a scutum on the male or the female. Their dorsum is covered by integument. Both male and female soft ticks suck blood several time during their life span.

## GENERAL INFORMATION ABOUT THE NUMBERS OF TICK SPECIES.

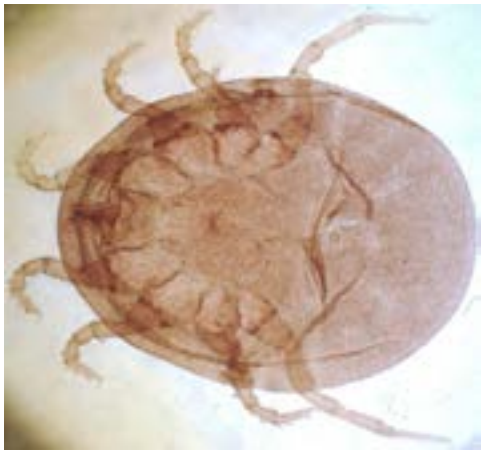
The differentiation of many species on morphological grounds is often a bit like shifting sand. Fortunately, for most control purposes, recognizing whether the tick is a hard tick or a soft tick is usually sufficient. Next the recognition of the occasional genus is important. It would probably be best to leave specific identifications to those who do it for a living or, at least, as a well practiced hobby.

### 49. *Argas*

This tick is classically identified by the sharp lateral margin of its body; this is very hard to see in the plastic embedded specimens.
The habits of most adult argasid are similar to bed bugs,
they sneak out of hiding places in the dark, take a blood meal, and race back to their hiding place. It is predominantly a pest of backyard poultry houses in Paksitan.

### 50. *Ornithodoros*

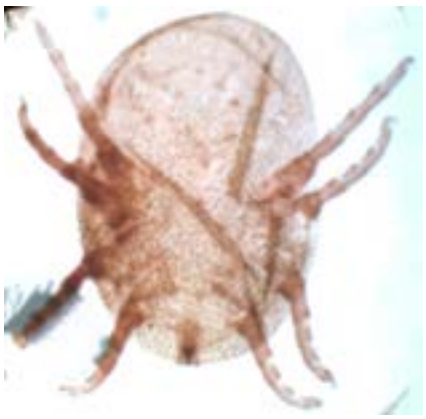
This argasid tick is superficially quite similar to Argas. The characteristic that is usually used to distinguish these two genera is the lack of a sharp lateral margin on Ornithodoros.



Again, this is difficult to see in this preparation. Note once again, however, that the mouthparts are underneath the body. The feeding behavior of this tick is much like *Argas;* the adult ticks come out of hiding places to feed.

**51. *Otobius***

These argasid ticks have different behavior than *Argas* and *Ornithodoros*. Larvae and nymphs are residents in the ear canals of cattle. The adults do not feed and are seldom seen. Usually, the location on the host will be sufficient to make a diagnosis of *Otobius*.



***52. Ixodes***

This tick is currently in vogue because *Borrelia burgdorferi*, the causative agent of Lyme disease, is transmitted by *Ixodes scapularis*. Unfortunately, this means that because clients will want to know if a tick is a potential vector, it will be necessary to recognize an *Ixodes* as an *Ixodes*. The fortunate part is that it is not too difficult; all one has to do is learn to recognize the "anal groove." Like an arch, the bases of which are at the posterior body margin, this groove curves around the anterior side of the anus. However, if you look at the specimen, you will note that the small size of this tick often makes this difficult.

Another approach is to look at the palps and to note that they are broadest at the junction of the second and third segments. Again, this will be a difficult task, but it is possible. The identification of a tick as an *Ixodes* is becoming a more and more common request, and veterinarians are expected to have some expertise. Thus, you should learn how this is done. Because the offending stage of *Ixodes* in cases of Lyme disease is usually a nymph, it is propitious that the same taxonomic criteria used for the identification of the adults as members of this genus hold for this stage as well.

The identification of a tick as *Ixodes* is not only of concern because people get Lyme disease. More and more cases of this infection with typical arthritic sequelae have been reported from dogs and other animals, thus, the responsibility for making a diagnosis extends beyond the client's health status to encompass the health of the pet as well. *Ixodes* spp. also transmit another pathogen that has zoonotic potential, *Babesia microti*, a protozoan parasite of rodents that also infects man. For a specific identification of any Ixodes species, it will be necessary to send the tick to an expert in tick identification. Only certain species transmit certain diseases, and therefore, for the certain identification of a vector competent species, a specific identification may be required.

**53. Nymph, *Ixodes scapularis***

This is the stage most commonly transmitting Lyme disease to man. The nymphs are very small, being only about 1.5 mm in length when unengorged. They have 4 pairs of legs as adult ticks do, but lack a genital opening. Note that even though the palps are long and thin, they are still widest at the junction between segments 2 and 3, a key character for *Ixodes* spp.

**54. Larval *Ixodes scapularis***

These stages are very small and only have 6 legs.

***55. Rhipicephalus***

This tick is a parasite of the dog. The key to the identification of this tick is the unornamented scutum, festoons on the posterior edge of the scutum, and a hexagonal basis capituli. Also, examine the coxae or plates, where the legs attach to the body. If the specimen is a male tick, note that these plates are about the same size from the anterior to the posterior of the tick. On *Dermacentor*, the coxae get progressively larger more posteriorly.

Ticks of the genus *Rhipicephalus* serve as vectors of the protozoan parasite, *Babesia canis*, and the rickettsia-like *Ehrlichia canis*. They are most important, however, as parasites of kennels where these three-host ticks can sometimes build up in massive numbers by feeding on *a* single species of animal, the dog.

**56. *Dermacentor***

This ixodid tick is characterized usually by its ornamented scutum. Also, notice that the basis capituli appears rectangular rather than hexagonal like *Rhipicephalus*. Think in terms of comparing this specimen with *Rhipicephalus*. Compare the shape of the basis capituli and the coxae on the ventral surface. Look for adanal shields on *Rhipicephalus*. Also, notice that both specimens have eyes and festoons.

Ticks of the genus Dermacentor commonly attack dogs, cats, and man. One species, Dermacentor andersoni, is an important vector of Rocky Mountain Spotted Fever. Dermacentor nitens is the vector of the equine parasite, Babesia caballi, in the extreme southern United States.

### *57. Boophilus (Now considered a subgenus of Rhipicephalus)*

The most important species of *Boophilus*, *Boophilus annulatus*, was eradicated from the southern and southwestern United States in the early part of this century. This species was important because it was the vector of "red water" fever, so-called not because it is a disease of cattle in areas of the Red River valley, but because of the hematuria caused by the protozoan parasite *Babesia bigemina*.

Species of *Boophilus* ticks have unornamented shields without festoons. They are distinguished from *Rhipicephalus* by the shape of the palpi and the lack of festoons. The basis capituli of this ixodid tick is hexagonal in shape.

### *58. Hyalomma*

This genus of ixodid ticks is most prevalent in Pakistan. Some species are important vectors of equine and bovine piroplasmosis.

### **59.** ***Amblyomma***

These ixodid ticks are usually ornate and have eyes and festoons. *Amblyomma americanum* the "lone star" tick is so called because of the large white area on its scutum. These ticks can be recognized by their long mouthparts and long second papal segment.

*Amblyomma americanum* tends to be a persistent biter of humans in all stages of its life cycle in the Western United States. It is an important vector of Rocky Mountain spotted fever and tularemia.

### **60.** ***Haemaphysalis***

These ixodid ticks have palpi with laterally flared second segments. Do not confuse these lateral flares with the hexagonal shaped basis capitulum of *Rhipicephalus*. These ticks are occasionally found on cats.

## 61. Larval Tick (seed tick)

Note that this tick has 6 legs. It is a larval stage.

**Points to be noted:**

- Only mouth parts of hard ticks are visible dorsally.
- In hard tick, females and nymph-only anterior part of abdomen covered with scutum.

**Exercise/Notes:**

Collect ticks from dog, cattle and horse and identify their genera

# MITES

There are three suborders of mites of veterinary importance: the Astigmata, the Prostigmata , and the Mesostigmata. These 3 groups can be differentiated by whether or not they have stigmata (spiracles), and if present, by where they are located. The astigmata have no stigmata. The prostigmata have stigmata near the gnathostoma (the mouthparts). The mesostigmata have stigmata located behind the third pair of legs, and some have a structure, the peritreme (a tracheal tube), that runs anterior from the stigma.

The astigmatids are small and designed for living in or closely attached to the hair of their hosts; *Sarcoptes* can be considered a typical member of this group. The prostigmatid *Demodex* is an atypical member of this group in that it is designed for life within hair follicles. Most prostigmatids live more superficially, but members of the family trombiculidae are parasites of the skin as larvae.

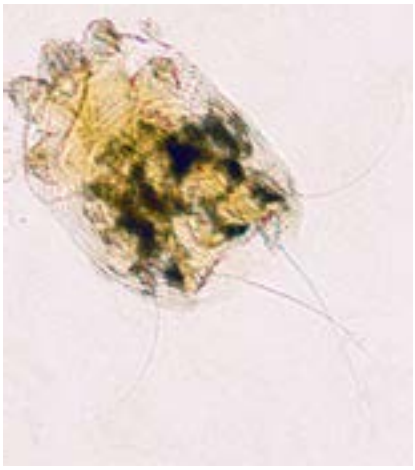
Mesostigmatids are usually larger and designed for an existence external to the host which they feed on in nests; of course some are parasites of other sites, e.g., Pneumonyssus and Pneumonyssoides live in lung parenchyma or nasal sinuses of their respective hosts.

**62.** ***Sarcoptes***

Take some time to become familiar with this astigmatid mite. It will be necessary to differentiate *Sarcoptes* from *Psoroptes* and *Chorioptes*, thus, take some time to learn basic features. Notice the number of legs that are present, and be able to recognize pretarsi, pedicels, and caruncles. The pretarsi can be used to separate these 3 genera, and they are pathogens with consequences that are serious enough to warrant learning the difference. One other feature worth noting is the location of the anus; it is at the posterior edge of the body. The position of the anus can be used to differentiate *Sarcoptes* from *Notoedres* which has its anus located on the dorsal surface of its body.

**63.** ***Notoedres***

This parasitic astigmatid mite is usually found on cats, rodents, and lagomorphs. It has long pedicels like *Sarcoptes* but it differs from *Sarcoptes* in the location of the anus.



**64.** ***Psoroptes***

This astigmatid mite causes mange in horses, cattle, and sheep. They can be differentiated from *Sarcoptes* and *Chorioptes* by the pretarsi. Note that the pedicel is relatively long and jointed. Remember, long and unsegmented in *Sarcoptes*, long and segmented in *Psoroptes*, and short in *Chorioptes*.

**65.** ***Chorioptes***

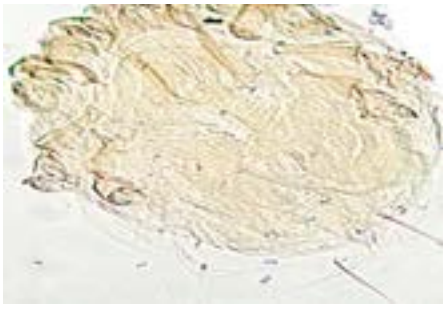
This astigmatid mite also infects cattle, sheep, and horses. Again look at the pretarsi and note that the pedicel is short. By now, it should be rather a simple task to differentiate *Sarcoptes*, *Psoroptes*, and *Chorioptes*.

**66.** ***Otodectes***

This astigmatid mite is found in the external ear canal of dogs and cats. It can be differentiated from Sarcoptes and Notoedres by the pretarsi. Otodectes has short pedicles, those of Sarcoptes and Notoedres are relatively long. Look at the 3 specimens (12, 13, and 16) and differentiate them.

**67.** ***Knemidokoptes***

This astigmatid mite infects gallinaceous birds causing scaly leg. Note that the female is very round in appearance and lacks pretarsi.



**68.** ***Myocoptes***

This astigmatid mite is a parasite of rodents. Note the general appearance of these mites, and the modification of the legs for hair clasping.

**69. *Demodex***

These prostigmatid mites live in the hair follicles of the mammalian host. In some animals they seem to multiply into huge populations causing mange in the host. From a diagnostic point of view, they are easy to identify by their wormlike appearance and short, stubby legs. Skin should be lift up during skin scrapping to get these mites.

**70. Trombiculidae, chigger**

The prostigmatid mites are parasitic only as larvae when they feed on various mammals or birds. Count the legs and note that only 6 are present. Learn to recognize the dorsal scutum and the two large plumose setae (they may be broken off on some specimens) that identify this larval mite as a trombiculid.

**71. *Dermanyssus***

This mesostigmatid mite usually is found in the nests or roosts of birds on which they feed. Note the location of the stigma and the peritrerne. The chelicerae of this mite are longer and more slender than those of *Ornithonyssus*.

***72. Ornithonyssus***

This is another mesostigmatid mite that feeds on birds and rodents. These mites tend to spend more time on their hosts than *Dermanyssus* from which they can be differentiated by their stouter chelicerae.

**Excersice/Notes:**
Skin scrapings of infected skin of two dogs, two cattle, two horses and two domestic poultry

# METHODS

## THE MICROSCOPES

The organisms that will be studied in this course tend, unlike viruses and bacteria, to be of a size that allows a great deal of structural study to be done with conventional microscopy. At your disposal in this class are 2 microscopes, the stereo- or dissecting-microscope and the compound microscope. It will be to your benefit to spend a little time familiarizing yourselves with these 2 very important tools for the examination of parasites.

Stereomicroscopes are basically a glorified magnifying glass giving between 10 to 40 magnifications. The stereoscopes in this laboratory have been designed to work with both incident and transmitted light. You can become familiar with the operation of this microscope by examining the various specimens in the supplied study set. At this time, do not try to learn features of identification, just concentrate on using the scope with maximum effectiveness.

Examine each specimen with the transmitted light that is produced by inserting the lamp into the hole in the back of the microscope base. Try both sides of the substage mirror at various angles the white side usually works better than the silvered side. You will find that transmitted light is worthless for viewing opaque specimens. Now try reflected light by inserting the lamp in the hole in the microscope standard. This provides light at a sort of standard angle and usually works fairly well if never brilliantly. Lastly, mount the lamp in the clamp atop the transformer and direct the light at the specimen from various elevations. You will find that some specimens that might have been virtually invisible by other methods become beautiful when shown 11 in the right light".

The compound microscope is a more intricate device than the stereo microscope. The microscopes in this laboratory, like most routine microscopes, are designed for observation using transmitted light. They have been designed to provide between 40 and 1000 magnifications. Later in the course, it will be necessary to learn to use the ocular micrometers to measure specimens, but at this time, concentration will be on the operation of the microscope itself.

Because of the more complicated nature of the compound microscope, it is necessary that the various lens elements be in proper orientation to produce the best possible image. The ultimate goal of the element arrangement is to produce a light path that has a few scattered rays as possible. This is done by the manipulation of the substage condenser and the field diaphragm located on the base plate of the microscope. A procedure for properly adjusting the light is outlined below, and it should be noted from the following that for the best possible observation, it is necessary to adjust the field diaphragm every time the objective lens is changed.

# USE OF THE STEREOMICROSCOPE

## Materials needed for exercise:

- 1 stereomicroscope
- 1 light source
- Opaque specimens (for example, plastic-embedded specimens of adult flies from study set 1-2)
- Transparent specimens (for example, slides 1-3 from study set 1-2)

## Procedure for opaque specimens:

- Place the light in the arm of the light stand, so that it is aimed at the specimen at an oblique angle (NOTE, the light may also be placed in the receptacle at the top of the microscope, in the back, but this does not allow the maneuverability you need).
- Turn on the light.
- Place the specimen on the glass plate on the base of the microscope.
- Adjust the intraocular distance to suit your eyes.
- Look through the microscope at your specimen; if you can't see it, adjust the power (upper knob on side) to the lowest power, and focus (lower knob at rear).
- To see details of your specimen in this position, adjust the angle of the light until the scratches are no longer apparent.
- Now try to get a better look at the mouthparts by moving your specimen to a different angle; you may also have to adjust the angle of the light to render the scratches invisible.
- Keep adjusting the angles of your specimen and of your light until you can see the specimen well (you may want to hold the light in your hand to quickly find a good angle, but be careful not to burn yourself as the light will become hot).
- If your specimen is very large, such as the horse fly (specimen 4 in study set 1-2), you may CARE-FULLY remove the glass plate from the stage of the microscope, placing it safely on a flat surface, and, and holding your specimen at various angles with your hand, find the best angle; PLEASE REPLACE THE GI.ASS PLATE when done.
- If you are still having trouble, get an instructor to help you.

## Procedure for transparent specimens:

- Place the light in the receptacle in the back of the microscope, at the bottom.
- Place the specimen on the stage of the microscope.
- Turn the mirror over so that the frosted glass is up and light is reflected up through the specimen.
- Adjust the power and the focus so that you can see the structures you want.
- If the spot of light being reflected is not big enough, you may back the light out of the receptacle slightly, so that the body of the lamp rests directly on the table.
- If needed, turn the mirror around until the light is best for what you want to see.

# USING YOUR OCULAR MICROMETER

In order to make a positive identification of eggs, oocysts, and larvae in stool samples, it is essential to determine the size of the specimen in question. To measure such specimens, you must use your ocular micrometer.
Each microscope is equipped with a precision made scale on a small glass disc in one of the four eyepieces. You can measure an object by rotating your eyepiece so that the scale falls over your specimen, noting the ocular units, and multiplying by the number of μm corresponding to the objective lens you are using.

## OCULAR MICROMETER CALIBRATION

| | |
|---|---|
| 4X objective | 1 ocular division - 25 *μm* with |
| 10X objective | 1 ocular division - 10.5 *μm* |
| 40X objective | 1 ocular division - 2.3 *μm* |
| 100X objective | 1 ocular division - 1 0 *μm* |

## MOUNTING MAGGOT SPIRACLES FOR IDENTIFICATION

**Materials:**

- Stereoscopic (dissecting) microscope and light preserved maggot specimens
- Fine forceps
- Razor blades
- Plastic petri dish
- Berlese mounting solution (see instructor for formula) slides
- Coverslips, marking pencil, slide box.

## Procedure:

- Place a maggot in the petri dish.
- Observe under the dissecting microscope, and perform the subsequent steps under the dissecting microscope.
- Note that the maggot is roughly conical, with the anterior end being narrow, and the posterior end being wide.
- With your forceps, hold the maggot at about midbody, and using the scalpel, slice off the last lmm of the posterior.
- Turn the slice so that the posterior surface is upward: you should see a pair of spiracles (refer to pp. 18-19 in your text).
- Holding the edge of the tissue with your forceps, trim off as much tissue as possible from around the spiracles so that they are lying flat: this is necessary to make a good permanent mount.
- Transfer the spiracles to a slide, and place a few drops of Berlese fluid on the spiracles, taking care not to make bubbles.
- Carefully lower a coverslip onto the preparation so that no bubbles are formed but that the spira-

cles are lying flat and are well covered by the Berlese fluid.
- Label the slide with your name and place it flat in the 60°C drying oven that is above the refrigerator in the front of the lab.
- In the next laboratory period, retrieve your slide and store it flat in your locker.

## FLEA DIRT CHROMATOGRAPHY

**Materials:**

- Flea dirt
- Whatman no. 1 filter paper (normal bond or notebook paper will also work)
- dilute detergent solution petri dish

**Procedure**

- Uniformly wet the filter paper with the detergent solution.
- Place the wet paper in the petri dish.
- Sprinkle a small amount of flea dirt on the paper.
- Observe the paper occasionally over the next 20 minutes for evidence of blood.

## SKIN SCRAPING AND KOH DIGESTION FOR DIAGNOSIS OF MITES

**Materials:**

- Gloves, skin from mange case, razor blades, scalpel handles and blades glycerin, 5% KOH (on front sink), protective goggles (on front sink) hot plate (on front sink), beakers (on front sink), watch glasses (on front sink) conical centrifuge tubes centrifuge. applicator sticks, sugar solution, s.g. 1.2 slides, coverslips, tap water bottle petri dish, Pasteur pipettes dropper bulbs dissecting needle fine forceps Berlese solution marker

**Procedure (note, skip to step 16 when you find a mite):**

- Prepare a clean razor or scalpel blade by placing a drop of glycerin on the blade.
- Wearing clean gloves, grasp the preserved skin firmly between your thumb and forefinger, pinching so that the epidermis is up.
- Scrape the pinched skin with your prepared blade until you reach the dermis, collecting the scrapings on your blade.
- Examine the bladeful of scrapings under your dissecting microscope for evidence of mites.
- If you are lucky and see a mite, you may now place a drop of Berlese in a slide, and transfer the mite, with a probe or fine forceps, from the blade to the drop of Berlese. Add a coverslip and examine the mite with the compound microscope.
- If you were unlucky at the above step and either did not see a mite or were unable to make an identification, transfer a small amount of the scrapings from the blade to a slide, using a dissecting needle. Then add a drop of glycerin, and cover with a coverslip.
- Observe under a compound microscope for evidence of mites. If you are lucky, identify your spec-

imen. If you cannot yet make an identification, continue with step 8.

- Transfer the remaining scrapings to a beaker, and add about 15-20 ml 5% KOH. (You may pour this over the blade to rinse the scrapings into the beaker.) YEAR GOGGLES for this step.
- Cover the beaker with a watch glass and heat the mixture on the hotplate for about 20 min.
- Remove the mixture from the hotplate, let cool about 5 min, remove the cover, and them carefully swirl and pour the contents into a 15 ml conical centrifuge tube. WEAR GOGGLES for this step.
- Spin (at max speed on yellow centrifuge or at "20" on large gray centrifuges) for 10 min, and carefully decant the tube into a sink with running water ...DO NOT SPLASH.
- With a Pasteur pipette, gently mix up the sediment and transfer a drop to a slide. Add a coverslip and examine for mites using the compound microscope. If you still cannot make an identification, continue with step 13.
- Add about 10 ml sugar solution to the sediment remaining in the centrifuge tube, mix well with an applicator stick, fill the tube to the top with sugar solution, and add a coverslip.
- Centrifuge as in step 11.
- Lift coverslip straight up so that a drop adheres to it, and place it on a slide; examine for mites under a compound microscope.
- If you wish to make a permanent mount of your mites, you may rinse off the slide and coverslip into a small petri dish with tap water, and under the dissecting scope, transfer mites to a clean slide. Add a drop of Berlese and a coverslip, and dry in a 60˚C oven.

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