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Field Guide to Collecting Parasites

Contents

TECHNIQUES

Recording data

- **Record host collector number.** Label parasites with this number as soon as they are removed from the host. Keep labels with the parasite specimens as they are being processed.

Example of label that goes in parasite vials. Be sure to write with indelible ink, or use a pencil and press down firmly.

Host *Genus species*
Collecting locality, Date,
Collector name and #

- Keep information regarding the parasites collected in a notebook. At a minimum the following information needs to be recorded: for each host specimen record the host collector number and indicate what parasites were collected from that host. It is important to indicate which hosts were searched for parasites **even if NO** parasites were collected
- For trips without a dedicated parasitologists this information can be written in the vertebrate collectors notebook On trips where there is a dedicated parasitologist then this information should be written in a a notebook that is dedicated to the parasites. An example of a data sheet for a parasite notebook is included on the next page.
- If there is a dedicated parasitologist on the expedition, then he/she should assign each vertebrate a parasite # (P#) , and put a label on the vertebrate specimen with this number. This is an important procedure because many of the vertebrates will be processed for parasites before the vertebrate biologists assigns a collector number. In this scenario the vertebrate collectors MUST record the parasite number in their notebooks so that the P# can be linked to the right collector #. At the end of each day the parasitologist should go through all of the vertebrate notebooks to record the associated collector # and host species in the parasite notebook.

Example data sheet for notebook used by a parasitologist

Parasite Collection Data Sheet **P#**_____

Collector/Field #_____ Date_____

Bird_____ Mammal_____ Reptile_____ Amph._____

Host species_____ Sex: M, F, Unkn

Host prep: Skin, Skull, Skeleton, Fluid: EtOH / Formalin

Lat / Long / Elev_____

Country / State _____

Specific locality _____

_____

For each parasite check (√) if the host was examined, even if no parasites were found. Indicate samples/parasites that were was collected. For endos- indicate where they were found (e.g. lungs, bladder, etc.). Use pencil or permanent ink.

Blood	**Endoparasites**
Flu Swab	Roundworms
Ectoparasites	Thorny-headed worms
Fleas	Tapeworms
Flies	Flukes
Lice	Other/Unkn
Mites	
Ticks	**Coccidia**
Other/Unkn	**Other/Unkn**

Notes:

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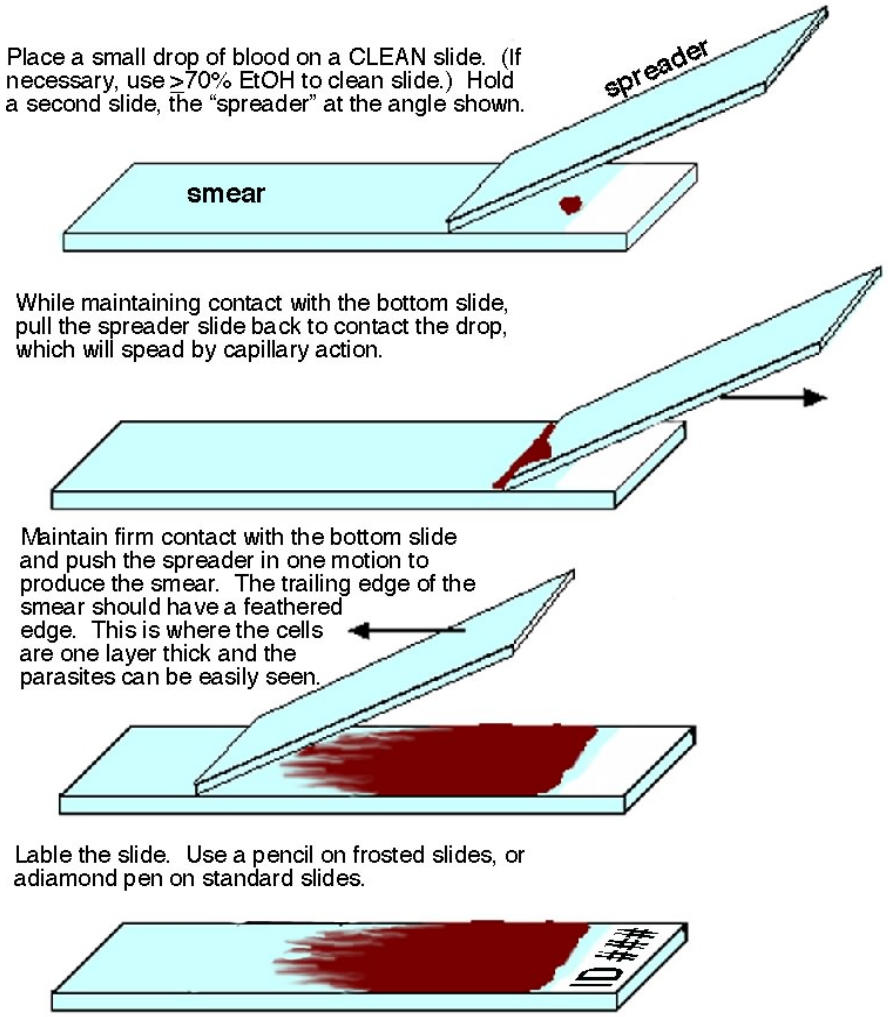
Blood samples

- Get drop of blood. Using a scalpel nick a blood vessel and collect the blood with a 30-70μl heparinized capillary tube.
  - **Birds**- Nick vessels in the tibio-tarsi. Another common methods is to nick the brachial artery, but this gets blood on the feathers, which is not desirable for birds that are destined to become museum skins. It is also possible to get blood by clipping toenails/claws.
  - **Mammals –** Nick vessels in the tip of the tail, or the artery at the base of the tail (ventral side). For bats, try nicking vessels in the tail membrane or along the leading edge of the wing. For tricky specimens, nicking the femoral vein, or the toe-pads may work.
  - **Herps –**Frogs, Toads, and Salamanders: nick the tail vein, ventral abdominal vein, or lingual venous plexus, or clip a toe-nail. Lizards and Snakes: nick the ventral tail vein, femoral artery, or clip the tip of the tail or toe-nail.

- Make 2-smears per vertebrate specimen (see diagram).
  - Each end of the "spreader" slide can be used only once. After that, the spreader slide can be used as a smear.



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- Preserve the rest of the blood in the capillary tube for DNA work by blotting it on filter paper. Several blood samples can go on each filter paper, as long as they are all labeled clearly. Store the filter papers in a cool dry place.
- Air dry smears as rapidly as possible - gently blow on slide as it rests face up on back of your hand.
- Fix IMMEDIATELY after the slides are dry by soaking in 100% methanol for 2-5 minutes. This is best done with a Coplin jar. Change the methanol in the jar every day or two.
- Store dry slides in a slide box. Keep specimens as cool and dry as possible.
- Send specimens to parasite specialist as soon as possible after returning from the field so that they can be stained.

Flu Screen

- Swab the mouth and the cloaca with a sterile cotton swab.
- Put the swab in a vial and clip off the swab stick with scissors so that the cotton end of the swab can be sealed in the vial. Label the outside of the vial with an ethanol proof marker.
- The solution that the swab goes into depends on circumstance, so prior to the trip it is best to check with the virologist who will screen the samples. If samples can be refrigerated/frozen in the field use “RNA later” (or equivalent) otherwise put samples in 95% ethanol.
- Keep vials as cool and dry as possible in the field, then freeze upon return from the field.

Ectoparasites

- Ectoparasites can transfer among dead hosts, so NEVER let dead animals contact each other. Keep live birds in paper bags. Keep dead birds in paper or Ziplock bags. NEVER re-use bags. For mammals and herps, thoroughly inspect and clean out cloth bags and traps before each use to prevent parasites from getting on the wrong host.

Fumigation (birds and mammals)

- Euthanize the bird/mammal. Note: if the entire body was exposed to chloroform for ≥ 15 min during euthanasia additional fumigation is not necessary, skip to ✶ below.
- Place the dead bird/mammal in a CLEAN tupperware.
- Place a cotton ball moistened with Ethyl Acetate in the tupperware. (If ethyl acetate is unavailable use chloroform, or fingernail-polish-remover made with ethyl acetate.) The cotton ball need not be soaked, but should be moistened enough to fill the chamber with fumes. Replace the cotton ball frequently. Ethyl acetate is harmless even if you breathe it, so there is no need for concern.

- Seal the tupperware and fumigate for ≥15 min (for large animals ≥ 20-30 min).
- CAREFULLY remove the bird/mammal from the tupperware. Be aware that parasites can easily fall off of the host at this stage. Inspect and remove parasites from the tupperware.
- **Birds -** over a large sheet of CLEAN paper (a cafeteria tray is really helpful for this procedure), ruffle all of the bird's feathers with one hand, while holding the bird with the other hand.

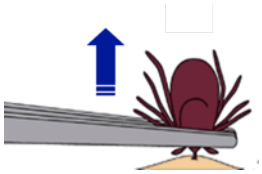
- ✶**Mammals -** place mammal on a large sheet of CLEAN paper and remove obvious ectoparasites such as ticks, bat flies, nose & ear mites, and burrowed parasites (see below), then use a metal-tined comb to brush the fur to remove fleas, lice and mites.
- Pick up ectoparasites from the paper using the tip of the forceps or a brush moistened in alcohol. DO NOT pick up parasites by grabbing them with the forceps as this can damage them; if you do grab them be careful. Dunk the forceps or brush tip into the vial of alcohol to dislodge parasites. CAREFULLY examine the brush after each bird/mammal for parasites that may be stuck between the bristles.

- Place ectoparasites from each individual host into a vial containing 95% ethanol.
- CLEAN the tupperware and collecting surface thoroughly (or use a new sheet of paper) between each animal in order to avoid erroneous host-parasite records.
- Label the vial with a cardstock label written with indelible ink that will not run in alcohol, or use a sharp pencil and press down firmly.

An alternative to fumigation with ethyl acetate is to "**dust**" with flea powder. Sprinkle a little flea powder on the bird/mammal; a robin-sized bird typically needs about a tablespoon of powder. Rub it into feathers for several minutes to distribute the dust, while holding the bird over a large sheet of CLEAN paper. Then ruffle the bird (or comb mammal) to dislodge parasites as described above.

Removing other ectoparasites

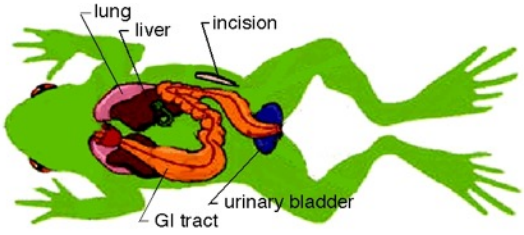
- **Feather mites** - hold the flight feathers of the bird over a light. Feather mites will look like grains of sand lodged between the feather barbs. If mites are present use a brush moistened with 95% ethanol to slide mites from between the feather barbs.
- **Mites –** some mites will be dislodged by ruffling feathers and combing fur (see above); however, other mites will need to be removed by other means, especially on herps. Mites that are around the nose, ears, eyes, and wing/tail membranes of bats, and mites under scales of herps can be removed with a brush or forceps. In cases where there are a lot of mites clumped together use a scalpel or scissors to cut off a small section of skin with the attached mites.



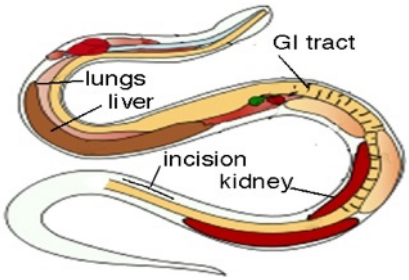
- **Ticks –** using forceps, grip the tick where it is attached to the skin, pull straight up. It is important to grab ticks as close to the skin as possible to keep their mouthparts intact. Put ticks in 70% ethanol for 1wk then move to 95%.
- **Burrowed parasites –** Some fleas and flies burrow under the skin near claws/nails, ears, or along the wing/tail membranes of bats. To remove these parasite use a scalpel and carefully cut around the skin near the parasite to remove it. Don't worry if you don't recognize the parasite when you remove it from the skin, it will probably look like a little white blob – that's okay.
- **Leeches** – pull leech from vertebrate. Kill and relax leech in a carbonated liquid such as carbonated water, soda pop, or beer, or you can use boiling water. It may take several hours – overnight before leech is limp, preserve in 70% ethanol.

Worms

- Consult vertebrate biologists to minimize damage done to the vertebrate specimens being collected.
- **Birds/Mammals**- dissect internal organs of specimens that are going to be skins, or if a skeleton remove organs without damaging any bones. Look primarily in the GI tract and liver. If time permits, other sites of interest in birds are the bursa (interesting flukes), and the eyes of raptors (interesting roundworms).



- **Herps**- For **amphibians** look for worms under the tongue, and then use forceps to remove the GI-tract, liver, lungs and urinary bladder through a small incision in the lower abdomen. For **reptiles** look for worms in the mouth and cloaca, then pull the GI-tract and attached organs through a small incision near the cloaca. If possible, pull until the lungs can be removed for dissection. Examine the GI-tract and lungs (look for small nematodes in the lungs).



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- During dissection keep the organs moist (or submerged) with physiological saline.
  - To make saline solution add 9g of NaCl to 1L water.
- When dissecting organs, slit the organ open along the length and then examine the contents. For the GI-tract it can be helpful to take a glass microscope slide and gently slide the edge of it along the opened GI-tract – this spreads out the intestines and make some worms more visible.
- Prepare the worms.
  - Roundworms (nematodes): relax in hot saline (NOT water), preserve in 70% ethanol.
  - Thorny-headed worms (acanthocephalans): be sure to remove worm with proboscis intact (even if it means cutting out part of the host tissue), relax in water until proboscis everts (may require several hours – overnight), preserve in 70% ethanol.
  - Tapeworms (cestodes) relax in water or hot saline until worm is dead and limp, preserve in 70% ethanol. (These worms can also be relaxed by soaking them in water overnight.)
  - Flukes (trematodes) relax in water or hot saline until worm is dead and limp, preserve in 70% ethanol. (These worms can also be relaxed by soaking them in water overnight.)
- When using hot saline to kill worms in the field.
  - Prepare a thermos with hot (near boiling) water. The thermos will stay hot enough to process worms for several hours.
  - Add enough salt to make a physiological saline solution (9g NaCL salt in 1L of water). Pre-packaging vials with the right amount of salt for the thermos makes this procedure very easy.
  - Pour the hot saline into a petri-dish with worms that have been removed from the vertebrate specimen. As the worms die, they should appear to relax in the solution.
- Move the dead worms into vials with 70% ethanol. To avoid damaging worms, use a pipette to move them, or a dissecting needle (not forceps).
- Dissecting hosts and preserving worms in the field provides the best quality specimens. However, if time is short, freeze GI-tracts of birds/mammals/herps and lungs from reptiles in liquid nitrogen for later dissection in the lab.

Coccidia

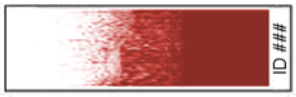
- Coccidia are described from oocysts. The oocysts are best preserved from fresh feces that are placed directly in 2-2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$) in a ratio of 1 volume of feces in ≥ 5 volumes postassium dichromate. In field collections, screw-cap scintillation vials work well, but one should not fill the vial all the way to the top; leave a layer of air between the top of the feces-dichromate mixture and the cap to allow the oocysts some atmospheric oxygen.
- for **birds** if there is fecal matter in the paper holding bags, you can tear the bag and put the piece of paper with attached fecal matter into the solution.
- It is easiest to take pre-measured vials of potassium dichromate to add to water in the field (2-2.5g of $K_2Cr_2O_7$ for 1L water).

BIRDS

Parasite Checklist

○ **Do NOT put birds in cloth bags** because it can cause erroneous parasite records when the bags are reused. Keep live birds in paper bags and only use a bag once. Dead birds can be placed in paper or ziplock bags.

○ Assign and **record host collector number.** Label parasites with this number as soon as they are removed from the host.



○ Sample **blood**
- 2 blood smears, fix in methanol
- 1 DNA sample on filter paper

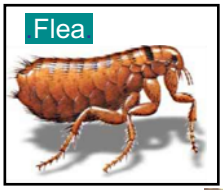
Note- sampling blood is best with live and freshly dead birds. If the bird has been dead >5 min it is not worth sampling blood for parasites.

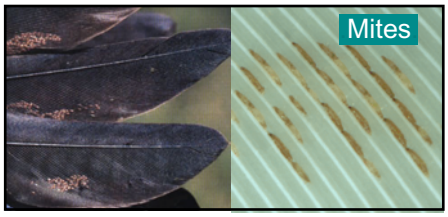
○ Euthanize bird

○ Swab for **flu** screen (preserve swab in buffer)

○ Fumigate bird (≥ 15min), then ruffle feathers to remove:
- **Fleas, Flies, Lice** and some **Mites** - Preservation: 95% ethanol.

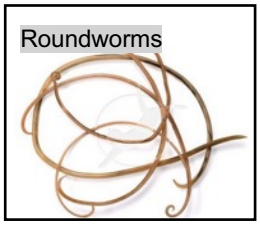
○ Visually search and remove ectoparasitic:
- **Feather mites** - Preservation: 95% ethanol.
- **Ticks** - Preservation: 70% ethanol, move to 95% after 1 week. This step is important to keep engorged ticks from exploding.





○ **Prepare bird specimen.**

○ **Collect worms.** If the bird is to become a **skin/skeleton**, dissect internal organs. Look primarily in the GI tract. If time permits, other sites of interest are: liver, bursa (interesting flukes), and the eyes of raptors (interesting roundworms).
- **Roundworms** - Identification: slender worms, head and tail pointed, often coiled. Preservation: relax in hot physiological saline (NOT water), preserve in 70% ethanol.



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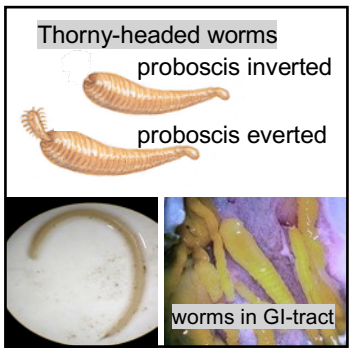
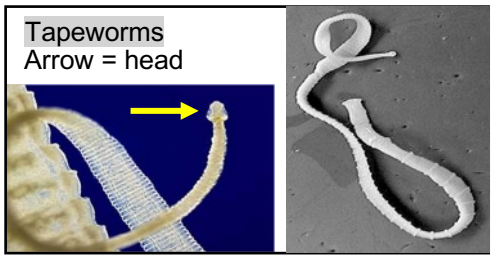
- **Thorny-headed worms** - Identification: stout worm that may appear segmented. If head of worm is imbedded in host tissue remove the section of host tissue with a scalpel to avoid damaging the proboscis. Preservation: relax in water until proboscis everts (several hours – overnight), preserve in 70% ethanol.



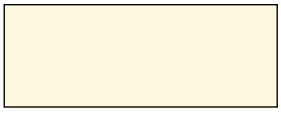
- **Tapeworms** - Identification: flat segmented worms. If head of worm is imbedded in host tissue remove the section of host tissue with a scalpel to avoid damaging the head. Preservation: relax in hot or cold water or hot physiological saline until worm is dead and limp, preserve in 70% ethanol.

- **Flukes -** Identification: flat worms, typically with two suckers. Preservation: relax in hot or cold water or hot physiological saline until worm is dead and limp, preserve in 70% ethanol.



- **Coccidia.** If there is at least 1ml of feces in intestinal tract collect a sample for coccidia screening. Preservation: potassium dichromate ($K_2Cr_2O_7$), there should be at least 5x as much $K_2Cr_2O_7$ as feces, leave space for air in top of vial.

- **<u>RECORD DATA</u>** Make sure all of the parasite specimens are labeled (see example on right). In a notebook indicate what categories of parasites were collected from each host. It is also important to indicate that hosts were searched for parasites even if **NO** parasites were collected.



- **Prioritizing specimens.** It is impossible to get all of the parasites from all of the collected vertebrates. The priorities for each expedition may be different, but here are a few rules of thumb.
  - Be opportunistic; if you see a parasite collect it, even if you were not planning to examine the host for parasites.
  - Get blood, flu and ectoparasites from as many birds as possible. These are the quickest and easiest procedures.
  - Processing worms takes the most time, so be more selective with this procedure. Try to get a broad sample of biodiversity by processing 2-5 individuals of each host species. Dissect what you can in the field (best case), and freeze the GI tracts of others for dissection in the lab.

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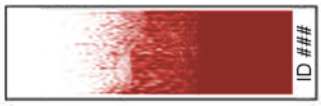
MAMMALS

Parasite Checklist

- ○ Thoroughly inspect and clean out cloth bags and Sherman traps before each use to prevent erroneous parasite records between hosts.
- ○ Assign and **record host collector number.** Label parasites with this number as soon as they are removed from the host.
- ○ Sample **blood.**
  - 2 blood smears, fix in methanol
  - 1 DNA sample on filter paper

  Note - sampling blood is best done with live or freshly dead mammals. If the mammal has been dead >5 min. it is not worth sampling blood for parasites.



- ○ **Euthanize mammal**. If the body of the mammal was exposed to chloroform for ≥ 15min then additional fumigation for ectoparasites is not necessary, otherwise **fumigate** it for ≥ 15min to kill ectoparasites.
- ○ Visually inspect and remove:
  - **Flies** - Very common on bats. Some bat flies do not have wings; they look more like spiders. Preservation: 95% ethanol.
  - **Mites** - Preservation: 95% ethanol.
  - **Ticks** (Acari: Ixodoidea). Preservation: 70% ethanol, move to 95% after 1 week. This prevents engorged ticks from exploding.
  - **Burrowed parasites**. Some flies and fleas burrow underneath the host's skin near claws and nails, and along the wing bones of bats. Excise these parasites with a scalpel. These parasites look like white blobs, not like a recognizable insects.

- ○ **Comb** through fur to remove:
  - **Fleas**, **Lice** and additional **Mites**: Preservation 95% ethanol.
- ○ **Prepare mammal specimen**
- ○ **Collect worms.** If the specimen is to become a **skin/skeleton**, dissect internal organs. Look primarily in the GI tract and liver.

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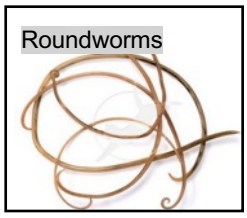
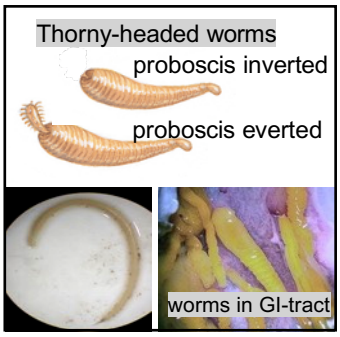
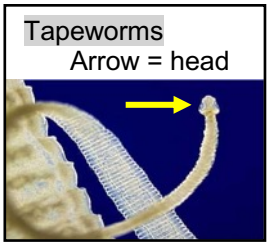
- **Roundworms** - Identification: slender worms, head and tail pointed, often coiled. Preservation: relax in hot physiological saline (NOT water), preserve in 70% ethanol.





- **Thorny-headed worms** - Identification: stout worm that may appear segmented. If head of worm is imbedded in host tissue then remove the section of host tissue with a scalpel to avoid damaging the proboscis. Preservation: relax in water until proboscis everts (may require several hours – overnight), preserve in 70% ethanol.



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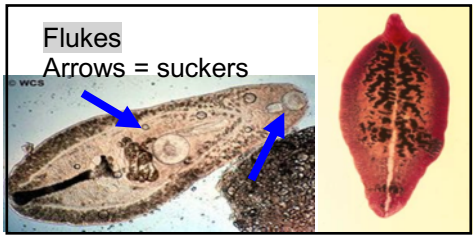

- **Flukes** - Identification: flat worms, typically with two suckers. Preservation: relax in hot or cold water or hot physiological saline until worm is dead and limp, preserve in 70% ethanol.

- **Coccidia** If there are 1-2mls of feces (about 5-10 small mammal pellets) in the collection bag, trap or intestine collect a sample. Preservation: potassium dichromate ($K_2Cr_2O_7$), there should be at least 5x as much $K_2Cr_2O_7$ as feces, leave space for air in top of vial.

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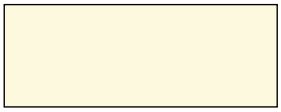

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HERPS

Parasite Checklist

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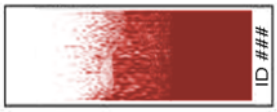
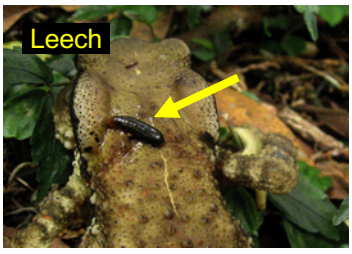
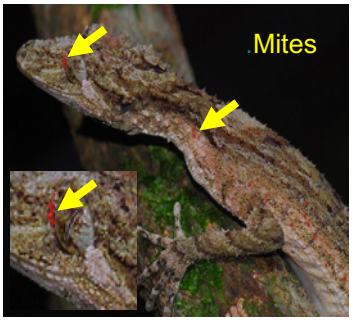




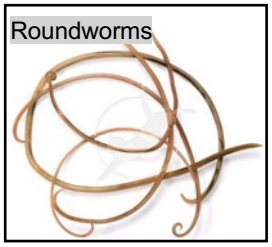
- Sample **blood**
  - 2 blood smears, fix in methanol
  - 1 DNA sample on filter paper

  Note - sampling blood is best done with live or freshly dead herps. If the animal has been dead >5 min. it is not worth sampling blood for parasites.



- Visually search to remove:
  - **Leeches** - Preservation: relax in carbonated water (soda or beer) or boiling water until leech is dead and limp (several hours – overnight), preserve in 70% ethanol.
  - **Mites** - Mites are usually found buried under the scales or near the eyes, mouth and nostrils; they often look like tiny black or red dots. Preservation: 95% ethanol.
  - **Ticks** - Preservation: 70% ethanol, move to 95% after 1 week. This step is important to keep engorged ticks from exploding.

- **Collect worms.** For **amphibians** look for worms in the mouth, GI-tract, lungs and bladder. For **reptiles**, examine the mouth, GI-tract, lungs (interesting nematodes), and cloaca.
  - **Roundworms** (Nematodes). Identification: slender worms, head and tail pointed, often coiled. Preservation: relax in hot physiological saline (NOT water), preserve in 70% ethanol.



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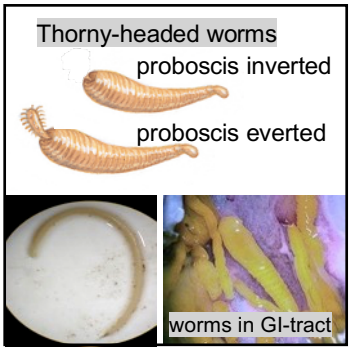
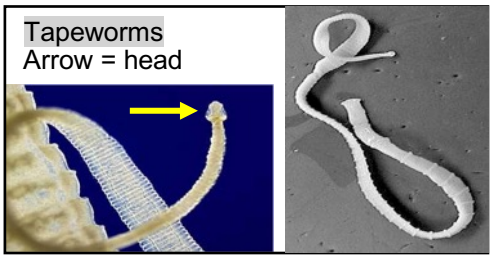
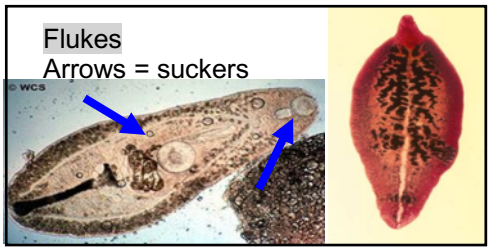
- **Thorny-headed worms** (Acanthocephalans). Identification: stout worm that may appear segmented. If head of worm is imbedded in host tissue then remove the section of host tissue with a scalpel to avoid damaging the proboscis. Preservation: relax in water until proboscis everts (several hours – overnight), preserve in 70% ethanol.



- **Tapeworms** (Cestodes). Identification: flat segmented worms. If head of worm is imbedded in host tissue then remove the section of host tissue with a scalpel to avoid damaging the head. Preservation: relax in hot or cold water or hot physiological saline until worm is dead and limp, preserve in 70% ethanol.



- **Flukes** (Trematodes). Identification: flat worms, typically with two suckers. Preservation: relax in hot or cold water or hot physiological saline until worm is dead and limp, preserve in 70% ethanol.



○ **Coccidia** If there is at least 1ml of feces in intestinal tract collect a sample for coccidia screening. Preservation: potassium dichromate ($K_2Cr_2O_7$), there should be at least 5x as much $K_2Cr_2O_7$ as feces, leave space for air in top of vial.

○ **<u>RECORD DATA</u>** Make sure all of the parasite specimens are labeled (see example to the right). In a notebook indicate what types of parasites were collected from each host. It is also important to indicate that hosts were searched for parasites even if **NO** parasites were collected.

○ **Prioritizing specimens**. It is impossible to get all of the parasites from all of the collected vertebrates. The priorities for each expedition may be different, but here are a few rules of thumb.

- Be opportunistic; if you see a parasite collect it, even if you were not planning to examine the host for parasites.
- Get blood and ectoparasites from as many herps as possible. These are the quickest and easiest procedures.
- Processing worms takes the most time, so be more selective with this procedure. Try to get a broad sample of biodiversity by processing 2-5 individuals of each host species. Dissect what you can in the field (best case), and freeze the GI tracts of others for dissection in the lab.

Materials

Recording data

- Notebook with >80% cotton paper (this can be a vertebrate notebook with space for recording parasite information, or a notebook dedicated to parasites)
- Pens with indelible ink for writing in notebook
- Permanent markers (VWR #
- Pencil (#2 or harder)
- Pre-cut paper labels on cardstock
- Parasite tags for vertebrates (if necessary)

General

- Scissors
- Bottles for solutions about five 500ml -1L bottles
- Bottle, one squirt bottle for filling vials with ethanol is very useful
- Scalpel + blades
- Disposable pipettes
- This guide to collecting parasites

Blood

- Capillary tubes (70ul heperinized tubes work well)
- Coplin jar
- Filter paper for DNA samples
- Methanol, 100%
- Microscope slide boxes
- Microscope slides, frosted
- Pencil (#2) for labeling microscope slides and filter paper
- Permanent markers for labeling buffer tubes/
- Scalpel with blades

Flu

- Ethanol, 95%
- Scissors
- Sterile cotton swabs
- Vials

Ectoparasites
- Comb with metal tines, ones designed for removing human head lice
- Cotton balls
- Disposable dropers/ pipttes for filling vials
- Ethanol, 70%, 95%
- Ethyl Acetate, chloroform, or fingernail polish remover (with ethyl acetate base)
- Fine tipped (00 or 000) paint brush
- Flea powder (if ethyl acetate is not available)
- forceps
- Forceps
- Handlens
- Mite-light, with batteries (small LED/flourescent ones work well)
- Paper bags for birds
- Paper, large sheets for ruffliing 11x17 is great, otherwise bring tape to put two 8.5x11 sheets togther
- Stapler + staples (bring several) for keeping birds in paper bags
- Tray
- Tupperware containers
- Vials

Worms
- Disposable pipttes for moving worms
- Ethanol, 70%
- Liquid nitrogen tank
- Petri-dishes
- Salt, prepakaged for mixing physiological saline
- Scalpel + blades
- Scissors, high quality for dissections
- Thermos
- Tray for dissections
- Vials
- Vials, cryo

Coccidia
- Pre-packaged potassium dichromate
- Vials, 5-7ml scintillation vials work well, for smaller samples 2ml screw-cap vials work well.

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