COLLECTION PROTOCOLS FOR HELMINTH PARASITES IN RODENTS

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COMMENTS ON THE DISTRIBUTION AND ABUNDANCE OF HELMINTHS

Generally we do not know in advance about the distribution (prevalence and abundance) of parasites in new host-parasite systems that are being sampled. Ideally we should aim (at least) for 30-50 rodents per species at a given location. Trapping effort should be scattered so that all animals do not come from a single habitat patch. This is necessitated because the distribution of parasites both across habitats and within host populations is patchy. This strategy should yield a picture of the common parasite species. Keep in mind that there are also uncommon or opportunistic parasite species. Often the opportunistic "rare" parasites are to be found in older host animals during mid to late summer. These can be rare on the level of the entire population, but they may be common within old age groups that would be soon disappearing from the general population. Consequently, if there are more animals than can reasonably be examined for parasites, the dissections should begin with the oldest animals. This to some extent may also dictate trapping strategy. Old breeding animals are more active and have larger home ranges than those that are young and non-breeding. Consequently it may be wise not to sit on one trapping line for an extended period of time waiting for the last juveniles; instead move traps to a new location when the older animals have been trapped after 1 or 2 days.

Be flexible, try to maximize the materials collected under the circumstances and constraints that develop in the field.

These instructions deal primarily for specimens to be preserved in ethanol. In special collections liquid nitrogen can be used for genetic samples, but ethanol preserved samples are equally good and can also be used for morphology. Therefore, preserving the helminths in ethanol is the basic rule. Do not use formalin because genetic analyses are usually necessary to define the cryptic species.

RODENT GASTROINTESTINAL TRACTS - GENERAL

Dissect/remove the whole gastrointestinal tract and place it on petri dish with a thin layer (max 0.5 cm) of tap water: cut oesophagus before stomach, rectum close to the end, cut ligaments connecting GI tract to the dorsal side of body cavity. In water, using petri dishes of appropriate size (not too small, and plastic ones are practical in field), first uncoil the small intestine by cutting the mesenteries, separate the small and large intestine, and caecum, and process separately (see next paragraph). 1) Cut the stomach separately at the conjunction of stomach and SI. 2) Cut

caecum separately at the conjunction of SI and caecum (warning: *Anoplocephaloides* tapeworms, short [0.5 – 2 cm], compact , can be just at the conjunction). 3) Cut large intestine (colon) after the caecum where the coiling of colon starts. Disregard the colon.

Stomach: Cut the stomach along the surface layer in a Petri dish with some water, and stir the stomach to spread the contents in water. Extra water may then be poured carefully away to see the contents better. Big nematodes are the normal findings, and their presence can often be observed already by the soft/loose structure of stomach wall.

Small intestine: Straighten carefully and simultaneously cut the mesenteries. Open lengthwise starting in the anterior by carefully sliding blunt tipped (iris) scissors to cut open and expose the lumen. Alternatively, dissecting can be started from the posterior end, allowing the dissector to encounter the posterior end of the worm first and thus more easily to save the scolex. This is recommended for newcomers. Try to slide the scissors along the intestine surface layer not to cut the potential tapeworms inside The SI can be cut into 2 or 3 segments to make the dissecting easier though the tapes may be cut accidentally this way. Stir opened SI in water in Petri dish to separate the helminths. Sometimes a tape scolex can be still attached to the SI wall; if so, let it relax for a while, and try again. Remove tapes to a separate dish in 1 cm of water (or more if a big tape) to relax. **When taken out of the intestine, tapeworms are contracted and compact, and they must be relaxed (extended to natural shape) to make the internal organs visible for microscopy**. Stir carefully the relaxing tape a couple of times so that possible intestinal debris will be detached from the surface. Change the water if there is too much "mud" and stir again. Too much debris on the surface of the tapeworm prevents the good microscopical visibility into the in internal organs. Record the location of the worm in the small intestine (first, second, or third part – this is important because various species/species complexes have specific locations in the intestine). If the relaxing tape has no scolex, look carefully for the scolex in the water of the dissecting Petri dish -- this will be helped by using a dissecting scope or magnifying loop. Scolices of all hymenolepidids are tiny, hardly visible by naked eye!

Caecum: Nematodes and small flukes may be present in the caecum. as well as short compact *Anoplocephaloides* tapes. It may be useful for a newcomer to run material through a small sieve to first discard some fine particulates. Often trematodes in the caecum of *Microtus* are covered with "greenish mud" and are difficult to separate from plant materials. Also some nematodes are relatively small and obscure (whitish, some millimeters). Dissecting microscope or good magnifying glass is recommended. If the caecum is large (*Microtus*), it can be washed in two or three parts.

Other organs: Helminths, especially larval tapeworms can be found in other organs, too. Most prevalent of these are larval *Taenia* species. There can be small or bigger cysts in liver, smaller or bigger (up to (1.5 cm) larvae free in the coeloma or thorax. There can also be small nematodes in the lungs (see later).

PROCESSING THE SAMPLES

Rodent cestodes. Following collection from the small intestine and caecum, each specimen should be held in water (filtered/tap, depending on circumstances) for an extended period, minimum 2 hours, but preferably much longer. If the tapes have been dissected in the evening, they can well be relaxed in water overnight. This allows the tapeworm to fully relax, which is necessary to examine the internal structure of the proglottids (segments). Relaxation is very important for big and thick tapes. Some Hymenolepidids are small and thin, especially the neck and scolex, and a shorter relaxation time is OK

Following relaxation and death in water, all tapeworms will be preserved in 70% ethanol. Preservation should be done flat. This is done in a petri dish of suitable size overnight. It is good to have also large Petri dishes because the big *Paranoplocephalas* are quite long, (up to 15 - 20 cm) when relaxed. The long ones can be fixed as a large circle, but be careful to uncoil the worm so that is really flat. Pour 70% ethanol into the Petri dish, and then carefully place the tape straight and flat on the dish. The big long tapes can be cut into two or three parts if the size of Petri dish does not allow fixing flat otherway. After fixing the tape long enough, the specimen is transferred to a vial with 70% ethanol. Use the appropriate size vial for the specimen so there is sufficient preservative (a ratio of about 5:1 in volume for preservative relative to the specimen is maintained). Some tapeworms in *Microtus* are quite large (up to 20 cm), so be certain to use the proper size vial - one that is large enough for the worm and a sufficient amount of ethanol. The preservative should be changed once after 24 hours, especially for the large-sized species.

Marmot and porcupine tapes are big, thick and abundant! Special care should be made when relaxing, washing and fixing them flat. A lot of surface "mud" will appear from these big tapes, and therefore the water should be changed a couple of times. Remember that studying and caring the material from one marmot or porcupine intestine can take the whole day. If you shoot them, be prepared to modify the daily program and be sure that you have tens of large petri dishes.

DIGENEA (FLUKES). Flukes can be relaxed in water, which often allows specimens to expel eggs that might otherwise obscure some organs. Preserve flukes in 70% ethanol.

NEMATODES. Nematodes should not be held in water for extended periods of time, as osmotic pressure will eventually cause the specimen to burst. Specimens should be washed in water or saline and then soon preserved in 70% ethanol. Keep parasites from different organ systems separate.

Be careful with the nematodes in lungs if they will be studied. There are two types: One forms greenish patches clearly visible on the surface of lung lobes, mostly in old breeding voles – fix the whole tissue patch in 70% ethanol. The second type, more difficult to observe, is in the big arteries of heart and lungs. These *Angiostrongylus* nematodes are 10-20 mm long, and can be seen as thin, dark, threadish formations when a lung lobe is teared apart from the heart. But do not play too much with lungs not contaminate them if lungs needed for hanta analyses.

LABELS AND NOTES: It is very important that all samples are well marked. The minimum is that there is paper label with a sample code number, written with pencil, in the vial, and the code number

certainly refers to the right host individual. If several sample vials are used for parasites from one host, it helps to have them marked (e.g. 22/1, 22/ 2, or 22a, 22 b). In the notes, the number of parasites found (best estimated by the umber of scolices if the worms have been cut during dissection), their location (organ, segment of SI), should be marked. Also negative hosts should be marked in the notes.

It is preferable to keep the vials in a cool place.
Keep the instrument clean between the individuals to avoid contamination of samples.

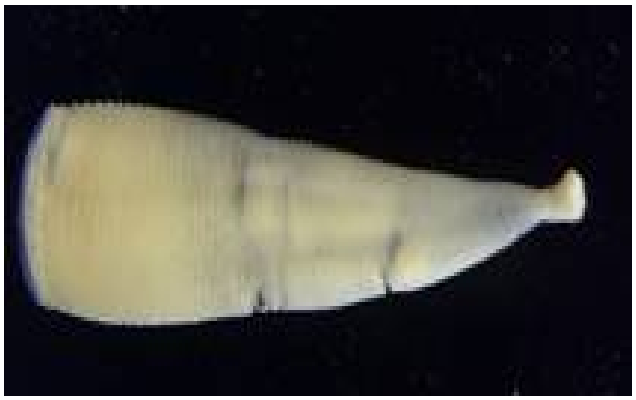
ALTERNATIVE WAYS

It is not always possible to dissect the rodents and intestines at the trapping locality the same day. The rodents, or worms in the intestines, stay OK for a couple of days in refrigerator If there is a freezer available, the intestinal tract can be detached and placed in small self-sealing (minigrip) plastic bag, air pressed out, and frozen. Normal freezer is OK. Frozen guts can be studied later and the material is good enough for microscopical and genetic studies. Frozen material can also be sent as air freight. Once frozen and thawed, tapeworms relax quickly when placed in water. Otherwise the procedures are as described above.

Fixing the whole intestine in ethanol is a possible but the least recommended method. The helminth material may be OK for genetics, but the worms remain contracted and are not good for microscopy. If the whole intestines will be preserved, enough ethanol should be used, and it must be changed next day, because intestinal material contains a lot of water, which dilutes the ethanol.

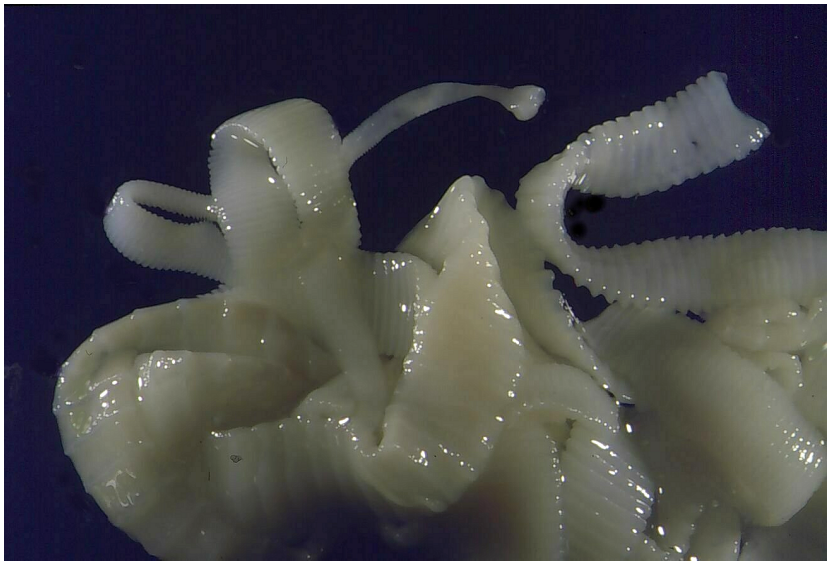
SOME EXAMPLES
The examples below deal only with anoplocephalines. Other typical cestode families in rodents are Hymenolepididae and Catenotaeniidae.

A typical specimen of *Anoplocephaloides dentata* species complex. The length of the tape can be 0.5-1.5 cm. Specimens of this group are found at the very end of small intestine or in caecum.



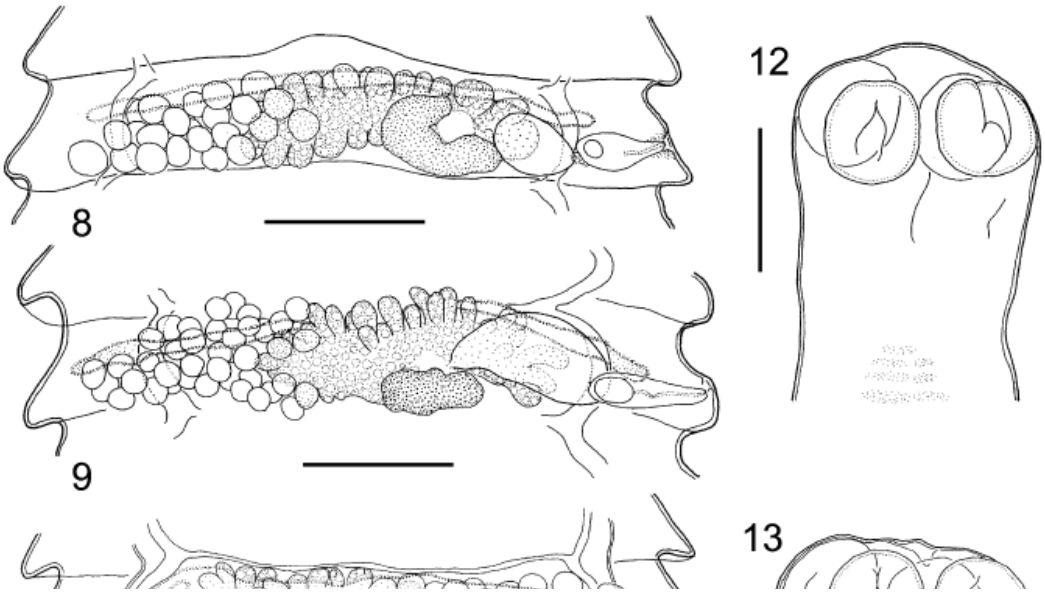
Typical specimens of *Paranoplocephala* species group, here *P. alternata*. *Paranoplocephala* species are usually found from the middle to ¾ of the small intestine.
Attention! Notice the thin neck posterior to the scolex (in low center). The neck can easily break and it is important to check that that the scolex is attached, and if not, the scolex should be searched on the petri dish.

Paranoplocephala batzlii exemplifies a big robust species in this group. The big paranoloplocephalas can be up to 15 – 20 cm long, and are easily observed through the intestine before dissection.

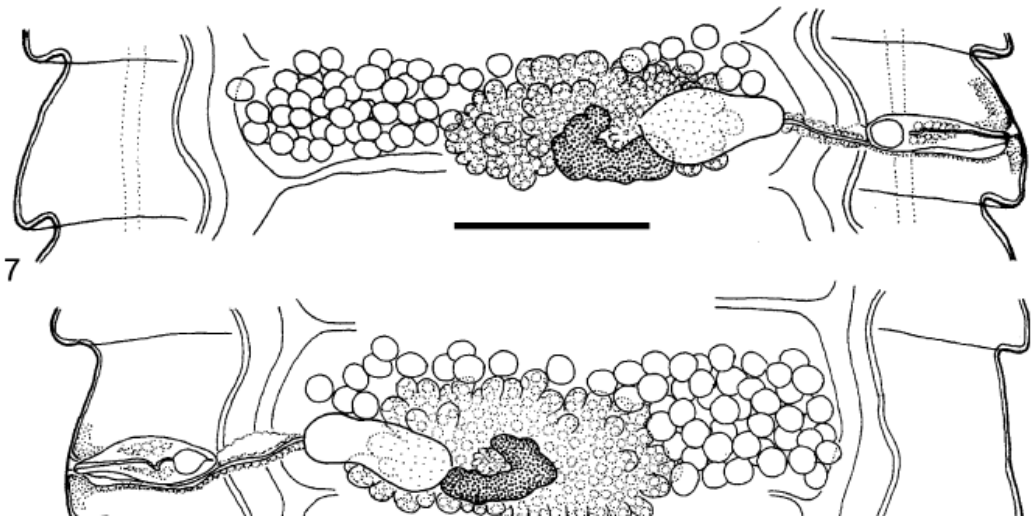


Examples of internal structure of proglottides in anoplocephaline tapeworms, indicating the size and location of genitalia (emphasizing why the proper treatment of samples is important).

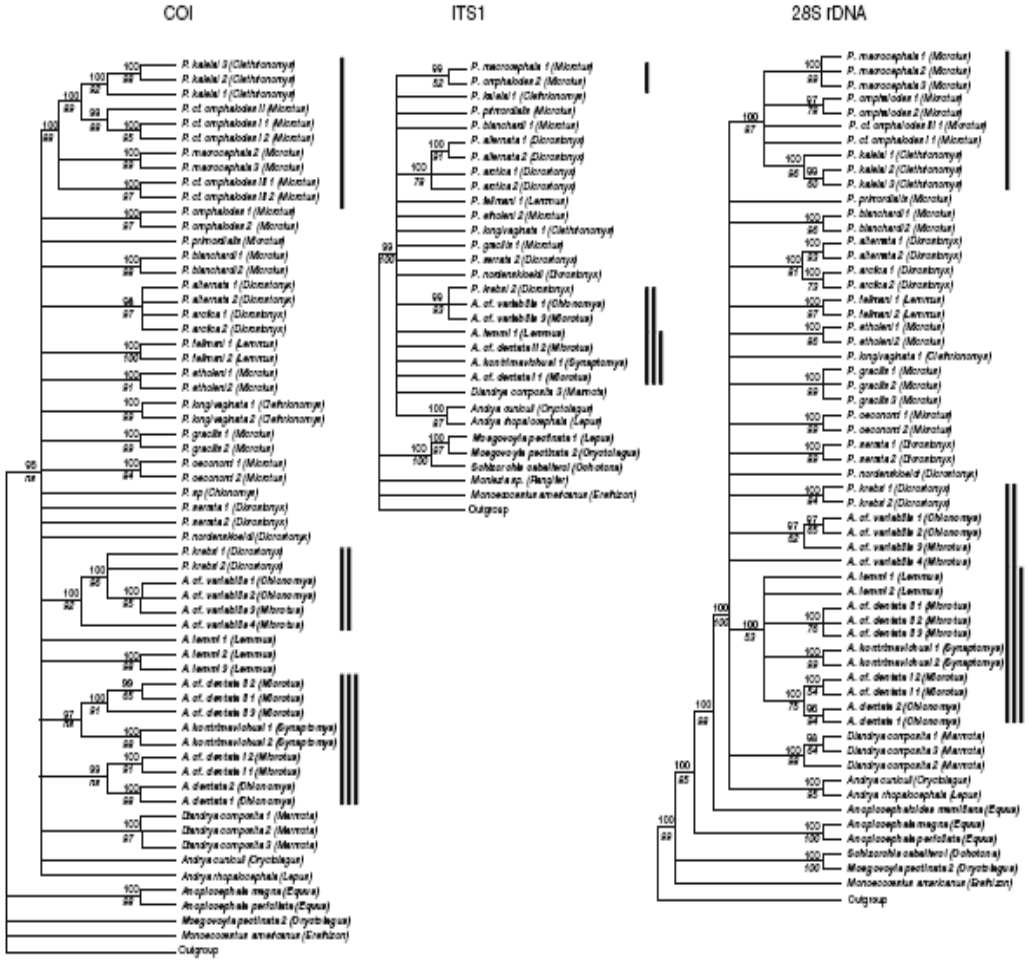
Microcephaloides, n.g., Haukisalmi et al., 2008.



Paranoplocephala buryatiensis, n.sp., Haukisalmi et al., 2007.



The phylogeny of anoplocephalines in arvicoline rodents (Wickström et al. 2005, Syst. Parasitol. 62:83-99), based on three genetic markers.



Figures 11. Phylogenetic trees for anoplocephaline cestodes produced by Bayesian analysis of individual sequence data-sets of COI (522 bp of which 204 informative), ITS1 (408 bp of which 227 informative) and 28S rDNA (1158 bp of which 223 informative). Bayesian posterior probabilities are shown above the branches. Branches with posterior probabilities < 95% have been collapsed. Bootstrap values (> 50%, based on NJ with ML distances) are shown in italics below branches. Other branches have < 50% bootstrap support (ns). Species with a tubular early uterus are shown in bold, species with a reticulated early uterus in normal font. The three subclades within the 'arvicoline clade' have been indicated by vertical lines (clade I = single line; clade II = double line; clade III = triple line).

If some one is interested in reading more on the topic, here some references on the taxonomy, phylogeny and phylogeography of anoplocephaline tapeworms in rodents.

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