The following guide is designed to provide basic information on the killing, fixation and preservation of crocodilian parasites.

**Acanthocephalans (spiny-headed worms)**

**Dissection and Preparation for Killing:** Acanthocephalans have been documented within the intestines of crocodilians. These parasites can be found attached to the intestinal lining by their anterior end (known as a proboscis) that is covered or armed with recurved hooks. This proboscis is one of the key characteristics for identifying acanthocephalans, so one must be extremely careful when removing these parasites from their hosts.

Once the acanthocephalans have been removed from the host, place in a petri dish of distilled water. Over some time, their proboscis will remain extended and they will slow down movement. At this time, pipette out the water leaving only a little bit covering the acanthocephalans.

**Killing and Fixation:** Acanthocephalans need to be killed in very hot or boiling 10% formalin or 70% alcohol. This assists in maintaining the proboscis extended, which is necessary for species identification. Once the excess water is extracted, pour the hot liquid into the petri dish. Boiling formalin is dangerous because it releases formaldehyde gas into the room. This can be minimized by boiling formalin under a fume hood or in a highly ventilated room. Further minimization can be accomplished by boiling only one tube at a time.

**Preservation:** Once the worms have been “stunned” by the hot formalin or alcohol, remove them with a small paint brush into a vial of 70% alcohol.

**Nematodes (roundworms)**

**Dissection and Preparation for Killing:** Nematodes are usually freely moving within the intestines, stomach, mesentery, etc. of crocodilians. They can be removed with tweezers or a small paintbrush and placed in a petri dish of saline water. The saline water assists in cleansing the nematode of any secretion or mucus.

**Killing and Fixation:** Nematodes need to be killed in a boiling or near boiling solution, which causes heat rigor. This causes the worms to become relatively straight as opposed to tying themselves in knots, which is easier for species identification. Please note that even with heat fixation, nematodes may die in a species-specific shape (eg coiled, straight).

To kill the worms, draw off (or pipette) all the saline water so the worms are left dry in the petri dish. Then poor a good amount of boiling water, boiling 70% ethanol or boiling glycerin alcohol (see below) over the worms. Worms killed in hot water need to be moved fairly quickly into fixative otherwise they may become “cooked”.

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**Crocodilian Parasite Preservation**

Prepared for the CSG by Dr. Marisa Tellez
Preservation: Once the nematodes have been stunned by the hot liquid, gently pick up them up with a probe or small paint brush and place them into a vial containing one of two fixatives: glycerin alcohol (see below) or 70% ethanol if glycerin alcohol is not available. Make sure there is enough space in the vial for the worms to extend.

If there are quite a few worms, it is always a good idea to preserve a few living worms in cool pure 95% ethanol for genetic analysis (best not to use denatured ethanol).

**Pentastomids (also known as tongue worms)**

**Dissection and Preparation for Killing:** Crocodilian pentastomids can be found in the esophageal tract or lungs. These parasites embed into their hosts using two pairs of hooks or claws on either side of the mouth. Low numbers of parasites do not seem to harm crocodilians, but if parasitic intensity is high, pentastomids have been associated with crocodilian mortality in farms.

The easiest way to remove pentastomids from lung tissue is to allow them to let go from the dissected tissue and wait for them to eventually surface to the outer area of the tissue. Using fine-point tweezers you can remove the pentastomids and place them in a petri dish of saline water to remove mucus. If pentastomids are still attached to tissue, you can use tweezers to slowly remove them from the tissue. Wiggling the pentastomids a bit loosens them from the tissue and they eventually let go. Do not pull hard on them as you may damage the specimen.

**Killing and Fixation:** Remove excess water from petri dishes, only leaving enough just covering the pentastomids. Then pour boiling 70% ethanol over the pentastomids.

Preservation: Once the parasites have been killed, remove them with tweezers and place in vials of glycerin alcohol (see Preservatives/Fixatives below).

**Trematodes (flatworms or flukes)**

**Dissection and Preservation:** Crocodilian trematodes have been documented in every organ. These parasites attach to their host by an oral sucker that surrounds the mouth, and another sucker on the ventral surface of the body. Like acanthocephalans and pentastomids, one must be very careful in removing trematodes. Using fine-point tweezers, you can slowly remove the trematodes from the host lining. You can also cut the host tissue around the trematode and place this on a large petri dish. You can then slowly tease away the host tissue from the trematode, making sure not to damage the oral sucker.

Once the trematodes have relaxed, pipette out excess water, leaving only a little in the dish to just cover them.

**Killing and Fixation:** Heat up 10% formalin to boiling, and pour over the trematodes.

Preservation: Once the formalin has cooled, move the trematodes with a paintbrush to a vial of 70% alcohol.
Preservatives/Fixatives

Glycerin Alcohol (from 70% ethanol)
70% Ethanol  90 parts
Glycerine  10 parts

Glycerin Alcohol (from 95% ethanol)
95% Ethanol  65 parts
Water  25 parts
Glycerine  10 parts

10% Formalin
40% Formaldehyde (by volume)  10 parts
Water  90 parts

Recommended Reading


