Bocas ARTS

Video transcript

How to preserve nemerteans

Svetlana Maslakova

- After you have examined live nemertean and documented features of external appearance and stylet armature, you are ready to preserve your samples for DNA-barcoding and histology.
- For preserving nemerteans it is very important to relax them first, so that they don't fragment into small pieces upon contact with fixative.
- To relax a nemertean you will want to gradually add magnesium chloride to seawater containing your specimen and pipette it to mix it in.
- Then you will want to wait for at least a few minutes to make sure your nemertean is not reacting to that by fragmenting, and then you can add a little bit more.
- Completely relaxing a nemertean can take anywhere between half an hour to several hours, depending on the size of the individual and the species.
- Once the nemertean is completely relaxed, you can now preserve it.
- It is a good practice to preserve a small portion of each individual for DNA-barcoding and save the rest for histology.
- To preserve a portion of nemertean for DNA extraction, you will want the middle part of the body.
- So you will make the first cut somewhere in the middle of the worm, and the second cut posterior to that.
- Now you will take a vial and fill it with 95% ethanol to preserve tissue for molecular work. Using a pair of forceps pick up the middle portion of the worm and put it in a vial. Close the vial and label it.
- Samples for DNA extraction stored in 95% ethanol should be kept as cold as possible, ideally in - 20^0C or - 80^0C freezer.
- To preserve tissue for histology, you will need a series of solutions:

• 10% formalin made up in seawater

• Bouin's fixative

• 30% Ethanol

• 50% Ethanol

• 70% Ethanol

- And you will also want a pair of forceps, a pipette and a small tray to handle formalin tissue.

- If you are handling multiple samples, you will want to rinse your tools between the samples so you don't contaminate DNA from one individual with DNA from another individual.
- For that you will need 10% bleach and water
- Histological fixatives such as formalin and Bouin's contain harmful volatile compounds and you should work with them in a chemical hood.
- You will pipette the anterior and posterior portion of the specimen into the little dish or tray and straighten it out as much as you can for sectioning.
- And then you are going to slowly drip formalin (this is 10% formalin buffered in seawater) on top of the specimen using a pipette for formalin. You can manipulate the specimen so it remains straight.
- After a few minutes it will harden up sufficiently so you can transfer it into a vial.
- You can fill it up with formalin and leave it in the hood for 12 to 24 hours.
- After 12 to 24 hours you can replace formalin with Bouin's.
- You will pipette formalin out of your vial into a labeled waste container designated for formalin and then you will replace it with Bouin's.
- Bouin's is not required for histological preservation but it makes staining and tissue preservation a lot better, so if you have access to it, use it.
- You can leave your specimens in Bouin's from 1 to 3 days.
- After 1 to 3 days in Bouin's, you'll want to replace Bouin's with 70% ethanol.
- It is better to replace it gradually through a series of ethanol concentrations: from 30% to 50% and then to 70%.
- If you place your specimens into 70% ethanol directly, the tissue will shrink very rapidly and it can be damaged.
- So pipette the Bouin's out of the vial into a labeled waste container dedicated to Bouin's solution. And pipette some of the 30% ethanol into the vial, and leave it from 30 to 60 minutes.
- After 30 to 60 minutes, you can replace the 30% ethanol with 50% ethanol and leave it for another 30 minutes.
- After another 30 minutes in 50% ethanol you can replace it with 70% ethanol.
- You should replace 70% ethanol daily or as often as possible until it no longer turns yellow.
- These specimens will be stable for many years and will yield superior histological results.