Bocas ARTS

Video Transcript

How To Make An Ascidian Spicule Prep

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- Some ascidians produce calcareous spicules that accumulate in their tunic or internal tissues.
- While not all species have unique spicules, they still have taxonomical value and help discriminate among species.
- The spicules are very tiny, usually between 15 and 100 microns, so we have to prepare them to be studied with a scanning electron microscope.
- So this is a didemnid colony and I am going to take a small piece.
- If the species is brittle, this means that it has a lot of spicules, so you need a small piece.
- But if it is softer, you might need a larger piece, this comes with practice.
- Now, I am going to take this piece, and burn it in an ethanol lamp.
- You have to burn it until it gets dark… so now it is burnt.
- I am going to put it inside this crucible, and there are still residuals of tissue in this preparation, so I am going to use bleach to get rid of this residue.
- After like 15 to 20 minutes in the bleach, it is time to wash the preparation.
- So the spicules are accumulated at the bottom, and I am taking only the liquid out; be careful not to disturb the spicules because they are very tiny and you do not want to pipette them out.
- After pipetting most of the liquid, you can wash them with water.
- Let them accumulate again on the bottom of the crucible for a few minutes, then we are going to repeat this procedure 2 or 3 times to make sure the bleach is completely out of our preparation.
- Bleach will make crystals on the spicules if it dries out.
- After the spicules are cleaned, we are going to pipette them to an Eppendorf, to store them.
- You are going to wait again for a few minutes for them to accumulate in the bottom of the Eppendorf.
- Now, I am going to take most of this water out, and store the spicules in ethanol.
- Be sure that you label your Eppendorf and match this number with the sample number in the spreadsheet in your computer.
- This is how the didemnid spicules look under the scanning electronic microscope, they are usually stellate but the number of rays may change.
- So you want to count the number of rays in an equatorial plane, take note of the shape of the rays they can be more conical like these ones, or more cylindrical, they can be short or longer, and they can have blunt tips or acute tips.

- This animal here is from the genus *Herdmania;* it is a solitary ascidian already dissected without its tunic and you can see that in the body wall, there are lots of needle-shaped spicules.
- They look like small white threads accumulated on the tissue, and the long white threads underneath them are the muscles.
- In this picture you can see that they also accumulate in the whole body wall.
- Although, these spicules are longer, and you can see them under the dissecting microscope, it is always good to make preps for the scanning electron microscope to see the details.
- The procedure will be exactly the same as what we have done for the didemnid
- Some species either Stolidobranchia have spines in their siphons, so this is something else that you have to prepare to study in detail under the microscope.
- And in this dissected specimen, here we have the oral siphon and the spines are usually found along the border, where the tunic enters the siphon, or also, on the lining of the siphon.
- You just have to take a small piece of the siphon with the border and make a slide to check on the optical microscope.
- Then you put a cover glass and check if the slide is thin enough to see the spines.
- Some spines inside the *Pyura*'s siphons, oral siphons, can be large enough that you can see them under the dissecting microscope.
- These white lines showed in the picture are the spines.
- When you see them under the optical microscope, they will show like this and it has some texture on the surface, you can measure its size and note its shape.