Bocas ARTS

Video Transcript

How To Dissect a Colonial Ascidian

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- In this video I am going to show you how to dissect a colonial ascidian.
- Since ascidians are fixed in formaldehyde, you might have to plan a little bit and take them out of the formaldehyde the day before of the dissection.
- You have to rinse them and maintain them underwater overnight.
- Even if this is water, it is better to use gloves to manipulate the animals.
- I am putting this animal here on a petri dish to make some slices, to be able to remove the zooids from the colony.
- You can make the slices either with a scalpel or a razor blade. These slices do not have to be too thin, and this way, you will have zooids on both sides of the slices.
- Now, I am going to put the slices on another petri dish to study them under the dissecting microscope.
- At this point, we can study the organization of the zooids inside the colony. If they are vertical or more oblique. How distant they are from each other. If there is a common cloacal channel or if both siphons go all the way to the surface of the colony. If there are any structures in the tunics such as sand, shell fragments or even algal symbionts.
- To remove the zooids from the colony, you have to use a probe. I do mine with entomological pins that are very thin, and you cut around the tunic and liberate the zooids.
- Some of those you can remove very easily, some take more time. Even if you break them and separate the thoraces from the abdomens, it is not a problem, you can save both and study part by part.
- Now we are going to move the zooids from this preparation towards a watch glass to stain them.
- We use this watch glass which is round with a round bottom so the zooids can accumulate in the center with enough volume of water and stain to be colored.
- And you just put 1 or 2 drops of stain, and mix it with the zooids and the water.
- Look through the microscope to be sure that the zooids are not floating and they are under the solution with the stain.
- It will take 1 or 2 minutes to stain the zooids and it’s better not to stain too much. If it is necessary, you can repeat the procedure.
- Now I am going to clean my zooids from the stain. Just pour more water on them, and taking the excess of stain with the pipette.
- Look through the microscope to be sure no zooids are going to be pipetted out.
- We are going to repeat that a few times until all the color is gone.

- When the preparation is cleaned, you can transfer it to another petri dish with a flat bottom to be studied under the dissecting microscope, and if the zooids are very small, you can prepare glass slides to study them under the optical microscope.
- After you finish your study, you might want to keep your zooids, so you just have to get them back, always with the pipette and put on a small Eppendorf like this or even smaller and maintain them in formaldehyde as you maintain your colony.
- So I am going to wait for them to reach the bottom, take the excess of water and complete the Eppendorf with formaldehyde.
- You are going to keep you Eppendorf with the zooids, the slices of the colony and the colony in the same jar.
- If you need to study your zooids again, you will only need to stain them because with time, the stain will fade in formaldehyde.
- Since didemnids have lots of spicules in their tunic, it is very hard to make slices to remove the zooids.
- So before, we are going to decalcify the colony with hydrochloric acid (HCl).
- Take a solution of hydrochloric acid that is weak like 4% or 5% and you are going to cover the material with this solution.
- Just take a piece of the colony, you do not want to lose all the spicules because they have taxonomic value.
- After some minutes, you are going to see bubbles coming out of the material. This is because the hydrochloric acid is dissolving the calcareous spicules.
- When everything is dissolved, it is possible to see the zooids spread along the tunic, and it will be much easier to make the slices.
- There will be zooids in both sides of the slices, since didemnids are very thin, the zooids are very small, and you have to work under the dissecting microscope.